SCUOLA NORMALE SUPERIORE

Pisa

CLASSE DI SCIENZE MATEMATICHE, FISICHE E NATURALI

CORSO DI PERFEZIONAMENTO IN NEUROBIOLOGIA

21st of January 2014

PhD Thesis:

Activity-Driven Plasticity Of Visual Cortex Circuits In Physiological And Pathological Conditions

candidate: Marta Pietrasanta

supervisor: dr. Matteo Caleo

ACTIVITY-DRIVEN PLASTICITY OF VISUAL CORTEX CIRCUITS IN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

Activity-Driven Plasticity Of Visual Cortex Circuits In Physiological And Pathological Conditions

INTRODUCTION

PART ONE: Mechanisms of OD plasticity in primary visual cortex

THE VISUAL SYSTEM AND THE CORPUS CALLOSUM

The Visual System

Understanding how the brain changes as a function of experience is an issue of great interest for neuroscientists. The relative contribution of genes and environment in brain development has long been debated. Many studies have addressed the role of experience in the development of the visual system, taking advantage of the relatively easy manipulation of visual inputs. Classic experiments have been performed in cats and primates, while rodents have become popular quite recently due to the advent of gene manipulating techniques and to the possibility of combining physiology with biochemical and molecular analysis. There are some differences in the anatomical organisation of visual pathways in the different species, even if the basic aspects are conserved in all mammals.

The sensory structures are represented by the eyes: light enters the eye by first passing the cornea and finally reaching the very back of the eye, the retina. The retina is responsible for converting light into neural signals that can be relayed to the brain. The retina is a very specialised sensory structure, consisting of a team of different types of neurons whose role is to collect light, extract basic information and pass the pre-processed image to visual structures in the brain. These cell types are photoreceptors, bipolar cells, horizontal cells, amacrine cells, and ganglion cells. They are arranged within the retina in precise layers.

Axons from the ganglion cells bundle together to form the optic nerves. Fibres from the nasal half of each retina turn towards the opposite side of the brain in a point called optic chiasm, while the fibres from the temporal half of each retina do not cross. In the rodent visual system the vast majority of the fibres cross at the chiasm (only 3-5% of the optic axons remain ipsilateral), while the percentage of decussating fibres is lower in carnivores and primates.

Past the chiasm, retinal ganglion cell axons run within the two optic tracts. Each optic tract carries a representation of the contralateral visual field. Retinal inputs terminate within two major subcortical visual structures, the superior colliculus (SC) and the dorsal geniculate nucleus (dLGN), a portion of the thalamus. In rodents, all ganglion cells project to the SC, and 40% of the retinal fibres send a collateral to the dLGN.

The dLGN is the structure that relays input to visual cortex. In each dLGN there is a retinotopic representation of the contralateral visual field. In primates and humans, the

dLGN contains six layers, each of whose receives inputs from one eye only. Indeed, retinal axons coming from the two eyes terminate in adjacent but not overlapping eye-specific layers that are strictly monocular (Hickey and Guillery 1974). In rodent the dLGN contains two patches, each receiving eye-specific input (Godement et al. 1984). The inner core is ipsilateral, surrounded by a contralateral patch.

Projections of neurons in dLGN reach the primary visual cortex, or V1, in the occipital portion of the brain. The V1 is a layered structure (layers I-VI). The major layer of inputs from dLGN is layer IV, then neurons in layer IV relay their information to layers II/III, that in turn communicate to layer V-VI. In carnivores and primates, inputs of each eye reach layer IV into alternating stripes, the ocular dominance (OD) columns (Hubel and Wiesel 1963).

In rodents there are no ocular dominance columns, because inputs coming from two eyes converge on the same postsynaptic target cell at the level of layer IV (Antonini et al. 1999).

V1 is the first station through the visual pathway where retinal inputs collected separately by the two eyes interacts directly. Although V1 receives input from the two eyes, information concerns always the contralateral half of the visual field. Many mammals have binocular vision, and their visual cortical neurons can respond to stimulation of both eyes, even if the response to one eye can be predominant (eye preference). The most binocular portion of the primary visual cortex is in correspondence of the cortical representation of the vertical meridian of the visual field, and lies at the V1/V2 border.

Together with the dLGN, another important input to V1 is the corpus callosum, the major fibre bundle in the brain interconnecting the two hemispheres, that sends a massive projection to the V1/V2 border. Its specific pattern of connectivity led many researchers to question whether the callosum has a role in determining cortical binocularity, as described more in detail below.

Besides ocular dominance columns, V1 has a columnar systems for orientation selectivity, and regions called "blobs" with specific responses for coloured stimuli, These columns communicate together by means of long-range horizontal connections. These connections allow individual cells to integrate information from a wide area of cortex (Gilbert 1992).

V1 cortex is responsible for creating the basis of a three-dimensional map of visual space, and extracting features about the form and orientation of objects. Once basic processing has occurred in V1, the visual signal goes to secondary visual cortex (V2), which surrounds V1. Secondary visual cortex is principally responsible for perceiving colours and forms.

Primary visual cortex contains two main types of neurons: pyramidal cells are projection neuron, while non-pyramidal cells represent local interneurons. There are several different

classes of pyramidal cells and interneurons, and their physiological, anatomical and molecular diversity is the subject of ongoing research.

The Corpus Callosum

From playing the piano to riding a bike, inter-hemispheric communication is a crucial tool that our brain uses to perform a variety of everyday actions, from very simple to complex behaviours. One of the most important pathways through which this communication is achieved is the Corpus Callosum, the major fiber bundle in the brain (Berlucchi et al. 1995; Innocenti et al. 1995; Gazzaniga 2000). In humans it contains about 170-190 millions of fibers that interconnect homologous cortical areas in the two hemispheres, as estimated by a pioneer work on human brains by Tomasch in 1954 (Tomasch 1954).

A number of experiments and observations suggest that the two hemispheres could inhibit each other via the callosal pathway, to achieve the segregation of lateralized functions such as language or face recognition (Gazzaniga 2000; Bloom and Hynd 2005). However, other experiments suggest that the callosal pathway provides an excitatory input to the opposite hemisphere that enables cortical integration (Bloom and Hynd 2005; Paul et al. 2007).

In vision, our main sensory system, the corpus callosum serves to bind together the separate representations of the two halves of the visual field (Berlucchi and Rizzolatti 1968; Innocenti 1980; Innocenti et al. 1995). Indeed, each hemisphere receives information from the opposite visual hemi-field; thus, the visual world is represented discontinuously in cortical maps, being split between the two hemispheres along the central vertical meridian. One key role of the callosum is to combine these two partial cortical maps of the visual field into a single, coherent representation (Houzel and Milleret 1999). Recent electrophysiological data obtained in the ferret confirm that callosal connections integrate the visual field across the vertical midline in a stimulus-specific manner (Makarov et al. 2008).

Anatomy Of Callosal Projections In The Visual Cortex

Consistently with a role in perceptual binding, callosal connections are strongly concentrated in a zone at the border between areas 17 and 18, corresponding to the representation of the vertical meridian in cats, macaques and humans (Van Essen et al. 1982; Clarke and Miklossy 1990; Houzel and Milleret 1999). Recent studies have employed diffusion tensor imaging to show evidence for callosal connections in human V1 (Dougherty et al. 2005; Putnam et al. 2010; Saenz and Fine 2010). The callosum links cortical loci that are in retinotopic correspondence (Lewis and Olavarria 1995; Olavarria et al. 2008a). Neuroanatomical tracing in cats shows that clusters of callosal boutons are

preferentially distributed in regions representing also the same orientation and not only the same visuotopic location in the opposite hemisphere (Rochefort et al. 2009).

In cats, inter-hemispheric fibres originate from a narrow transition zone between area 17 and 18 (Innocenti 1980) and, according to the general rule that retinotopic loci are callosally connected, callosal terminals in the opposite hemisphere are particularly concentrated in this area 17/area 18 border (Blakemore et al. 1983; Payne 1990; Payne and Siwek 1991). Tracing studies show that callosal axons display 2 or 3 clusters of synaptic boutons in layers 2-3 and the upper part of layer 5 (Rochefort et al. 2009).

In rodents, different from cats and primates, the entire extent of the primary visual cortex contains callosal cells (Olavarria and Van Sluyters 1995). However, their terminals are still particularly concentrated in a quite narrow stripe at the area 17/18 border (Jacobson 1970; Cusick and Lund 1981). Recently, Mizuno et al. (Mizuno et al. 2007) have confirmed this findings in mice by labelling callosal axons via in utero electroporation of green fluorescent protein. These experiments have also shown that axonal arborisations of callosal cells are mainly located in layers 1-3 and layer 5 (Jacobson and Trojanowski 1974; Mizuno et al. 2007).

Callosal cells do not constitute a homogenous population, since they have different morpho-chemical phenotypes (Innocenti 1980; Voigt et al. 1988; Buhl and Singer 1989). In cats, the vast majority of callosal neurons are large pyramidal cells, and immunohistochemistry observations and studies using the selective uptake of radiolabeled transmitters have failed to identify GABA containing callosal neurons (Elberger 1989). Nevertheless, experiments with retrograde transport of horseradish peroxidase injected into border region of the opposite hemisphere report the occasional observation of transcallosal non-pyramidal cells in cats (Peters et al. 1990). Also, transiently during early rat development immunocytochemical staining reveals numerous GABA-positive fibres in the callosum, which largely disappear at later stages (Kimura and Baughman 1997). In keeping with these results, our group has shown that in juvenile rat visual cortex only 1% of callosally projecting neurons display GABA immunoreactivity (Restani et al. 2009).

In monkeys, chimpazees, and humans, callosal axons of distinct size interconnect functionally different cortical areas (Caminiti et al. 2009; Tomasi et al. 2012). The axons originating from each cortical site cover a considerable range of conduction velocities, dispersing in time the action potentials transmitted to the other hemisphere. A wide range of temporal delays might expand the number of neuronal ensembles that transcallosal connectivity can activate (Caminiti et al. 2009).

<u>Binocularity</u>

In rodents, as in other mammals, visual input from each eye is transmitted first to the dorsal lateral geniculate nucleus (dLGN) through retinogeniculate projections and then to V1 via the geniculocortical pathway. In the rat visual system, the vast majority of the retinal fibres cross at the chiasm [only 3–10% of the optic axons remain ipsilateral; (Lund 1965; Cowey and Perry 1979)]. This means that ipsilateral eye input to V1 would be little and binocular cells should consequently be rare. However, most cortical neurons (> 80%) in the lateral segment of V1 (mapping the central part of the visual field) are binocular (Fagiolini et al. 1994; Caleo et al. 1999a; Caleo et al. 1999b; Di Cristo et al. 2001). The origin of binocularity has not been clarified. Using morphometric measures, Coleman et al. suggested that the contralateral ocular dominance (OD) in the primary visual cortex of the mouse can be accounted for solely by the relative density of feed-forward geniculo-cortical inputs from the two eyes. Indeed, the contralateral eye pathway exhibits a higher degree of convergence on geniculate neurons than the ipsilateral eye pathway (Coleman et al. 2009). Work by other authors suggest that binocularity might be provided via other pathways, as described in more details below (see below: The role of corpus callosum in cortical binocularity).

Binocularity is a key property of visual neurons, not only because it the base of stereopsis [for a review see R. Blake "Binocular Vision" (Blake and Wilson 2011)] but also because neurons in a plastic visual cortex can adapt to a changing visual experience with modifications in binocularity. This property is therefore used as an index of cortical plasticity.

How the inputs coming from the two eyes combine in binocular neurons has long been debated. Psychophysical observations report that binocular viewing is superior to monocular when it comes to threshold tasks such as contrast detection, a superiority that lead to the idea of binocular summation (Blake and Wilson 2011). Conversely, other authors report that doubling of the input to the visual cortex in binocular vs. monocular viewing hardly results in a noticeable difference in our subjective perceptual experience (Moradi and Heeger 2009). Only at low contrasts and/or for brief stimulus presentations does binocular stimulation affect psychophysical performance: visual acuity (Cagenello et al. 1993; Zlatkova et al. 2001), contrast sensitivity (Legge 1984), orientation discrimination (Bearse and Freeman 1994), or perceived contrast (Baker et al. 2007). These effects are negligible at higher contrasts, leading to the idea that cortical contrast normalisation may involve suppressive rather than additive interactions in V1.

Pioneering electrophysiological experiments to investigate how the two eyes inputs combine in binocular neurons have been performed during depth perception studies in cats, but yielded contradictory results. Some neurons showed additivity between responses evoked through the two eyes, while other displayed inhibitory interactions (Pettigrew et al. 1968; Bishop et al. 1971; Ferster 1981). In rodents, a recent study aimed at describing binocular interactions in V1 neurons has been recent published, and shows with intracellular recordings that binocular inputs to layer 2/3 pyramidal neurons are integrated sub-linearly in an amplitude-dependent manner (Longordo et al. 2013). To our knowledge, more in depth analyses on binocular interactions in rodents are not published.

SHAPING THE BRAIN: EXPERIENCE-DEPENDENT PLASTICITY

To date it is well accepted that, after an initial period during which nervous system development is strictly controlled by genetic programmes, successive aspects of brain development require neuronal activity to reach complete maturation (Zhang and Poo 2001; Sengpiel and Kind 2002).

Many years have been spent by neuroscientists to address the question "nature versus nurture", that is the debate on what in brain development is genetically determined and what could be changed by environment and experience. One way by which environment could change the brain is via patterns of electrical activity generated by experience.

There are two categories of electrical activity that have been studied by neuroscientists: spontaneous versus experience-dependent activity. Each one is predominant during a particular stage of development.

Spontaneous activity plays a fundamental role in the early stage, when there is the initial anatomical and physiological development and when vision is not present yet. Its role is crucial in the refinement and maintenance of retino-thalamic inputs and segregation of eye-specific afferents within the dLGN.

Subsequently, patterns of activity generated by experience represent the major force driving circuit maturation in the visual system. Two main experimental paradigms have been employed to study experience-dependent plasticity: total deprivation of vision from birth (dark rearing, DR) or unbalanced vision through the two eyes via either experimental strabismus or monocular eyelid suture (monocular deprivation, MD). Specifically, MD produces a loss of cortical responsiveness to the deprived eye, but only when performed during an early "critical period" of development (see below).

Visual Experience And Development: Effects Of Dark Rearing

A widely used paradigm to study the role of vision in cortical development is to rear animals in complete darkness. Animals kept in darkness from birth show abnormal functional and anatomical maturation of the visual cortex, that appears immature far beyond the end of the critical period (Benevento et al. 1992; Fagiolini et al. 1994; Gianfranceschi et al. 2003). Visual deficits of dark reared (DR) rats include an extremely low spatial resolution (visual acuity), as measured electrophysiologically and behaviourally (Fagiolini et al. 1994; Gianfranceschi et al. 2003). Cortical neurons of DR animals exhibit rapid habituation, i.e. the visual response tends to disappear after repeated stimulus presentations (Sherman and Spear 1982; Fagiolini et al. 1997). Receptive fields of cortical cells are larger than normal, and neurons have a reduced orientation selectivity. By Visual Evoked Potential (VEP) technique it has been found that also other parameters of visual responses are changed: the latency of visual response appears increased while temporal resolution is significantly reduced (Pizzorusso et al. 1997a). In addition, changes have been reported in the spontaneous discharge of cortical units of DR animals, and they are linked to alterations in intracortical inhibitory mechanisms (Benevento et al. 1992). Indeed, the mean rate of spontaneous activity was increased in DR animals (Gianfranceschi et al. 2003). The increased spontaneous activity, the loss of orientation selectivity and the larger receptive fields indicate that intracortical inhibitory mechanisms could be seriously compromised. Indeed, dark-rearing resulted in a significant decrease in the density of GABA-immunoreactive neurons in all cell layers of primary rat visual cortex. Inhibitory currents are also reduced (Benevento et al. 1995; Morales et al. 2002). Deficits in inhibition could also explain the relatively prolonged duration of responses to moving stimuli, an effect resembling the prolonged discharge found in GAD65 KO animals (Hensch et al. 1998).

As discussed before, inhibitory circuit maturation is a key factor in determining critical period plasticity. It is well established that DR animals retain a great potential for plasticity, i.e. the critical period is extended. In DR animals, MD performed beyond the end of the critical period is effective in producing a shift in OD distribution (Cynader and Mitchell 1980; Mower 1991; Gianfranceschi et al. 2003). This potential for plasticity decrease, however, if normal visual experience is allowed. Similarly, most of the cortical cells become orientation selective when dark rearing is followed by light experience (Cynader and Mitchell 1980). Given that activity could switches on various molecules (Nedivi et al. 1993), visual-driven input may switch on a molecular cascade that finally leads to maturation of the visual cortex. More than ten years ago, various groups have demonstrated that among the molecules involved a key role is played by neurotrophic factors (Maffei et al. 1992; Berardi et al. 1994; Pizzorusso et al. 1997b; Huang et al.

1999). Experiments showed reduced expression and activity of neurotrophic factors in DR animals (Castren et al. 1992; Cotrufo et al. 2003). Two important experiments demonstrated physiological rescue of DR effects by neurotrophins. First, transgenic mice overexpressing BDNF were kept in darkness from birth, and visual cortex maturation and susceptibility to MD were analyzed. The authors found that visual acuity, receptive field size, critical period for OD plasticity and inhibitory transmission in DR BDNF mice were identical to those of normal, light-reared mice (Gianfranceschi et al. 2003). Second, enriched environment promotes the same rescue effects from DR as BDNF-overexpressing mice (Bartoletti et al. 2004). As reported in the literature, animals kept in an enriched environment shown a precocious expression of BNDF (Cancedda et al. 2004). Therefore, BDNF overexpression rescues effects of DR, and it is sufficient to promote visual cortex maturation also under conditions of visual deprivation, likely acting at the level of GABAergic circuits.

Monocular Deprivation And Critical Period

Wiesel and Hubel in the early 1960s pointed up that cortical neurons had an eye preference, and cells driven by the same eye were grouped together, originating the columns of ocular dominance (Hubel and Wiesel 1963). Visual cortex circuits can adapt to a changing environment, selecting during development the appropriate pattern of connectivity to respond to different visual experiences. Indeed, one of the most important properties of visual neurons, binocularity, can be modified in response to an altered visual experience. Hubel and Wiesel started to manipulate early in life afferent visual input, by closing one eye, and they obtained a shift in binocularity, the "ocular dominance shift": the loss of visually driven activity in V1 through the closed eye, and a dramatic increase in the number of neurons responding preferentially to stimuli presented to the open eye (Wiesel and Hubel 1963). This susceptibility to MD changes with age: it begins 5-10 days after onset of vision, it is most robust during a specific time window, then it declines and it is absent or minimal in the adult age (Wiesel and Hubel 1963; Fagiolini et al. 1994; Gordon and Stryker 1996). Later, OD shift has been reported in all mammals studied (Berardi et al. 2000). The brief time window during which MD produce maximal shift, i.e. when experience is crucial for shaping the brain, is called critical period (Hubel and Wiesel 1970; Berardi et al. 2000; Hensch 2005). The critical period can be defined as "a strict time window during which experience provides information that is essential for normal development and permanently alters already present neuronal connections", by mechanisms that are activity-dependent (Hensch 2005).

MD leads to anatomical and functional effects. In addition to the shift in OD, MD renders the closed eye amblyopic, i.e. with a lower visual acuity, even if retina is physically

healthy. Moreover stereoscopic vision is affected and contrast sensitivity drops down (Hensch 2004; Medini and Pizzorusso 2008). Anatomical changes comprise an expansion of territories driven by the open eye, and a subsequent reduction of those driven by deprived eye (Katz and Shatz 1996). Moreover, geniculate neurons receiving input from the deprived eye are shrunken (20-25 %) and those driven by open eye are hypertrophic (10-15 %) (Sherman and Spear 1982). Studies by Stryker and colleagues show that anatomical changes occurred following the detection of functional effects. Indeed, a OD shift is already detectable after a short period of MD (1 day), while changes in thalamo-cortical arbors lag behind (4 days) (Antonini and Stryker 1993; Antonini and Stryker 1996; Antonini et al. 1998).

As discussed before, the critical period is the window of time during which OD plasticity is maximal. During life, the ability of the visual cortex to modify binocularity according to visual experience changes. A few days of monocular deprivation (MD) in juvenile mice are sufficient to induce a marked shift in OD in the visual cortex (Gordon and Stryker 1996). The effect is strongest during a relatively brief phase at the end of the fourth postnatal week, and the time between postnatal day 28 and postnatal day 32 is generally considered as the critical period for OD plasticity in the mouse visual cortex (Gordon and Stryker 1996). OD plasticity can also be induced before this period or after, but differs quantitatively and qualitatively. Several laboratories had started using transgenic mouse models to investigate the molecular and cellular mechanisms underlying the time course of the critical period and the mechanisms of OD plasticity.

Hensch's group was the first to shed light on the role of local, inhibitory cortical circuits in OD plasticity (Hensch et al. 1998). He took advantage of a knockout (KO) mouse, lacking the 65 kDa isoform of glutamic acid decarboxylase (GAD65), the GABA biosynthetic enzyme. The other isoform, GAD67, localizes to cell somata and dendrites, and accounts for most of GABA synthesis in interneurons. Indeed, GAD67 KO die at birth, with GABA concentration less than 10% of wild-type value. In contrast, GAD65 is found primarily in the synaptic terminal, where it serves as a reservoir of GABA, because it can be recruited when needed to provide additional GABA synthesis, for example following intense activity. GAD65 KO mice survive and develop normal morphology and normal adult concentration of GABA, because GAD67 expression is normal. Only after an intense neuronal activation, GABA release in GAD65 KO mice is reduced with respect to wild-type. Interestingly, extracellular recordings revealed prolonged discharge in GAD65 mice, i.e. a tendency to continue to fire even after stimuli have passed the cell's receptive field. Hensch and coworkers found that in these KO mice MD was ineffective: cells continued to respond preferentially to the contralateral, closed eye following MD (Hensch et al. 1998).

Remarkably, mechanisms of synapse modification in vitro, as LTP and LTD, were not impaired in these mice, demonstrating no general deficit in activity-dependent plasticity. Enhancement of intracortical inhibitory transmission by benzodiazepines (diazepam) restored OD plasticity in GAD65 KO mice (Hensch et al. 1998). Notably, rescue of plasticity is possible at any age, indicating that critical period onset is dependent on a certain level of inhibitory transmission (Fagiolini and Hensch 2000).

Consistent with this view, the onset of the critical period can be accelerated by premature enhancement of GABA-mediated transmission (Fagiolini and Hensch 2000; Fagiolini et al. 2004). In transgenic mice overexpressing BDNF, inhibitory circuits mature precociously, and the critical period for plasticity begins and ends earlier than normal (Huang et al. 1999).

An interesting experiment of Hensch's group was to analyze if a particular GABAergic receptor was involved in OD plasticity. They used a mouse "knockin" mutation to alpha subunits that renders individual $GABA_A$ receptors insensitive to diazepam. Using this approach, they found that only $GABA_A$ receptors containing α1 subunit are responsible for critical period plasticity (Fagiolini et al. 2004).

These receptors are preferentially localised at somatic synapses opposite to parvalbumin-positive (PV+) large basket cell terminals. This class of interneurons is particularly important for critical period plasticity. Specific blockade of fast-spiking fidelity in these cells decreases the OD shift after MD (Hensch 2005). Parvalbumin (PV)-expressing interneurons also take up OTX2, a transcription factor transcribed and translated in the retina and transported to the visual cortex (Sugiyama et al. 2008). In the absence of OTX2, the critical period does not start, a defect that can be rescued by intracortical infusion of OTX2. OTX2 has interesting links with other cellular events associated with critical-period onset. Interestingly, data from Xenopus suggest that the effects of IGF-1 on the development of cortical inhibition are mediated through OTX2 (Carron et al. 2005). OTX2 appears to enter PV+ interneurons through an interaction with dense aggregates of the extracellular matrix (ECM) known as perineuronal nets that, with age, enwrap these neurons (Sugiyama et al. 2009). Perineuronal nets of extracellular matrix provide more efficient inhibitory neurotransmission. When perineuronal nets are disrupted, perisomatic inhibition of the target is reduced (Saghatelyan et al. 2001) and OD shift can be induced by MD beyond the critical period (Pizzorusso et al. 2002). The key role of GABAergic transmission in the regulation of OD plasticity is not restricted to mouse, as it has been demonstrated that OD columns are disrupted in cat by manipulating inhibitory transmission (Hensch and Stryker 2004).

Altogether, a possible interpretation of all these experiments proposes a dual role for inhibitory networks in plasticity: a certain threshold of inhibition is necessary to trigger

plasticity, but a higher level of inhibition, reflecting maturation of circuits, is responsible for critical period closure (Huang et al. 1999).

Cellular Mechanisms Underlying OD Plasticity

Wiesel and Hubel proposed a mechanism in which OD plasticity results from competitive interactions between the two eyes for the control of cortical units. This idea was supported by the fact that binocular lid suture was not effective to shift OD columns in mammals (Wiesel and Hubel 1965; Sherman and Spear 1982; Gordon and Stryker 1996). In favour of a competitive view, an experiment performed by Stryker's laboratory showed that an imbalance in the electrical activities of the two retinas is sufficient to shift OD also in visual deprivation conditions (Chapman et al. 1986). A competition-based model of OD plasticity is related to heterosynaptic mechanisms, involving interactions between two sets of inputs (i.e. from the two eye) where open eye inputs drive down the synaptic efficacy of the deprived inputs (Miller et al. 1989; Harris et al. 1997). Active geniculate neuron corresponding to the open eye compete better than less active neurons, driven by closed eye, so they become functionally and structurally strengthened. Competition between thalamic inputs might be mediated via the acquisition of a neurotrophic factor from target structures (Maffei et al. 1992).

Data published since 1990 proposed an alternative view of the processes underlying MD, suggesting the idea that OD plasticity is due to different, homosynaptic mechanisms, related to specific forms of synaptic plasticity. These mechanisms engage separately each eye's pathway (Blais et al. 1999; Heynen et al. 2003; Frenkel and Bear 2004). First, there is a depression of responsiveness of deprived eye, following by a potentiation of open eye responses. This model is known as Bienenstock-Cooper-Munro model (BCM theory) and postulates a bidirectional change of synapses: they can undergo homosynaptic long term potentiation (LTP) but also homosynaptic long term depression (LTD) (Kirkwood et al. 1996; Bear and Rittenhouse 1999; Sengpiel and Kind 2002; Heynen et al. 2003).

Homosynaptic depression occurs only at active synapses. Loss of responsiveness of deprived eye was proposed to be the result of homosynaptic depression, where spontaneous, residual activity coming from closed eye contribute to synaptic depression. To test this hypothesis, the effect of very brief MD by lid suture has been compared with that of monocular silencing by intra-ocular injections of TTX (Rittenhouse et al. 1999). Results showed that lid suture was more efficient in causing depression of deprived eye responses than blockade of all retinal activity by TTX.

Other evidence indicates the involvement of a phenomenon of homosynaptic depression in the effects of MD. In particular, brief MD sets in motion the same molecular and

functional changes as the experimental model of homosynaptic LTD. Prior synaptic depression by MD also occludes subsequent induction of LTD (Heynen et al. 2003). These data support the view that homosynaptic LTD accounts for the loss of responsiveness of deprived eye during MD.

A delayed modification induced by MD is an experience-dependent potentiation of open eye responses. There is evidence supporting the idea that long-term potentiation of the synapses driven by the open eye is important for ocular dominance plasticity. First, alphaCAMKII activity appears to be required for both LTP in vitro and MD plasticity in vivo (Kirkwood et al. 1997; Taha et al. 2002). Second, a form of in vitro LTP (white matter - layer II-III LTP) is developmentally regulated with a decline over time that mirrors that of the critical period for ocular dominance plasticity (Kirkwood et al. 1996). Monocular inactivation by TTX is also able to enhance potentiation of uninjected eye, as shown by Visual Evoked Potentials (VEPs) (Frenkel and Bear 2004).

Other data are at odds with the view that MD effects during the critical period are entirely ascribable to LTP- and LTD-like mechanisms. For example, in GAD65 knockout mice, that are not sensitive to brief MD, there is no impairment of LTD in vitro (Hensch et al. 1998). Moreover, brain-derived neurotrophic factor (BDNF) prevents LTD in V1, but BDNF-overexpressing mice are sensitive to MD at least during an early phase of postnatal development (Huang et al. 1999; Jiang et al. 2003).

Thus, is still unclear whether MD effects are completely modelled by homosynaptic mechanisms. Other form of plasticity might come into play.

In the last years, another form of synaptic plasticity has emerged as a complement of Hebbian mechanisms, based on the concept of homeostasis. Indeed, neurons are able to maintain their responsiveness and synaptic strength within a certain range, despite perturbations of the levels of neuronal activity (Burrone and Murthy 2003; Turrigiano and Nelson 2004; Davis 2006). Neurons in the visual cortex could use two strategies to counteract the reduced visually-driven activity during MD. First, deprived neurons could reduce the threshold for LTP induction (Kirkwood et al. 1996; Bear 2003); second, visual responsiveness could be enhanced directly by increasing synaptic strength or intrinsic excitability (Desai 2003; Maffei et al. 2004; Turrigiano and Nelson 2004), a mechanism known as homeostatic response compensation.

A homeostatic response could include multiple mechanisms, acting by activity-dependent regulation of intrinsic firing properties (Marder and Prinz 2003; Zhang and Linden 2003), or through presynaptic and postsynaptic forms of excitatory synaptic plasticity (Turrigiano and Nelson 2004; Davis 2006), balancing of excitation and inhibition through neuronal

networks (Maffei et al. 2004), or also using compensatory change in synapses number (Wierenga et al. 2006). All these changes seem to act in order to restore the neuronal firing rates to normal levels after perturbation.

Synapses in the CNS require several hours to show a homeostatic response (Davis 2006; Sutton et al. 2006). It is interesting to note that presynaptic changes in neurotransmission release could affect dynamics of short term plasticity, whereas postsynaptic modification in receptor number could scale synapse responsiveness without affecting short term kinetics of presynaptic output (Abbott and Nelson 2000; Turrigiano 2007).

Concerning visual cortex plasticity, a recent study has found evidence for homeostatic mechanisms using two-photon calcium imaging in vivo (Mrsic-Flogel et al. 2007). The authors investigated how MD shifts the magnitude of deprived and nondeprived eye responses in individual neurons. At the level of the entire population, OD shifts are explained by a reduction of deprived eye responses and a delayed increase of closed eye inputs. However, Mrsic-Flogel and colleagues found that in neurons devoid of open eye-input, the responses of deprived eye were unexpectedly stronger after MD. These findings demonstrate that the weak input of deprived eye is not able per se to induce response depression, which instead seems to be dependent on the input of the other eye. Second, increased visual drive in cells responding predominantly to the deprived eye is difficult to reconcile with Hebbian rules of synaptic plasticity. These results are best explained by homeostatic mechanisms. In favour of this homeostatic interpretation, authors found that the proportion of monocular, closed eye-driven cells remained constant after MD. In addition, most neurons in monocular cortex increased their responsiveness. Thus, in the deprived visual cortex compensatory mechanisms seem to exist that maintain firing rates within a certain range during MD.

Other studies have indicated a role for inhibition in actively suppressing responses from the deprived eye during MD. For example, studies in cats have shown that weak inputs from the closed eye can be reinstated (at least to some extent) by localised blockade of intracortical GABA-ergic inhibition [(Duffy et al. 1976; Sillito et al. 1981; Mower and Christen 1989) but see (Khibnik et al. 2010)]. MD also potentiates inhibitory feedback between inhibitory basket cells and star pyramidal neurons in layer IV (Maffei et al. 2006). These data suggest that deprived eye responses are somehow masked or suppressed by inhibitory circuits.

In summary, it is likely that distinct mechanisms concur to produce the OD shift after MD. Decorrelated input through the closed eye causes weakening of deprived-eye synapses, possibly via Hebbian rules (Heynen et al. 2003), or via enhanced inhibition (Maffei et al.

2006), during the first few days of MD. Later a compensatory upscaling of responses occurs. This is consistent with the finding that the level of deprived-eye response depression stabilises or even reverses (Frenkel and Bear 2004) after 3 days of MD. Further studies are required to clearly understand the mechanisms underlying homeostatic compensation of neuronal responsiveness. These mechanisms may include rearrangements in intracortical inhibitory circuitry or changes in synaptic strength/intrinsic conductances of individual cells (Mrsic-Flogel et al. 2007).

Plasticity Of Interneurons

Classically, shifts in binocularity have been measured mainly as population responses or by extracellular recordings, that are more likely to sample activity from large excitatory pyramidal neurons.

Given the pivotal role of inhibition in determining the plastic potential of the cortex shaping the critical period, it is of great interest to understand the role of interneurons in the plastic changes induced by MD.

Parvalbumin-positive (PV+) fast-spiking basket cells are key regulators of inhibition in the cerebral cortex. Fast-spiking cells are the most numerous subtype of cortical interneurons (Gonchar et al. 2007; Xu et al. 2010). These cells form powerful, proximally localised synapses on the soma and axon initial segment of postsynaptic cells, exerting fine control over the timing and probability of target cell discharge.

Parvalbumin (PV+) interneurons receive very dense and strong synaptic input from nearby pyramidal cells with diverse feature selectivities (Hofer et al. 2011). PV+ neurons provide fast, strong but untuned feedforward inhibition to excitatory neurons, while somatostatin (SOM+) neurons provide orientation and direction selective but delayed and weak inhibition (Ma et al. 2010).

Anatomical work by Mainardi et al showed, by measuring c-fos activation in response to visual stimulation of the deprived eye, that PV+ cells are particularly refractory to long term MD, suggesting that different cortical subpopulation may show different response to MD.

Two recent works on this topic were performed with in vivo two-photon imaging of calcium responses in mice expressing a fluorescent protein marking interneurons (Gandhi et al. 2008; Kameyama et al. 2010), and a third one with in vivo intracellular recordings to measure the OD of individual fast-spiking interneurons (Yazaki-Sugiyama et al. 2009).

Three studies found that long-term MD causes interneurons, similar to excitatory neurons, to shift their responsiveness toward the nondeprived eye. The studies did not agree, however, on how interneurons change OD after short-term MD. Using intracellular recordings, Yazaki-Sugiyama et al. (Yazaki-Sugiyama et al. 2009) found that inhibitory

neurons paradoxically show a shift toward the deprived eye, supporting a role of GABAergic inhibition early after MD in suppressing responsiveness to the nondeprived eye. This is not supported by the two other studies using calcium imaging, however. One shows that inerneurons undergo a delayed shift toward the open eye (Gandhi et al. 2008), whereas the other shows that inhibitory and excitatory neurons both shift toward the open eye within the same time course (Kameyama et al. 2010). It is of importance to clarify the causes of these contradictory results to understand how interneurons alter their responsiveness during OD plasticity and what the consequences are for the responsiveness of excitatory neurons.

The importance of inhibition during OD plasticity is further confirmed by the evidence that blocking inhibitory inputs by intracellular injection of GABA antagonists increases the binocularity of neurons in the primary visual cortex (V1), whereas reducing inhibition after MD causes a reversal of the eye bias of excitatory neurons (Yazaki-Sugiyama et al. 2009). This shows that OD plasticity is accompanied by a change in the balance of excitation and inhibition at the single-cell level. Another recent work on the balance between excitation and inhibition in MD has been published by Huizhong Whit Tao and collaborators (Ma et al. 2013). The authors showed that in layer 4 neurons, after long term MD, excitation and inhibition driven by the closed eye are equally decreased, while the open eye increase in responsivity can be explained by a decrease in inhibitory inputs.

Molecular Mechanisms In Visual Cortex Plasticity

The molecular network involved in visual cortex plasticity remains only partly understood. Starting from the pioneering experiments on neurotrophins, several studies have been performed to gain knowledge of the molecules mediating the action of experience in plasticity (Berardi et al. 2003; Medini and Pizzorusso 2008).

In the '90s, Maffei's group and other laboratories put forward the idea that competition in OD plasticity might depend on the uptake of limiting growth factors, such as neurotrophins (NT), secreted by cortical neurons and retrogradely transported (Maffei et al. 1992; Bonhoeffer 1996; Harris et al. 1997; McAllister et al. 1999). Accordingly, exogenous delivery of NT in the visual cortex during MD prevented competition, rescuing cortex from MD effects (McAllister et al. 1999; Berardi et al. 2000). It has also been clarified that, with the exception of neurotrophin 3 (NT-3), all neurotrophins influence MD, but not all factors play an identical role on visual neuron properties (Lodovichi et al. 2000). In particular, neurotrophin 4 (NT-4) and NGF prevent the shift induced by MD, and they have no effects on spontaneous or visually-driven activity (Gillespie et al. 2000; Lodovichi et al. 2000). In contrast, BDNF is less effective in preventing OD shift, and it changes both spontaneous and visually-evoked activity of cortical neurons.

A complementary strategy consisted in antagonizing the action of endogenous neurotrophins. Blockade of NGF led to alterations in visual acuity and binocularity of cortical neurons. In addition it induced a shrinkage of geniculate neurons, demonstrating a key role for neurotrophins in normal visual cortex development and plasticity (Berardi et al., 1994). Blockade of the TrkB ligands BDNF and NT-4 desegregated OD columns (Berardi et al. 1994; Cabelli et al. 1997). Other key studies were conducted in BDNF-overexpressing mice. These animals maintain normal cellular pattern and expression of BDNF in visual cortex, but have an accelerated postnatal rise of this neurotrophin (Huang et al. 1999). BDNF-overexpressing mice showed an acceleration of development of visual function, such as visual acuity, and of the critical period time course. Notably, a strong link was established between BDNF and intracortical inhibition, because GABAergic circuit maturation is accelerated by overexpressing BDNF (Huang et al. 1999).

One link between experience-dependent plasticity and NT action is represented by experience-dependent production and release of NT (Bozzi et al. 1995; McAllister et al. 1999). NT can modulate electrical activity, at pre and post-synaptic level, increasing neurotransmitter release, depolarizing neurons but they also act on gene expression (Sala et al. 1998; Kafitz et al. 1999; Lodovichi et al. 2000; Poo 2001). This reciprocal regulation between activity and neurotrophins might be the reason because only active synapse in MD are reinforced (Caleo et al. 1999a; Kovalchuk et al. 2002).

It is well known that N-methyl-D-aspartic acid (NMDA) receptors are particularly implicated in mediating changes in synaptic efficacy. These receptors are not only transmitter and voltage-dependent, but they also permit Ca^{2+} influx, which is an intracellular signal. NMDA receptors involvement in visual cortical plasticity started with experiments showing that blockade of NMDA receptors abolished MD effects (Bear et al. 1990; Roberts et al. 1998; Sawtell et al. 2003). Interestingly, NMDA receptors are developmentally regulated: in visual cortex, NMDA subunit composition varies from prevalent presence of NR2B to a dominant present of NR2A, paralleling visual cortex development and critical period plasticity. NR2B is responsible of long-lasting current, while NR2A originates currents with faster kinetics. Dark rearing, which freezes visual cortex development and plasticity, delays expression of NR2A subunit, suggesting that NR2B/NR2A switch has a role in visual cortex maturation and CP regulation. However, in mice with deletion of NR2A subunit, OD plasticity is weaker but restricted to normal CP, while orientation selectivity fails to mature (Fagiolini et al. 2003). Thus, NR2A might be not necessary for CP regulation, but might be involved in other properties of cortical maturation.

Electrical activity, neurotrophins, and NMDA receptors can set in motion three kinases important for OD plasticity: cAMP-dependent protein kinase (PKA), extracellular-signal-regulated kinase (ERK), α Ca2+/calmodulin dependent protein kinase II (α CAMKII) (Beaver et al. 2001; Di Cristo et al. 2001; Taha et al. 2002; Cancedda et al. 2003). Each kinase has its own signalling pathway, but they can also converge, for example via phosphorylation of the same downstream target (e.g., CREB). The overlap and crosstalk among these pathways explain why blockade of only one of these molecules affects OD plasticity. However, level of regulation could be of two types: cytoplasmatic, local and rapid, targeting molecules involved in synaptic transmission or cytoskeleton motility; or nuclear, delayed, engaging gene regulation.

Long-lasting modifications require changes in gene expression, also in visual cortex (Mower et al. 2002; Taha and Stryker 2002). It has been found that visually-driven activity activate transcriptional factors, such as zif268 or CREB (Caleo et al. 1999b; Pham et al. 1999; Mower et al. 2002). Many other sets of genes are modulated by visual experience or deprivation, and one important molecular cascade regulated by activity is the IGF-1 receptor pathway (Nedivi et al. 1993; Tropea et al. 2006). An experience-regulated factor is not always necessary for OD plasticity, in fact mice knock out for zif268 respond with normal shift after MD (Mataga et al. 2001). To date the action of only a few factors has been found to be necessary for MD (Pham et al. 1999; Mower et al. 2002). ERK inhibition prevents synaptic plasticity and MD effects in visual cortex, as exogenous application of IGF-1 (Di Cristo et al. 2001; Cancedda et al. 2003; Tropea et al. 2006). A challenge is represented by addressing the gene expression profile in single, identified types of neurons, following experience.

In the latter years, it became clear that neurons modify gene expression patterns in response to experience-dependent activity. These mechanisms could explain the way by which the brain produces long term changes in its circuits. Histone phosphoacetylation, seems to be important for synaptic plasticity in hippocampus and for activity-dependent gene transcription (Alarcon et al. 2004; Korzus et al. 2004). Recently, the regulation of chromatin structure has emerged as one mechanisms regulating visual cortex plasticity. The experiments have demonstrated the involvement of histone phosphoacetylation in OD plasticity (Putignano et al. 2007). The authors found that these modifications occurred within minutes, following visual experience. A mediator seems to be ERK, because ERK inhibition prevents visually-driven phosphoacetylation. Remarkably, this effect is developmentally regulated: in adult mice visual experience is able to activate ERK and other kinases, but it is unable to promote histone phosphoacetylation at a level comparable to that found in juvenile animals. Accordingly, restoring histone acetylation by

pharmacological drugs is able to promote OD plasticity in adult mice (Putignano et al. 2007).

Recent data describe also the involvement of miRNAs in the development and plasticity of the visual cortex. The miR-132 has been identified as an important molecular transducer of the action of visual experience on developing visual cortical circuits, possibly acting through modulation of dendritic spine plasticity (Mellios et al. 2011; Tognini et al. 2011).

Plasticity has not only intracellular effectors, because it is known that the extracellular environment also contributes to regulate plasticity. One of the factors involved in experience-dependent plasticity is represented by tissue plasminogen activator (tPA). Biochemically, it is a serine protease and it is an immediate early gene induced by electrical activity (Qian et al. 1993). Proteolysis by tPA increased in V1 after two days of MD during the CP, but not in adulthood or in GAD65 knock out mice (Mataga et al. 2002). Accordingly, tPA inhibition impaired OD shift induced by MD (Mataga et al., 1996) and prevented recovery from MD following reverse occlusion (Muller and Griesinger 1998). Targets of tPA include extracellular-matrix proteins, growth factors, membrane receptors, cell-adhesion molecules (Endo et al. 1999; Wu et al. 2000; Nicole et al. 2001), that could all be involved in cortical plasticity. A further evidence for a key role of tPA in plasticity is represented by data showing an increase of spine motility in young animals after tPA delivery (Oray et al. 2004).

Another component of extracellular matrix (ECM) which has been investigated is represented by chondroitin-sulfate proteoglycans (CSPGs). These molecules are organized in typical structures, named perineuronal nets (PNNs), around soma and dendrites of parvalbumin-positive neurons. PNNs increase during development and their organization in the visual system is complete at the end of the critical period (Hockfield et al. 1990; Koppe et al. 1997; Pizzorusso et al. 2002). Dark rearing prevents PNNs formation (Pizzorusso et al. 2002), while axonal sprouting and regeneration are inhibited by CSPGs (Bradbury et al. 2002). CSPGs exert an inhibitory action in cortical plasticity, indeed degradation of CSPGs in adulthood by chondroitinase ABC is able to restore OD plasticity and to promote recovery from amblyopia (Pizzorusso et al. 2002; Pizzorusso et al. 2006). In addition, treatment with chondroitinase ABC is also able to increase spine density (Pizzorusso et al. 2006). It is interesting to note that ECM proteolysis could be regulated not only exogenously, but also autonomously by particular rearing conditions. Enriched environment promotes amblyopia recovery and decreases PNNs number (Sale et al. 2007).

To date it has been accepted that the adult brain has a decreased ability to repair and that myelin exerts an active inhibitory role in these processes (Schwab 2004). Interestingly, the

receptors for myelin-associated growth inhibitors, Nogo-66 receptor (McGee et al. 2005) and PirB (paired immunoglobulin-like receptor B) (Syken et al. 2006; Atwal et al. 2008), are also implicated in closure of the critical period, and in mice deficient for either receptor, OD plasticity can be readily induced in adulthood. Thus, myelinization is able to inhibit not only recovery from injury, but could also promote the decrease of plasticity observed at the end of the CP (Sengpiel 2005).

Plasticity In Adulthood

Since the classical experiments of Hubel and Wiesel in 1960s, plasticity is thought to occur only during a brief time window early in life, the critical period, as discussed above. However, recent evidence suggests some residual plasticity also in adult animals. This seems to be true not only for the visual system, but also for other models, such as the somatosensory cortex that shows persistent plasticity in some cortical layers also in mature animals (Diamond et al. 1994).

Several laboratories have reported OD plasticity in the visual cortex of adult mice, using intrinsic signal imaging, activity reporter gene Arc, and Visual Evoked Potentials (VEPs). Some but not all, authors reported OD plasticity in adult mice using extracellular unit recordings (Gordon and Stryker 1996; Hensch et al. 1998; Sawtell et al. 2003; Lickey et al. 2004; McGee et al. 2005; Tagawa et al. 2005; Hofer et al. 2006b; Tohmi et al. 2006; Heimel et al. 2007). Interestingly, OD plasticity in adult mice has been reported in urethane- but not barbiturate-anesthetized animals (Fagiolini and Hensch 2000; Pham et al. 2004). Using VEPs, plasticity has been measured in awake, head-restrained mice (Fagiolini and Hensch 2000; Sawtell et al. 2003; Pham et al. 2004).

Important data were reported a few years ago by Sawtell et al. (Sawtell et al. 2003) demonstrating not only that OD plasticity occurred in adult mice but also that the mechanism is different from that found during the critical period. First, they measured in normal mice the ratio of VEPs elicited by visual stimulation of the contralateral versus the ipsilateral eye. As expected, they found a strong contralaleral eye bias. After brief MD in adult mice (3 days, a period that is effective in shifting OD in juvenile mice), the contra-ipsi ratio was unchanged. However, a slightly longer MD (5 days) was able to induce a shift in OD. Hence, they studied daily VEPs amplitude in chronically implanted animals, to identify the mechanism: the adult OD plasticity was almost completely due to a potentiation of absolute amplitude of VEP of ipsilateral, open eye, that developed gradually after the third day of MD. This stands in contrast with the effects of MD in juveniles. Indeed, in young animals 3 days of MD were effective in shifting OD mainly by depression of contralateral, deprived eye inputs, while there was only a delayed component of ipsilateral, open eye response potentiation. Thus, the mechanism by which OD plasticity occurs in adults seem

to be different from juveniles. However, the same group has also demonstrated that functional NMDA receptors are necessary for adult plasticity, suggesting common molecular effectors. It is noteworthy that effects induced by adult MD seem to be different depending on whether the contralateral or ipsilateral projection is analysed. Ipsilateral eye inputs undergo weakening after MD of ipsilateral eye, demonstrating that input depression is still possible in adult mice, despite the developmental downregulation of LTD (Tagawa et al. 2005; Hofer et al. 2006b).

An important point to investigate is the structural rearrangement consequent to MD in adulthood. In juvenile animals, functional changes come first, and thalamocortical axon remodelling takes some days to develop (Antonini and Stryker 1993; Antonini et al. 1999). Progress in neuroscience techniques, such as the introduction of 2-photon microscopy, has allowed to study changes at the level of dendritic spines. In juvenile mice, it was found that spine motility increased after 2 days of MD, while after 4 days there was a significant spine loss, consistent with the initial strong reduction of responses elicited by the deprived eye (Gordon and Stryker 1996; Mataga et al. 2004; Oray et al. 2004; Hofer et al. 2006b). Additional days of MD led to formation of new connections, in keeping with the subsequent strengthening of open eye inputs in juvenile mice (Mataga et al. 2004). In adulthood, spine motility is still present in normal mice (Holtmaat et al. 2005). However, spine loss after MD is not observed, consistent with the absence of depression of deprived eye inputs (Sawtell et al. 2003; Hofer et al. 2006b). Rather, there is a slight spine gain, consistent with potentiation of the open eye (Mataga et al. 2004). A direct link between spines and adult plasticity has been provided by an experiment in which pharmacological enhancement of spine density resulted in reinstatement of OD plasticity in adulthood (Cerri et al. 2011).

Hofer and collaborators demonstrated that, in the adult animal, MD doubled the rate of spine formation, thereby increasing spine density. This effect was specific to layer-5 cells located in binocular cortex, where most neurons increase their responsiveness to the non-deprived eye. Restoring binocular vision returned spine dynamics to baseline levels, but absolute spine density remained elevated and many monocular deprivation induced spines persisted during this period of functional recovery. However, spine addition did not increase again when the same eye was closed for a second time. This absence of structural plasticity stands out against the robust changes of eye-specific responses that occur even faster after repeated deprivation. Thus, spines added during the first monocular deprivation experience may provide a structural basis for subsequent functional shifts (Hofer et al. 2009).

The dynamics of inhibitory synapse turnover in adult V1 accompanying OD plasticity are very different from what has been described for excitatory synapses (van Versendaal et al.

2012). In adult V1, MD causes the loss of inhibitory synapses on distal apical dendrites of layer 2/3 pyramidal neurons and results in increased responsiveness to the non-deprived eye. Surprisingly, restoration of binocular vision is again associated with inhibitory synapse loss, and increases the responsiveness to the previously deprived eye.

The Hofer group also showed that the adult cortex can retain memory of prior MD induced plasticity. They found that a transient shift in OD, induced by monocular deprivation (MD) earlier in life, renders the adult visual cortex highly susceptible to subsequent MD many weeks later. Irrespective of whether the first MD was experienced during the critical period or in adulthood, OD shifts induced by a second MD were faster, more persistent and specific to repeated deprivation of the same eye. The capacity for plasticity in the mammalian cortex can therefore be conditioned by past experience (Hofer et al. 2006b).

In the literature it is described also a different form of interocular plasticity of vision in adult mice, in which MD leads to an enhancement of the optokinetic response (OKR) selectively through the non deprived eye. Over 5 days of MD, the spatial frequency sensitivity of the OKR increased gradually, reaching a plateau above pre-deprivation baseline (Prusky et al. 2006). Indeed, deprivation of vision in one eye in adult mice induces a "sensory learning" process: a use-driven improvement of visual acuity and contrast sensitivity through the nondeprived eye after daily testing in a virtual-reality optomotor setup (Greifzu et al. 2011). The persistence of this interocular plasticity has been studied also in the ageing brain. Data collected from old mice show a general impairment of the visual function, and a decrease of the optomotor response-induced plasticity in old MD mice (Lehmann et al. 2012).

The vast majority of papers stress the importance of thalamic-driven MD plasticity. Conversely, at least in adult animals, OD plasticity cannot be conceptualised solely as a local process. The influence of a small phototrombotic lesion in the somatosensory cortex was sufficient to compromise both MD plasticity and the open eye sensory learning. Anti-inflammatory treatments restored sensory learning but not OD plasticity. This indicates that "whole brain sickness" prevents learning, ruling out the idea of cortical plasticity as a purely local phenomenon (Greifzu et al. 2011).

<u>Promoting Adult Plasticity</u>

We have already discussed about the possibility to enhance visual cortex plasticity by removing inhibiting factors, such as components of ECM or myelin-associated molecules (Pizzorusso et al. 2002; McGee et al. 2005; Pizzorusso et al. 2006). Enhancing plasticity in adulthood is of high interest because is strictly connected to functional recovery. The

same factors that promote OD plasticity could for example be exploited to allow recovery from amblyopia.

It has been demonstrated that plasticity and recovery can be obtained by chronic administration of fluoxetine, that reinstates ocular dominance plasticity in adulthood and promotes the recovery of visual functions in adult amblyopic animals (Maya Vetencourt et al. 2008). In adult rats, enriched environment (EE) is sufficient to lead to complete recovery from early MD (Sale et al. 2007). Enhanced environmental stimulation is able to restore juvenile-like plasticity in the adult visual cortex, allowing the induction of an OD shift in response to MD, with a mechanisms comprising enhanced serotoninergic transmission, decreased intracortical inhibition and increased BDNF levels (Baroncelli et al. 2010). EE has been shown to be able to reactivate plasticity also in aged rats (Scali et al. 2012).

Similarly, ten days of visual deprivation (dark rearing) in adult rats are effective in restoring susceptibility to MD, as measured by evaluating the ratio of VEPs elicited by the two eyes (He et al. 2006). A period of dark rearing followed by reverse suture is effective in promoting recovery from amblyopia consequent to early MD (He et al. 2007). However, this recovery is not complete, at least at the behavioural level (Pizzorusso et al. 2006; Sale et al. 2007).

In conclusion, plasticity in adult mice was found by many laboratories and with different techniques. Plasticity in adulthood seems to be qualitatively and quantitatively different with respect to juvenile animals. The potential for plasticity remains maximal during the critical period (Hofer et al. 2006a; Medini and Pizzorusso 2008). Differently from mice, an OD shift can not be induced in rats past the critical period (Fagiolini et al. 1994; Guire et al. 1999). In the adult rat visual cortex, plasticity can be reinstated pharmacologically, by manipulating the rearing environment or by degrading CSPGs (Pizzorusso et al. 2006; He et al. 2007; Sale et al. 2007; Maya Vetencourt et al. 2008).

PHYSIOLOGY OF CALLOSAL CONNECTIONS IN THE VISUAL CORTEX

Electrophysiological observations have shown that callosal inputs can provide both excitation and inhibition to the contralateral side (Payne et al. 1991; Sun et al. 1994). On one hand, the removal of the callosal input to the opposite visual cortex (via cooling or GABA injections in one hemisphere) results in a decrease of neuronal responsivity in a fraction of the recorded cells, suggesting a callosal excitatory contribution to these neurons. On the other hand, a subset of neurons show an increase in the response

magnitude, compatible with the removal of a callosally driven inhibition (Payne et al. 1991; Sun et al. 1994).

These physiological data showing transcallosal inhibitory and excitatory effects are corroborated by the intra and extra-cellular results obtained in cats by different groups showing that callosal fibres mainly evoke a direct excitation of neurons in the opposite hemisphere but can also produce a disynaptic inhibitory postsynaptic potential via a local GABAergic cell (Toyama et al. 1974; Innocenti 1980).

The type of information transmitted by the corpus callosum to the visual cortex has been studied more recently by electrophysiological, optical imaging and psychophysical approaches. Recordings of local field potentials before, during and after inactivation by cooling of the opposite hemisphere demonstrated that callosal input modifies visual responses in a complex and stimulus-dependent manner (Makarov et al. 2008). Specifically, callosal influences more frequently depress the responses elicited through the thalamo-cortical pathway (indicative of interhemispheric inhibition), but facilitatory events are also observed (Makarov et al. 2008). This callosal excitation is mainly between neurons tuned to the same orientation, consistent with anatomical evidences of direct monosynaptic connections linking neuronal clusters representing the same orientation in the two sides of the brain (Rochefort et al. 2009). Conversely, transcallosal inhibition is both between iso-oriented and cross-oriented neurons. It is possible that this effect is mediated via local interneurons and spread of GABAergic inhibition across columns of different orientations (Makarov et al. 2008).

The callosum also modulates visual response properties, like orientation and direction selectivity across the midline. In particular, in the cat, callosal connections contribute to the strength and specificity of the orientation and directional response in cortical neurons (Schmidt et al. 2010). Cortical domains preferring cardinal contours seem to receive a strong inter-hemispheric input, that is lost after cooling of the contralateral hemisphere (Schmidt et al. 2010).

Another key function of the visual callosal connections is to create trans-hemispheric neuronal assemblies by synchronising the activity of neurons in the two hemispheres. Indeed, section of the corpus callosum or inactivation of one side substantially impacts functional coupling of the two hemispheres (Engel et al. 1991; Munk et al. 1995; Knyazeva et al. 1999; Carmeli et al. 2007).

Callosal axons terminate in discrete clusters of synaptic boutons corresponding to distinct cortical columns. Specifically, combined optical imaging and tracing studies in the cat have shown that callosal axons interconnect columns coding for the same stimulus

orientation in the two hemispheres (Rochefort et al. 2009). The anatomical structure of callosal axons is such that the target columns tend to be activated almost synchronously when action potentials travel in callosal fibres (Innocenti et al. 1994; Tettoni et al. 1998). Thus, interhemispheric interactions may serve a key role in the stimulus-specific synchronisation of activity in the two hemispheres (Innocenti 2009). This idea has been confirmed directly by studies of functional coupling of the two striate cortices. In the cat, neurons in V1 of the two hemispheres correlate their discharges when stimulated by iso-oriented stimuli (Engel et al. 1991). Interhemispheric correlation occurred for cells displaying the same orientation preference and with receptive fields close to the vertical meridian. This synchronisation is critically dependent on interhemispheric interactions, as it is abolished by section of the callosum. Coherence in activity between the two hemispheres may be involved in perceptual binding, i.e. in binding stimulus features across the midline of the visual field (Engel et al. 1991).

In ferrets and humans, EEG inter-hemispheric coherence has been studied during stimulation with moving gratings. Stimulation of the two hemispheres with gratings of identical orientation increased EEG coherence, specifically in the beta-gamma frequency band. In the animal studies, it could be shown that this synchronisation is specifically due to interhemispheric projections, as transection of the corpus callosum erases the increased coherence (Kiper et al. 1999). In the human studies, the increase in coherence was seen with a single grating extending across the vertical meridian (Knyazeva et al. 1999), and with bilateral iso-oriented gratings positioned close to the vertical meridian (Knyazeva et al. 2006). This strongly indicates a role for splenial connections in synchronization, as the two representations of the central visual field in the two hemispheres are strongly linked by callosal axons in humans (Clarke and Miklossy 1990). Importantly, a reduction in interhemispheric synchronisation was found in acallosal children (Koeda et al. 1995).

These studies are important because they demonstrate that coherence analysis of scalp EEG recordings can be reliably used to explore the functionality of corticocortical connections (Knyazeva and Innocenti 2001). This has opened the way to investigate callosal connectivity in human and animal models of brain pathologies (Jalili et al. 2007).

Callosal connections also play a role in determining cortical binocularity and in other functions such as depth perception, horizontal disparity tuning, contrast sensitivity and transfer of adaptation (Mitchell and Blakemore 1970; Maffei et al. 1986; Berardi et al. 1988; Schmidt et al. 2010). Spatial and temporal characteristics of the visual information transmitted through the callosum are similar to those of a low pass filter, indeed high spatial and temporal frequencies are attenuated, and callosal neurons have reduced

sensitivity to low contrasts (Berardi et al. 1988). A recent study in human has confirmed the importance of callosal communication in processing high contrasts. Indeed, after rTMS silencing of the left visual cortex the authors found a selective increase, in the opposite hemisphere, of field potentials evoked by high contrast stimuli (Bocci et al. 2011).

<u>Development Of The Callosum: Role Of Spontaneous Activity And Visual Experience</u>
The development of the corpus callosum is a slow process that spans many years in humans; the fibers appear at 10-11 weeks of gestation but the maturation continues until myelination is completed during puberty (Hellige 1993). In cats, the callosum is fully developed between 1-3 months of age (Kennedy and Dehay 1988). In rodents maturation of the callosum is complete just after eye opening (postnatal day 15) (Mizuno et al. 2007), but the process of myelination continues into adulthood (Yates and Juraska 2007).

Visual callosal axons are initially exuberant, but during development they undergo a phenomenon of partial elimination (Innocenti and Caminiti 1980; Innocenti 1981). During the first two postnatal months in cats, the callosal efferent zones become progressively restricted to their adult locations in visual cortex (Innocenti 1981). Also in the rat parietal cortex, the major factor in the progressive restriction of the callosal projection is the withdrawal or degeneration of axon collaterals, rather than the selective death of many of the cells that initially project to the opposite side (O'Leary et al. 1981). Initial exuberance of neuronal connectivity followed by a later phase of axon pruning is a common theme in neural development (Innocenti and Price 2005).

Like any other brain structure, the callosal pathway can undergo plastic changes during its early formation and maturation. It has been shown that the development of visual callosal connections is strongly dependent upon neural activity even before eye opening (Innocenti et al. 1986; Mizuno et al. 2007). Neonatal enucleation experiments show that activity is required for the refining of callosal projections. Electron microscopy on sections from enucleated rats show that eye presence is necessary for the development and/or maintenance of callosal terminals forming multiple synaptic contacts (Sorensen et al. 2003). Eye presence is also important during a window of callosal plasticity (from postnatal day 4 to 6 in rats and mice) to specify callosal maps in a non-NMDA receptor-mediated process (Olavarria and Hiroi 2003; Olavarria et al. 2008b). NMDA receptors seem to be required mainly for the initial elaboration of callosal arbor development (Olavarria et al. 2008b). In mice, Mizuno et al. (Mizuno et al. 2007) explored the role of spontaneous activity in callosal development, showing that a decrease in firing activity of callosal neurons leads to an impaired growth of axon and their arbors. Conversely

interfering with firing of callosal target neurons has only a limited effect on the pattern of callosal terminals (Mizuno et al. 2007). During development it has been also demonstrated the presence in the rat cortex of a substantial, but transient population of functional GABAergic transcallosal neurons. These GABAergic neurons are detectable perinatally but don't seem to persist into adulthood, and could work as pathfinding or differentiation cues (Kimura and Baughman 1997). There are also a few identified molecular determinants responsible for the callosal fate of excitatory projecting neurons in mouse cortex. In the absence of Fezf2, a zinc finger transcription factor, cortical neurons adopt the axonal targeting of callosal neurons and their typical strong spike frequency adaptation in response to intracellular current injection. Fezf2-/-neurons also acquire the expression of a known callosal marker, the chromatin remodeling protein Satb2 (Hattox and Nelson 2007; Chen et al. 2008a; Lodato et al. 2011).

Modulation of visual experience also affects the development of callosal connections. Rearing animals in complete darkness from birth exaggerates the partial elimination of callosal projections, with fewer terminating callosal axons at the area 17/18 border (Lund and Mitchell 1979; Frost and Moy 1989). Similarly, bilateral eyelid suture causes a clear reduction of callosal connections, with a 50% reduction of the total number of callosal neurons (Innocenti et al. 1985). Conversely, monocular enucleation produces an abnormally wide distribution of callosal cells at the 17/18 border. This latter effect is similar to that described in cats reared with convergent or divergent strabismus, or monocular eyelid suture. All these manipulations produce a widespread distribution and exuberant number of callosal terminals (Innocenti and Frost 1979; Berman and Payne 1983; Milleret 1994).

There are also data showing that visual experience can influence the functional properties of callosal neurons in adult cats. For example, a study demonstrated that MD in adulthood is able to induce functional changes in visual callosal map, leading to an increase of receptive field size and to a loss of orientation selectivity (Watroba et al. 2001).

Role Of The Callosum In Developmental Maturation Of The Visual Cortex

The experiments described so far demonstrate that spontaneous activity and sensory experience can modify the fine connectivity of the corpus callosum. The question arises whether there is a role for inter-hemispheric communication in visual cortical development. The visual cortex is immature at the time of eye opening and gradually develops its functional and structural properties during a critical period early in life (Berardi et al. 2000). During this time window, experience refines a number of visual properties. Among these, an important marker of maturation is the increase of visual acuity, that in rats reaches

adult values around postnatal day 35. In parallel with the maturation of acuity, there is a progressive loss of the potential for plasticity in the cortex. This is usually demonstrated by a downregulation of the effect of a period of monocular eyelid suture (monocular deprivation, MD) on eye preference of cortical cells (Fagiolini et al. 1994; Berardi et al. 2000). Many studies have described the role of visual experience in visual cortex maturation (Sherman and Spear 1982; Fagiolini et al. 1994; Katz and Shatz 1996). Total lack of visual experience by dark rearing, for example, halts maturation of visual acuity and prolongs the period of sensitivity to MD (Sherman and Spear 1982; Fagiolini et al. 1994). While the role of visual experience in cortical maturation is well established, our group has recently addressed the specific role of callosal connectivity in functional development of the visual cortex. Specifically, Caleo et al. (Caleo et al. 2007) produced a unilateral, prolonged silencing of activity in the developing rat primary visual cortex by taking advantage of the clostridial enzyme botulinum neurotoxin E (BoNT/E). BoNT/E is a metalloprotease that enters the cytosol of nerve terminals close to the site of delivery and specifically cleaves the synaptic protein SNAP-25 (synaptosomal-associated protein of 25 kDa), causing a prolonged blockade of transmitter release (Schiavo et al. 2000; Davletov et al. 2005; Bozzi et al. 2006)

Caleo et al. unilaterally injected BoNT/E into the visual cortex of rat pups at the time of eye opening (Caleo et al. 2007). BoNT/E injection resulted in a selective blockade of activity in the injected, but not contralateral, cortex that persisted at least 2 weeks, thus spanning most of the "critical period" for cortical development (Fagiolini et al. 1994). This experimental approach is ideal to dissect the role of the interhemispheric connections during cortical development, because the uninjected cortex experiences normal vision through the retino-thalamic pathway and only lacks callosal input. This transient unilateral silencing of intrinsic cortical activity prevented functional cortical maturation on both sides. The injected cortex displayed deficits in visual acuity and an extension of the critical period for ocular dominance plasticity. Remarkably, these same effects were detectable in the visual cortex of the opposite uninjected side, pointing to a crucial role for inter-hemispheric connections in postnatal development. Thus, maturation of the blocked cortex was superimposable to that of the opposite side, which only lacks callosal input and maintains normal afferent activity through the direct retino-geniculate pathway. The very similar developmental deficits observed ipsilateral and contralateral to the activity blockade indicate a fundamental role for callosal linkages in coordinating the process of cortical maturation (Caleo et al. 2007). This finding is consistent with the well-known role of the callosum in synchronizing activity in the two halves of the brain (Engel et al. 1991; Munk et al. 1995; Knyazeva et al. 1999; Carmeli et al. 2007).

Explanations for these bilateral effects after unilateral silencing may implicate the lack of a sustaining callosal input to the opposite visual cortex during activity blockade in one side (Caleo et al. 2007; Berlucchi 2011). In teleological terms, parallel development of the two sides of the brain is needed to ensure a match in information processing between the cerebral hemispheres; the results of these experiments show that transcallosal pathways mediate this coordinated maturation.

To corroborate this idea, Pinto et al. (Pinto et al. 2009) took advantage of a mouse model with conditional deletion of the AP2γ transcription factor. Deletion of AP2γ during development results in a specific reduction of upper layer neurons in the occipital cortex, particularly callosally projecting neurons (Pinto et al. 2009). As a result, adult AP2γ conditional knockout mice display reduced size of the corpus callosum. At the functional level, this phenotype was coupled to a profound reduction in visual acuity. As the reduced visual acuity was reminiscent of a physiologically more immature state of the visual cortex, Pinto et al. tested the hypothesis of whether this was also accompanied by maintenance of a higher degree of plasticity, as is the case at more immature stages. Indeed, ocular dominance plasticity triggered by a brief period of MD was retained in adult AP2γ–/– mice. These data provide further support for the hypothesis that callosal projections act as an important determinant for the functional maturation of visual cortex (Pinto et al. 2009).

Reports in the literature suggest that the role of the callosum in cortical maturation might be well conserved across species. Indeed, transection of the callosum in kittens during an early phase of postnatal development (but not at later stages) produces a reduction in behaviourally measured visual acuity, supporting a role for interhemispheric communication in cortical maturation (Elberger 1984). Monkeys that received unilateral lesions of primary visual cortex in infancy display impairments of stimulus detection in the intact visual hemi-field (Moore et al. 1996). There may be a similar early sensitive period in humans, when callosal integrity appears to be particularly important for the development of visual acuity. Indeed, children born preterm show correlation between white matter microstructure and visual acuity (Lindqvist et al. 2011). In keeping with the idea of a "critical period" for the role of the callosum in acuity maturation, adult patients experiencing callosotomy have no impairments of visual acuity Afraz et al. 2003. It is important to mention that functional maturation of other cortical properties during development appears to proceed independent of callosal influences. For example, development of orientation selectivity in ferret visual cortex is not affected by activity blockade in the contralateral hemisphere (Chapman and Stryker 1993).

The development of visual acuity is dependent on proper callosal function only during an early critical period, but the importance of the callosal pathway in integrating cerebral processing is still apparent in adults. Patients with unilateral occipital cortex injury show reduced spatial and temporal sensitivities in the sighted hemi-field (Hess and Pointer 1989; Rizzo and Robin 1996). Moreover, patients with hemianopia show impairments in figure detection tasks also in the intact hemi-field, suggesting that this deficit may be caused by loss of interhemispheric interactions (Paramei and Sabel 2008).

The Role Of The Corpus Callosum In Cortical Binocularity
The particularly high concentration of callosal terminals at the area 17/18 border, close to the vertical meridian, prompts for a role of the callosum in binocularity.
In cats, callosal neurons are highly binocular cells (i.e. the respond equally to a stimulus presented to the ipsilateral or the contralateral eye) (Hubel and Wiesel 1967; Berlucchi and Rizzolatti 1968) and a number of experiments have been performed to probe a role for the corpus callosum in eye preference. However, they have yielded contradictory results. Section of the callosum had no effect (Minciacchi and Antonini 1984) or lead to a dramatic reduction in binocularity (Payne et al. 1980; Blakemore et al. 1983; Elberger and Smith 1985; Yinon et al. 1992) in cat visual cortex. The discrepancies in these results may be consequence of technical aspects, including age at which the callosal section is performed and time elapsed between surgery and recording.

In rodents, visual cortex responsiveness is biased towards the contralateral eye (Fagiolini et al. 1994; Gordon and Stryker 1996; Hensch et al. 1998; Sawtell et al. 2003), due to the high percentage (over 95%) of retinal fibres crossing at the chiasm (Sefton 1995), as compared to about 50% of fibers (from the nasal hemiretina) in cats, monkeys and humans. In rats and mice, the contralateral bias is progressively reduced getting closer to the highly binocular V1/V2 border (Gordon and Stryker 1996; Caleo et al. 1999b), that maps the vertical meridian, and where callosal projections are particularly dense (Cusick and Lund 1981; Mizuno et al. 2007). A pioneer experiment addressing the role of interhemispheric communication in rodents has ben performed by Diao and collaborators in albino rats (Diao et al. 1983). Their data that show a dramatic shift of eye preference (due to loss of ipsilateral eye responses) after cooling of the opposite cortex (Diao et al. 1983). More in depth analyses on the role of the callosum in rodents binocularity are, to our knowledge, not published.

PART TWO: Tetanus neurotoxin–induced epilepsy in mouse visual cortex

CLOSTRIDIAL NEUROTOXINS

All the most poisonous toxins are neurotoxins. Given the essential role of the nervous system in animal physiology, even a minor biochemical modification of a few neurons may result in a profound modification of behaviour. In general, neurotoxins block in one way or another the transmission of the synaptic impulse. Clostridial neurotoxins (CNTs) are a family of eight proteins endowed with a metalloprotease activity, all of them sharing a common structure and mechanism of action. The mouse LD_{50} values of CNTs are between 0.1 and 1 ng toxin/kg body weight, thus they are the most toxic substances known (Schiavo et al. 2000). This family is composed by seven serotypically distinct botulinum neurotoxins (BoNTs), indicated with letters from A to G, and the tetanus neurotoxin (TeNT). BoNTs and TeNT are the causative agents of respectively botulinum and tetanus. The opposite clinical symptoms of tetanus and botulism (tonic versus flaccid paralysis) result from different sites of action of TeNT and BoNTs, rather than from a different mechanism of action. BoNTs and TeNT enters the nervous system via presynaptic terminals of the α-motor neuron at the neuro-muscular junction (NMJ) where BoNTs exert their toxic activity, leading to blockade of acetylcholine release and flaccid paralysis. In contrast, TeNT undergoes retrograde transport into the spinal cord, where it blocks inhibitory inputs to α-motor neurons, thus triggering spastic paralysis.

Structurally, TeNT and BoNTs share a common organisation, with a heavy (H, 100kDa) and a light chain (L, 50 kDa) linked by a disulphide bond and non-covalent interactions. After entering the general circulation through infected food or wounds, the carboxy-terminus of the heavy chain (HC) binds with extraordinary affinity and specificity to nerve terminals. Following internalization, the amino-terminal portion of the heavy chain (HN) inserts into the membrane of the endosome at acidic pH and assists the translocation of the L chain into the cytosol. Finally, the L chain is endowed with a zinc-endopeptidase activity specific for the exocytosis complex SNARE, thus blocking neurotransmission. These steps are described in details below.

Binding Of Clostridial Neurotoxins To Neurons

Binding of clostridial toxins to synaptic terminals involves a first step of interaction with polysialogangliosides and a second interaction with protein receptors. The involvement of polysialogangliosides in binding has been shown by several experiments, for example

pre-incubation of BoNT with polysialogangliosides partially prevents intoxication at the NMJ (Verderio et al. 2006). Proteins exposed to the cell surface (especially during the endo/exocytosis cycle) are also involved in toxin binding.

Protein receptor molecules have been so far identified only for a subset of clostridial toxins (Dong et al. 2003; Rummel et al. 2004; Dong et al. 2006; Mahrhold et al. 2006). BoNT/ B and BoNT/G have been found to bind to synaptotagmins (Syts) I and II, two proteins integral to the synaptic vesicle membrane (Dong et al. 2007; Rummel et al. 2007). The Syt domain recognized by BoNT/B is inside the synaptic vesicle lumen and it is therefore exposed at the synaptic terminal during exocytosis (Schiavo 2006). This accounts for the well known activity-dependent uptake of BoNTs by synaptic terminals. Another synaptic vesicle protein, SV2, acts as the protein receptor for BoNT/A (Dong et al. 2006; Mahrhold et al. 2006). Again, binding of BoNT/A occurs within a lumenal loop of SV2, accounting for accelerated uptake of the toxin following nerve stimulation (Hughes and Whaler 1962; Keller et al. 2004; Dong et al. 2006). In spite of these recent discoveries, many questions about the membrane binding of BoNTs remain unanswered. For example, protein receptors do not seem to play a major role in the binding of BoNT/C and D (Tsukamoto et al. 2005). Furthermore, recent data indicate that BoNTs may interact with more than one single protein ligand. Indeed, BoNT/A and BoNT/B appear to associate with synaptic vesicle protein complexes comprising SV2, synaptotagmin, synaptophysin, VAMP-2, and several subunits of the vesicular ATPase (Baldwin and Barbieri 2007). There is also evidence that a growth factor receptor, the fibroblast growth factor (FGF) receptor 3 can bind BoNT/A (Jacky et al. 2013).

At the NMJ, TeNT internalization relies on a specialized clathrin-mediated pathway, which is independent of synaptic vesicle recycling (Deinhardt et al. 2006a). Nevertheless, in the central nervous system, activity seems to enhance TeNT internalization (Matteoli et al. 1996).

TeNT HC binds to a lipid-protein receptor complex containing the ganglioside GD1b (Deinhardt et al. 2006a). Furthermore, a recent report suggested the possibility that both the oligosaccharide and sialic binding sites of TeNT recognise different ganglioside species (Chen et al. 2008b).

To date, only one paper reports a protein identified as TeNT receptor in the CNS, the vesicular protein SV2A/B (Yeh et al. 2010a). Other papers describe the involvement of additional molecules in TeNT binding. The toxin binds the glycoprotein Thy-1 but this interaction is unlikely to be crucial for the biological activity of TeNT as Thy-1 knockout mice retain sensitivity to this toxin (Herreros et al. 2001). Interestingly, Yeh and colleagues

(Yeh et al. 2010a) found an unexpected preferential action on excitatory versus inhibitory neurotransmission, by exposing cortical neurons to low concentrations of TeNT. In contrast, in spinal cord cultures, TeNT preferentially acted on inhibitory neurons resulting in the expected pathological symptoms of hyper excitability (Yeh et al. 2010a).

Internalization And Intracellular Trafficking

After the binding to the neuronal membrane, BoNT L-chain undergoes translocation into the cytosol induced by a drop in the vesicle pH (Matteoli et al. 1996; Deinhardt et al. 2006a). Thus, most of the BoNT action remains localised to the delivery site. However, recent data report evidence for long distance anterograde and retrograde axonal trafficking of BoNT/A (Antonucci et al. 2008b; Restani et al. 2011b). Moreover, BoNT/A and E undergo retrograde transport in primary motor neurons (Restani et al. 2012).

Conversely, once bound, TeNT is internalized into non-acidified vesicles that also harbour growth factor receptors (Bohnert and Schiavo 2005). The TeNT-harbouring vesicle is sorted via a Rab 5/7 dependent pathway and transported back to the cell body of the motor neuron (Deinhardt et al. 2006b). TeNT then undergoes transcytosis by being released from the motor neurons such that it enters upstream inhibitory neurons, where it translocates to the cytosol to cleave SNAREs and inhibit transmitter release (Curtis and De Groat 1968; Schwab et al. 1979).

Proteolytic Activity

The L-chains of the CNT are remarkably specific proteases acting at single, different peptide bonds. TeNT, BoNT/B, D, F and G cleave only VAMP (vesicle-associated membrane protein), with loss of most of its cytosolic domain. BoNT/A and E cleave only SNAP-25 within its C-terminus, and BoNT/C cleaves both SNAP-25 and syntaxin. SNAP-25 and syntaxin reside on the plasma membrane facing the nerve cell cytosol. VAMP, SNAP-25 and syntaxin form the trimeric SNARE complex by winding one to another to form a stable, four-helix, coiled-coil structure. Several SNARE complexes then assemble into a rosette that brings the synaptic vesicle membrane close enough to the cytosolic face of the presynaptic membrane to permit their fusion, with subsequent release of the vesicle neurotransmitter content into the synaptic cleft (Jahn et al. 2003; Montecucco and Molgo 2005). Proteolysis of one SNARE protein prevents the formation of a functional SNARE complex and, consequently, the release of neurotransmitter, thus blocking neuronal communication (Rossetto et al. 2006).

Given its ability to interfere with neurotransmission, CTS are extensively exploited for the study of cellular physiology. Moreover, BoNTs are also employed in clinical neurology for the treatment of several disorders characterised by hyper-excitability of peripheral nerve terminals (Caleo and Schiavo 2009).

TeNT and BoNTs do not normally cross an intact blood brain barrier. However, several investigators have deliberately introduced these toxins into the brains of experimental animals to interfere with central neurotransmission (Caleo et al. 2007; Antonucci et al. 2010).

In particular, injections of TeNT into the brain have been used to produces epileptic foci (Jefferys and Walker "Tetanus toxin model of focal epilepsy", Models of Seizures and Epilepsy; Elsevier 2006).

EPILEPSY: A QUICK OVERVIEW

Epilepsy has an estimated frequency between 4 and 10 per 1,000 individuals per year, and, if not treated, is associated with progressively impaired cognition and function, brain damage, and other neurologic deficits (Duncan et al. 2006; Sorensen and Kokaia 2013). It is a common end point of many forms of acquired brain pathology, such as tumors, infection, stroke and traumatic brain injury. It can also be the result of mutation of a single gene, and it can be one component of neuro-developmental disorder (Goldberg and Coulter 2013). Epilepsy genes are isolated from two principal sources: the cloning of the inherited disorders in human, mouse, and fly, and the incidental finding of epilepsy phenotypes following targeted mutagenesis (Noebels 2003).

Many strategies have been developed to generate animal models to study this pathology, including generation of transgenic strains, lesional models, kainate and pilocarpine injections, and kindling induced seizures. Despite many years of research, the common mechanisms generating epilepsy as a response to so many different brain conditions are not clear, and the current treatments are mainly aimed at controlling seizures with antiepileptic drugs (AEDs), the mainstay for epilepsy treatment. Nevertheless, approximately 35% of all epilepsy patients experience recurrent nonprovoked spontaneous seizures (Duncan et al. 2006). Unfortunately, other treatment possibilities for patients with drug-refractory epilepsy are limited.

Although many models of Temporal Lobe Epilepsy (TLE, the most frequent form of the pathology) are available, there is a growing need for models of cortical epilepsy, especially

generated in mouse, the election animal model of the recent years due to the powerful transgenic techniques available in this species.

The biochemistry of the TeNT makes it an ideal candidate to generate non lesion-induced cortical hyper-excitability.

THE TETANUS TOXIN MODEL OF FOCAL EPILEPSY

When injected into the brain parenchyma, TeNT induces a focal, chronic epileptic syndrome, apparently by interfering with GABA release (Empson et al. 1993).

Data examining inhibitory postsynaptic currents in slices from TeNT-injected rat hippocampi show a transient loss of inhibition that likely plays a key role in triggering hyper-excitability (Empson et al. 1993; Whittington and Jefferys 1994). Because GABAergic inhibitory cells constitute only about 20–30% of cortical neurons, and VAMP (the TeNT target molecule) is expressed in both excitatory and inhibitory neurons (Yeh et al. 2010b), a selective action of TeNT in interneurons is still debated.

A number of experiments were aimed at generating epilepsy in the hippocampus, which models TLE, or in the neocortex, which models focal neocortical epilepsy, secondary generalized seizures, and epilepsia partialis continua (Jefferys and Walker "Tetanus toxin model of focal epilepsy", Models of Seizures and Epilepsy; Elsevier 2006). The toxin-induced epileptic activity has been found in any vertebrate tested, but the animal model of election for these type of studies has quickly become the rat.

In the version of the model induced by intrahippocampal injection in adult rats, spontaneous generalised seizures are very frequent (about 30 per day). They are characterised by initial behavioural arrest followed by forelimb clonus with rearing and falling. Most (~90%) animals do gain remission of seizures after 6 to 8 weeks. The remaining approximately 10% continue to have seizures indefinitely. However, none of the rats returns to normal. They all have enduring impairments of learning and memory (George and Mellanby 1982; Jefferys and Williams 1987; Mellanby et al. 1999) and a loss of the aversion that rats normally have to novel objects (Mellanby et al. 1977; Mellanby et al. 1999). They also retain enduring changes in neuronal structure and function, including reduced synaptic responses and changes in several intrinsic neuronal properties (Brace et al. 1985; Vreugdenhil et al. 2002).

When tetanus toxin is given intrahippocampally in the neonate, the initial seizures stop within about 1 week, but inter-ictal spiking continues almost indefinitely. When these rats

become adults, they can exhibit unprovoked seizures, but the incidence appears to be low (Lee et al. 1995; Lee et al. 2001). As with the adult model, learning is impaired many months after the injection of toxin (Lee et al. 2001). In both adult and neonatal models, this finding shows that neither gross neuronal lesions nor the presence of anticonvulsant drugs is necessary for problems in learning and memory associated with temporal lobe epilepsy.

In contrast to the intra-hippocampal administration, when the toxin is injected into the neocortex, there is little sign of seizures remission over periods of many months (Louis et al. 1990; Brener et al. 1991; Nilsen et al. 2005). Thus intracortical delivery of TeNT reliably produces spontaneous seizures.

Notably, focal neocortical epilepsy in the rat induced by TeNT delivery in the motor cortex appear to be resistant to conventional AEDs (Nilsen et al. 2005).

A detailed in vitro analysis of the hyper-excited phenotype shows that TeNT injection generates both interictal and the seizure-like spontaneous epileptic discharges (Jefferys 1989). Moreover, TeNT induced hyper-excitability displays high-frequency cortical activity, particularly in the 250–600 Hz (fast ripple) band, that has been implicated in playing a crucial role in epileptogenesis and seizure generation in humans (Jiruska et al. 2010).

Despite its large use for generating neuronal hyper-excitability, the time-course of TeNT proteolytic action in the brain and of the consequent epilepsy development has not been precisely determined. In addition, it remains unclear whether TeNT effects can propagate to distant, synaptically connected areas when delivered into the CNS. This is particularly relevant in view of the ability of TeNT to undergo bidirectional axonal transport and transcytosis (Salinas et al. 2010).

AIM OF THE THESIS

AIM OF THE THESIS

The rodent visual cortex is the model of election to study the development, plasticity and pathology of cortical circuitry, given its well known physiology and the ease of manipulation of its primary sensory input.

One of the most used paradigm to test the plastic potential of visual cortical neurons is the experience-induced shift in one of their main properties, i.e. binocularity. Binocularity arises from the interaction of the visual stimuli coming from the two eyes and converging on primary visual cortex neurons. Given the anatomical organisation of the rodent visual pathways (with a massive percentage of optic nerve fibres crossing at the chiasm), the high binocularity of visual neurons mapping the vertical meridian is quite unexpected.

The aim of the first part of my thesis is to clarify how binocularity is generated in primary visual cortex, including in the study the role of a major but often neglected cortical input: the corpus callosum. By silencing either the callosal or the thalamic route I elucidated the contribution of each pathway to cortical binocularity, leading to a model of cortical circuitry.

Then, I clarified how the inputs coming from the two eyes integrate in binocular neurons, and how plasticity induced with the classic paradigm of monocular deprivation influences this interaction. Furthermore, I probed the role of the callosum in the plastic shift of binocularity induced by monocular deprivation.

In the second part of my work, I took advantage of the well known visual cortex physiology and of the properties of the tetanus neurotoxin (TeNT) to characterise a model of neocortical epilepsy in mouse.

Despite its wide use in generating cortical hyper-excitability, in the literature there were missing data describing the time course and site specificity of TeNT action in the cortex, and of the emerging hyper-excited phenotype.

Moreover, TeNT induced hyper-excitability had been previously described in rats, while the fine genetic techniques available in mice encourage the development of models of diseases in this species.

Here, I used biochemical, neuroanatomical and in vivo electrophysiological techniques to describe in details the proteolytic action of the TeNT in the brain parenchyma and the development of epileptiform activity.

The transient focal impairment of neurotransmission in the visual cortex of one hemisphere also renders callosal communication asymmetric, generating a new model to study the role of inter-hemispheric communication in all the pathological conditions that disrupt the balance between the two hemispheres.

MATERIALS AND METHODS

PART ONE: Mechanisms of OD plasticity in primary visual cortex

ANIMAL TREATMENT

Long-Evans juvenile (P20-P31) and adults (2 months old) hooded rats were used in this study. Animals were reared in a 12 hr light/dark cycle, with food and water available *ad libitum*. All experimental procedures conformed to the European Communities Council Directive n° 86/609/EEC.

The number of animals used for each experiment is indicated in the figures.

After surgery, ophthalmic antibiotic (when needed) and paracetamol were administered.

Monocular deprivation was performed at P21 by eyelid suture under isoflurane or avertin [1.25% W/V Tri-Br-Ethanol (Sigma-Aldrich, Milan, Italy) in 2.5% V/V tertiar-amyl alcohol (Baker, Milan, Italy) in water] anesthesia. MD animals were carefully inspected every day to make sure that the lid suture remained intact.

IN VIVO EXTRACELLULAR ELECTROPHYSIOLOGY

P27-P31 rats were anesthetized with urethane (8 ml/kg; 20% solution in saline, i.p.; Sigma) and placed in a stereotaxic apparatus. Both eyes were fixed by means of adjustable metal rings surrounding the external portion of the eye bulb, and optic disk locations were projected onto a tangent screen to determine the vertical meridian. Body temperature during the experiments was constantly monitored with a rectal probe and maintained at 37°C with a heating blanket. Electrocardiogram was also continuously monitored. A portion of the skull overlying the binocular visual cortex was carefully drilled and the dura was removed. A glass micropipette (2 MΩ) filled with NaCl (3M) was inserted into the binocular portion of primary visual cortex (V1, coordinates were as follows: 4.5 - 4.7 mm lateral and in correspondence with lambda).

VEPs Recordings

For each penetration the electrode was inserted in the cortex and positioned at a depth of 150 or 400 µm into the cortex, respectively where the positive and negative EEG deflection triggered by visual stimulation had their maximal amplitude.

Visually Evoked Potentials (VEPs) were recorded in response to abrupt reversal of a horizontal square wave grating (0.07 c/deg, 90% contrast, 1 Hz), generated by a computer on a display (Sony) by a VSG card (Cambridge Research System). The display was

positioned in front of the rat's eyes to include the binocular visual field. Signals were amplified (10,000 fold), bandpass-filtered (0.1 – 500 Hz) and fed to a computer for storage and analysis. At least 50 events were averaged in synchrony with the stimulus contrast reversal. VEPs in response to a blank stimulus were also frequently recorded to estimate noise. Transient VEPs were evaluated in the time domain by measuring the peak to trough amplitude of the major positive (for superficial recordings) or negative (for deep recordings) component. Transient VEPs were recorded binocularly and for each eye, occluding the other eye with a black patch during the acquisition.

Binocularity in naïve and monocularly deprived (MD) animals was measured in V1 as the ratio between VEPs evoked by the contralateral and the ipsilateral eye (C/I ratio). The C/I ratio was measured at 150 and 400 µm of cortical depth and averaged for each penetration, usually three penetrations were done and averaged per each animal.

Latency of the major component of the response was measured stimulating the contralateral eye with 15% contrast and ipsilateral eye with 30% contrast to get a C/I ratio ≈ 1. In this condition, the population of neurons activated by the two eyes is roughly the same, and any possible confounding factor in the latency measurement is minimized.

Single-unit Recordings

The position of receptive fields of single units were mapped using a hand-held stimulator. Only cells with receptive fields within 20° of the vertical meridian were included in the analysis. All of the cells were recorded from supragranular layers (i.e. at a depth less than 800 µm from the cortical surface). The visual stimulus consisted of a computer-generated bar (contrast, 90%; thickness, 3°; speed, 28°/sec) presented on a monitor (Sony, 40 x 30 cm; mean luminance 15 cd/m2). Signals were amplified 25,000-fold, bandpass filtered (500-5000Hz), and conveyed to a computer for storage and analysis. Action potentials were discriminated from background by a voltage threshold, that was set as 4.5 times the standard deviation of noise, as described (Antonucci et al. 2007; Caleo et al. 2007; Resta et al. 2007).

Peak response was determined from peristimulus time histograms (PSTHs; bin size = 33 msec) of the cell response to the stimulus, averaged over 20 consecutive stimulations as described (Lodovichi et al. 2000; Antonucci et al. 2008a). Peak response was evaluated as the peak firing rate (spikes per second) in the cell response to the stimulus.

Ocular dominance (OD) was evaluated from peak responses according to the methods of Hubel & Wiesel (Hubel and Wiesel 1962). Neurons in ocular dominance class 1 were driven exclusively by stimulation of the contralateral eye; neurons in ocular dominance class 2/3 were binocular and preferentially driven by the contralateral eye; neurons in ocular dominance class 4 were equally driven by the two eyes; neurons in ocular

dominance class 5/6 were binocular and preferentially driven by the ipsilateral eye; neurons in ocular dominance class 7 were driven only by the ipsilateral eye.

INTRACORTICAL INJECTIONS

Unilateral stereotaxic injections of muscimol (1µL, 30 mM) or saline (0,9% NaCl) were made into left primary visual cortex of P27-31 rat during in vivo electrophysiology experiments (urethane anesthesia: 8 ml/kg; 20% solution in saline, i.p.; Sigma). A portion of the skull overlying the binocular visual cortex was carefully drilled before starting electrophysiological measurements, and the dura was left intact. Coordinates were as follows: 4.5 - 4.7 mm lateral and in correspondence with lambda, great care was taken in injecting at the same coordinates used for recording in the contralateral side.

Injections were performed by means of a back-filled glass pipette (40 µm tip diameter) mounted on a three-axis micromanipulator and connected with a PE tube to a syringe. The solution was slowly delivered at a depth of 0.6 – 1 mm from the pial surface. After muscimol delivery, we waited 40 minutes before measuring binocularity again.

The same protocol was used under avertin anesthesia for Cholera Toxin subunit B (CTB) intracortical injections (see below: Retrograde Labeling of Callosal Cells).

INTRAVITREAL EYE INJECTIONS

Eye injections of tetrodotoxin (TTX; 1 µL of a 3 mM solution; Sigma) or saline (0.9% NaCl) were performed during in vivo electrophysiology experiments (urethane anesthesia: 8 ml/ kg; 20% solution in saline, i.p.; Sigma) by means of a handheld back-filled glass pipette (40 µm tip diameter) connected with a PE tube to a Hamilton micro-syringe. Eyes were fixed by means of adjustable metal rings surrounding the external portion of the eye bulb. The pipette was inserted at the ora serrata avoiding blood vessels, in correspondence of the temporal portion of the eye, and the solution slowly delivered into the vitreous. In 5 minutes, a single TTX injection was sufficient to induce a tonic dilation of the pupil and loss of direct pupillary reflex to eye illumination, confirming the silencing of retinal ganglion cells activity [see (Caleo et al. 1999a)]. The efficacy of the TTX injection was confirmed by the loss of visually evoked responses through the injected eye.

OSMOTIC MINIPUMP IMPLANT

Rats were monocularly deprived for 4-7 days and muscimol (10–30 mM solution) or saline were concurrently infused by osmotic minipumps into the visual cortex ipsilateral to the closed eye. Rats under avertin anesthesia were placed in a stereotaxic apparatus, the skin was cut open and the skull carefully cleaned. A small hole was drilled in correspondence of V1 (coordinates 4.5 - 4.7 mm lateral and in correspondence with lambda) and the minipump was carefully inserted beneath the skin in the back of the animal. The custom made infusion cannula releasing muscimol or saline was inserted in the cortical parenchyma through the opened hole. After the implant, the skull was covered in acrylic dental cement (Paladur), and the skin re-sutured. Topic antibiotics and paracetamol in the drinking water were administered after the surgery.

At the end of the deprivation period, minipumps were cut apart from the implant under isoflurane anesthesia, and removed from the back of the animal. The animals were then placed in complete darkness for 26–48 hr to allow washout of muscimol without interfering with monocular deprivation induced plasticity.

RETROGRADE LABELING OF CALLOSAL CELLS AND IMMUNOHISTOCHEMISTRY

Cholera Toxin subunit B (CTB, 1% solution in water; Sigma-Aldrich) was injected intracortically in P28 rats (naïve, n = 4; monocularly deprived, n = 4). In monocularly deprived animals, injections were contralateral to the occluded eye. Two days after CTB, rats were deeply anesthetized and perfused with 4% paraformaldehyde. Brains were cryoprotected in 30% sucrose and cut in the coronal plane with a freezing microtome. After a blocking step (5% Horse serum, 2.5% albumin, 0,3% Triton X-100, in PBS), coronal sections (40 μm thick) were reacted overnight with anti-CTB (goat polyclonal; Calbiochem; 1:3000 dilution) and anti-GABA (rabbit polyclonal; Sigma-Aldrich; 1:1,000) antibodies. Bound primary antibodies were revealed by donkey anti-goat Alexa 568 and donkey anti-rabbit Alexa 488 secondaries (Invitrogen; 1:400 dilution). We examined 80–135 CTB-positive cells (four to five sections) per animal with an Olympus confocal microscope and Metamorph software. We restricted our analysis to cells located in superficial layers and at the border between area 17 and 18.

PREPARATION OF ANIMALS FOR OPTICAL IMAGING AND WHOLE-CELL RECORDINGS

Four weeks old C57BL/6J mice were used. Animals were anesthetized with urethane (8 ml/kg; 20% solution in saline, i.p.) and placed in a stereotaxic apparatus. Betamethasone (0.05 mL per mouse, i.m.) was injected to prevent cortical and mucosal edema, and artificial tears were used to protect the cornea. Body temperature during the experiments was constantly monitored with a rectal probe and maintained at 37°C with a heating blanket.

The skull was exposed and the bone above V1 was carefully drilled. Before opening the craniotomy a small dental cement (Paladur) crown was performed at the borders of the skull to create a small bath and keep the cortical surface always covered by physiological solution (NaCl 0.9%) After preparing the recording pipettes, a small rectangular craniotomy (about 500x300 µm) was cut in the bone with a surgical blade above the binocular portion of V1, identified by optical imaging measurements (see below).

INTRINSIC OPTICAL IMAGING

The intrinsic optical imaging (IOI) response was measured before and after injection of fluorescent muscimol in the dorsal lateral geniculate (dLGN) ipsilateral to the recording site, to confirm the decrease in cortical responsiveness after geniculate silencing.

A vasculature ("green") image was acquired under 540 nm illumination before starting the imaging session. During IOI, the cortex was illuminated with monochromatic light of 630 nm wavelength. Images were acquired using a cooled 50-Hz CCD camera connected with a frame grabber (Imager 3001, Optical Imaging Inc., Germantown, NY, USA), defocused ca. 500–600 μm below the pial surface. Data frame duration was 200 ms and a spatial binning of 3x3 was applied over the images, which were 4.35x4.35 mm, with a pixel resolution of about 25 μm.

Visual stimulation during IOI. Corneas were protected with artificial tears. 20x20 degrees squared spots were projected along the vertical meridian, the spots displayed squared drifting gratings (duration: 8 s, spatial frequency: 0.05 cycles/deg, speed: 2 cycles/s, contrast: 90%, mean grating luminance: 19 cd/m2). Stimulus orientation was randomly alternated during the 8 s of stimulation every 45 degrees, every second [for details see (Iurilli et al. 2012) (Medini 2011)].

Image analysis. All image frames obtained during stimulus presentation were divided by the average image of the first 10 frames acquired just before stimulus presentation. The relative decrease of reflectance, averaged over the stimulus presentation period, was then outlined. The spot area was taken as the image area where the visually evoked decrease

in reflectance was higher than 50% of the peak decrease. This region was then overlaid with the vasculature "green" image and above this region the craniotomy was performed.

IN VIVO WHOLE-CELL RECORDINGS

Borosilicate patch pipettes (6–8 MΩ, 1μm tip, 2mm outer diameter, without filament) filled with intracellular solution (135 mM K-gluconate, 10 mM HEPES, 10 mM Na phosphocreatine, 4 mM KCl, 4 mM ATP-Mg, 0.3 mM GTP, pH 7.2, 291 mOsm osmolarity) were lowered with a 30° inclination to the pia applying ~300 mmHg of positive pressure until the layer of interest was reached (Margrie et al. 2002). At that point, positive pressure was lowered to 30 mmHg, and cells were searched for in voltage-clamp mode. On approaching a cell, pressure was relieved and light suction was applied to allow Gigaseal formation. A small suction usually led to whole-cell configuration. The membrane potential (Vm) signal and its responses to visual stimulation were low-pass filtered at 10 kHz, digitized at 20 kHz and acquired using the program Patchmaster (HEKA). No holding current was used.

Visual stimulation: The screen was positioned at 30 cm from the mouse eyes. 1 second of baseline was acquired before and after the stimulus appearance. The stimulus presentation lasted for 1 second [drifting grating: 0.05 c/deg, contrast 90%, of different directions (every 90°)] and were separately presented to the two eyes. Sweeps were averaged over 20 presentations. Each stimulus direction was presented to the ipsilateral eye, to the contralateral eye and with both eyes open.

Analysis of visual responses: Data were analyzed using custom-made software written in MatLab by Umberto Olcese (IIT, Genova). Ocular dominance was quantified by computing an ocular dominance index (ODI) for each recorded neuron defined as $(C - I)/(C + I)$, where C and I are the peak responses to stimulation through the contralateral and ipsilateral eyes, respectively. This index varies from -1 to +1 for cells that are driven solely by the ipsilateral and contralateral eye, respectively, and is 0 when cells are perfectly binocularly driven [as described in (Medini 2011)].

INTRA-GENICULATE INJECTIONS

10 animals (four weeks C57BL/6J mice) received unilateral muscimol injection in the dorsal lateral geniculate (dLGN) before the intracellular recording of V1 neurons. A craniotomy was opened centered around coordinates 2.3 mm anteroposterior and 2.1 mm

mediolateral from bregma, 3 mm below dura. An injecting pipette (20 μm tip) was lowered at the above mentioned coordinates and 0.5 - 1 μL of fluorescent muscimol (1.6 mM in saline, TMRX-conjugated, Molecular Probe) were slowly delivered in the thalamic nucleus via a device for the control of pipette internal pressure. After 20 minutes the intrinsic optical imaging was performed to visually probe the decrease in V1 activity, and the patch experiment was performed.

At the end of every experiment animals were perfused with 4% paraformaldehyde. Brains were cryoprotected in 30% sucrose and cut in the coronal plane with a freezing microtome. The 100 μm slices were briefly incubated with Hoechst (1mg/ml, diluted 1:500 in PBS) and inspected to evaluate the accuracy of the injection.

PART TWO: Tetanus neurotoxin-induced epilepsy in mouse visual cortex

ANIMAL TREATMENT AND TeNT INJECTIONS

Adult (age > postnatal day 60) C57BL/6N mice were used in all the experiments. Experiments were conducted in conformity with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

For TeNT injections, animals were anesthetized with Avertin (1.25% W/V Tri-Br-Ethanol [Sigma-Aldrich, Milan, Italy] in 2.5% V/V tertiar-amyl alcohol [Baker, Milan, Italy] in water) and placed in a stereotaxic apparatus. Coordinates for injection were 0.0 mm anteroposterior and 2.7 mm lateral to the lambda suture. Using a glass micropipette, a solution of TeNT (Lubio, Lucerne, Switzerland; 200 nL total volume; 0.1–0.2 ng) in phosphate-buffered saline (PBS) containing 2% rat serum albumin (RSA) was injected at a cortical depth of 600 lm. Control animals received RSA alone (for more details, see "*Intracortical injections*").

IMMUNOBLOTTING

Visual cortices of adult mice that received unilateral TeNT injections in the primary V1 were dissected 1, 7, 14, and 21 d after treatment. Proteins were extracted (Viegi et al. 2002) with lysis buffer (1% Triton X-100, 10% glycerol, 20 mM Tris-HCl, pH 7.5, 150 mM

NaCl, 10 mM EDTA, 0.1 mM Na$_3$VO$_4$, 1 g/ml leupeptin, 1g/ml aprotinin, and 1 mM PMSF). The total protein concentration of the samples was assessed with a protein assay kit (Bio-Rad) using a bovine serum albumin-based standard curve. Protein extracts were separated by electrophoresis, and blotted (total proteins loaded per lane were 10 μg to detect intact VAMP2, VGlut1 and GAD65/67 and 50 μg to detect cleaved VAMP2). Filters were incubated for 2 hours with blocking solution (4% milk, 0.2% Tween in tris-buffered saline TBS) and then with primary antibodies overnight at 4°C. The primary and secondary antibody solution contained 2% milk, 0.1% Tween- 20, and either 1:1000 anti-VAMP2 mouse monoclonal antibody (Synaptic Systems) or 1:100 anti-cleaved VAMP rabbit polyclonal antibody (produced by Ornella Rossetto) or 1:10000 anti-α-tubulin mouse monoclonal antibody (Sigma-Aldrich) in TBS. For the detection of VGlut1 and GAD56/67 we used 1:4000 VGlut1 Synaptic System polyclonal antibody and 1:5000 GAD65/67 Sigma polyclonal antibody. HRP-conjugated secondary antibodies were used 1:20000 (Jackson Immunoresearch Goat-Anti-Mouse and Goat-Anti-Rabbit). Filters were then reacted with Immun-Star™ HRP Chemiluminescent Kit (BIO-RAD) and proteins bands were visualized on Kodak photographic films. Quantification of Western blot experiments was performed as described previously (Caleo et al. 2007) (Mainardi et al. 2010). Films were digitized with a scanner and band optical densities (OD) were measured with ImageJ software. Data from each sample were normalized to the internal control run on the same gel, and summarized results were presented as a percentage of controls.

IMMUNOHISTOCHEMISTRY

Anatomic analysis was conducted in an independent subset of animals injected with TeNT (n = 4) or vehicle (n = 4) 55 days after injection. Animals were perfused and immunostaining was performed as described [see: Retrograde Labeling of Callosal Cells and Immunohistochemistry and (Restani et al. 2011a)]. Primary antibodies were: 1:500 anti-intact VAMP2 mouse monoclonal (clone 69.1; Synaptic Systems, Heidelberg, Germany); 1:200 anti-NeuN mouse monoclonal (Millipore, Billerica, MA, U.S.A.); 1:400 anti-Iba-1 rabbit polyclonal (Wako, Osaka, Japan). The number of cells immunoreactive to Iba1 (ionized calcium adaptor binding molecule 1, specific marker for microglia) and neuronal nuclei, specific marker for neurons (NeuN) was quantified with Stereo Investigator software (MicroBrightField, Williston, VT, U.S.A.), using a three-dimensional counting box (150 x 800 x 30 μm) encompassing layers I–VI of V1. For each animal, at least six sections were examined blind to experimental treatment.

LOCAL FIELD POTENTIAL (LFP) RECORDINGS

We performed bipolar recordings by placing a couple of Nichrome wire electrodes to sample the local electrical activity originating in the TeNT-injected V1 (Di Garbo et al. 2011). Under avertin anesthesia, mice were placed in a stereotaxic apparatus, and then two electrodes were positioned epidurally (2.5 and 3.5 mm lateral and 0.0 mm anteroposterior to lambda); a ground screw was placed in the occipital bone. Recordings began 3 days after surgery; the animal was habituated for 1 h to the test cage before a 1-h recording session, using a digital acquisition system. Quantitative analysis of electrographic epileptiform activity was done using a custom-made application, based on LabView. We used an automatic quantification in which all large amplitude events (>4 times the standard deviation [SD] of the baseline) were considered epileptiform spikes (Antonucci et al. 2008a). Clusters of these high-amplitude spikes lasting for >4 s were classified as ictal events. Trains of spikes lasting <4 s or isolated spikes were counted as interictal events.

RESULTS

PART ONE: Mechanisms of OD plasticity in primary visual cortex

ROLE OF THE CALLOSUM IN BINOCULARITY

In rodents the entire extent of the primary visual cortex contains cells that send their axons to the opposite visual cortex through the corpus callosum (Lewis and Olavarria 1995). Their terminals are particularly concentrated in a quite narrow stripe at the area 17/18 border (Cusick and Lund 1981; Mizuno et al. 2007), in correspondence of the representation of the vertical meridian, the cortical region of highest binocularity (Gordon and Stryker 1996; Caleo et al. 1999b).

To test whether the corpus callosum was involved in determining binocularity in naïve young rats, we measured eye preference before and after acute inactivation of the callosal input to the binocular portion of the primary visual cortex (V1), in correspondence of the cortical representation of the vertical meridian. Inactivation of the callosal input was performed by a single intracortical injection of muscimol, a $GABA_A$ agonist, in the hemisphere contralateral to the recording site.

By silencing the callosal input to the cortex we were able to infer its contribution to V1 neurons.

Eye preference was measured as the ratio between the Visually Evoked Potentials (VEPs) triggered by the ipsilateral or the contralateral eye, the contra/ipsi (C/I) ratio.

VEPs represent the integrated response of cortical neurons to patterned visual stimuli and are routinely used to measure and assess changes in binocularity (Porciatti et al. 1999; Frenkel and Bear 2004) (Sale et al. 2007; Maya Vetencourt et al. 2008). All experiments were performed under urethane anesthesia.

The VEP ratio in naïve juvenile rats (post natal day, P28; n=13) was C/I = 1.6 (± 0.07 s.e.), indicating, as expected in rodents, a cortical preference towards the contralateral eye. Saline injection in the opposite visual cortex did not affect eye preference in naïve animals (n=5; paired t-test n.s.; Fig.1A). On the other hand, silencing callosal communication resulted in a robust shift in eye preference in favor of the contralateral eye, increasing the C/I ratio from C/I=1.69 to C/I=2.26 (n=8; paired t-test p=0.007; Fig.1B). These results indicate a role for the callosum in cortical binocularity.
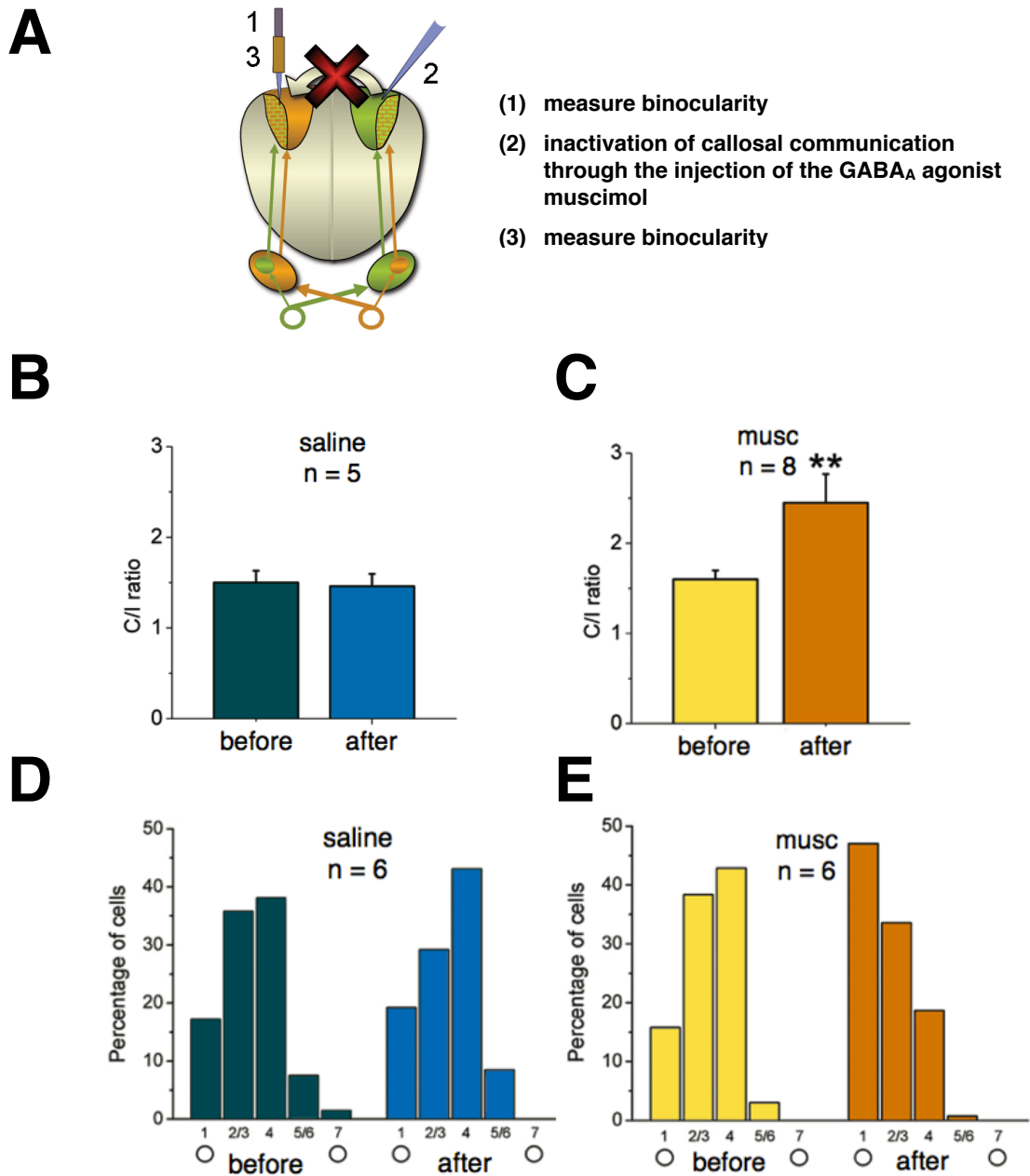
A

(1) measure binocularity

(2) inactivation of callosal communication through the injection of the GABA$_A$ agonist muscimol

(3) measure binocularity

Fig.1 (**A**) Experimental scheme. (**B,C**) Contra/Ipsi (C/I) VEP ratio before and after acute saline (**B**) or muscimol (musc, **C**) injection in the visual cortex opposite to the recording site. Saline injection has no effect on cortical binocularity (n=5; C/I pre = 1,540 ± 0,208 s.e.; C/I post saline = 1,591 ± 0,189 s.e.; paired t-test n.s), while after muscimol injection there is a robust increase in the C/I ratio, indicating a shift in binocularity in favor of the contralateral eye (n=8; C/I pre = 1,694 ± 0,114 s.e.; C/I post musc = 2,257 ± 0,313 s.e.; paired t-test **p<0.01). Data are mean ± SE. (**D,E**) OD distributions before and after injection of saline (**D**) or muscimol (MUSC; **E**) into the opposite hemisphere. Saline has no effect on binocularity (n= 6; c2 test, p = 0.48), whereas rats injected with muscimol show a clear increase in the number of units driven exclusively by the contralateral eye (n = 6; c2 test, p < 0.001). Before saline, n = 135 cells; after saline, n = 130 cells; before muscimol, n = 133 cells; after muscimol, n = 134 cells.

SPIKING DATA: REDUCTION OF IPSILATERAL EYE RESPONSES AFTER CALLOSAL SILENCING

To further investigate the mechanisms by which the callosal input contributes to binocularity, we analyzed the evoked spiking activity of cortical cells before and after silencing of the visual cortex contralateral to the recording site, to block callosal input to the recorded cortex (Fig.1D,E).

Ocular Dominance (OD) was evaluated according to the method of Hubel and Wiesel (Hubel and Wiesel 1962). Neurons in OD class 1 were driven exclusively by stimulation of the contralateral eye; neurons in OD class 2/3 were binocular and preferentially driven by the contralateral eye; neurons in OD class 4 were equally driven by the two eyes; neurons in OD class 5/6 were binocular and preferentially driven by the ipsilateral eye; neurons in OD class 7 were driven only by the ipsilateral eye.

The spiking activity of cortical neurons was recorded extracellularly before and after the injection of either saline or the $GABA_A$ agonist muscimol into the contralateral hemisphere. We found that saline injection had no effect on the OD distribution of cortical neurons (n = 6; c2 test p = 0.48; Fig.1D), but muscimol injection significantly shifted the OD distribution toward the contralateral eye, increasing the proportion of class 1 cells and leading to a corresponding decrease of binocular units (n = 6; c2 test p < 0.001; Fig.1E). The spiking results are consistent with the VEP data and demonstrate that acute silencing of callosal input shifts cortical OD towards the contralateral eye.

The next question was whether the shift of the C/I ratio after the removal of the callosal input was due to an enhancement of contralateral eye driven responses or to a decrease of the ipsilateral eye responses. To answer this question we analyzed the absolute peak firing rates of cell units. As shown in Fig.2 the contralateral eye driven peak responses (spikes/second) display no alteration after muscimol injection, but the ipsilateral eye driven responses show a robust decrease after the removal of the callosal input (n = 6; post ANOVA Dunn's test p < 0.01; Fig.2).

These data demonstrate that after the inactivation of the callosal communication there is a significant reduction of the ipsilateral eye driven responses, suggesting that the callosal pathway represents a major route by which ipsilateral eye inputs arrive to the cortex.
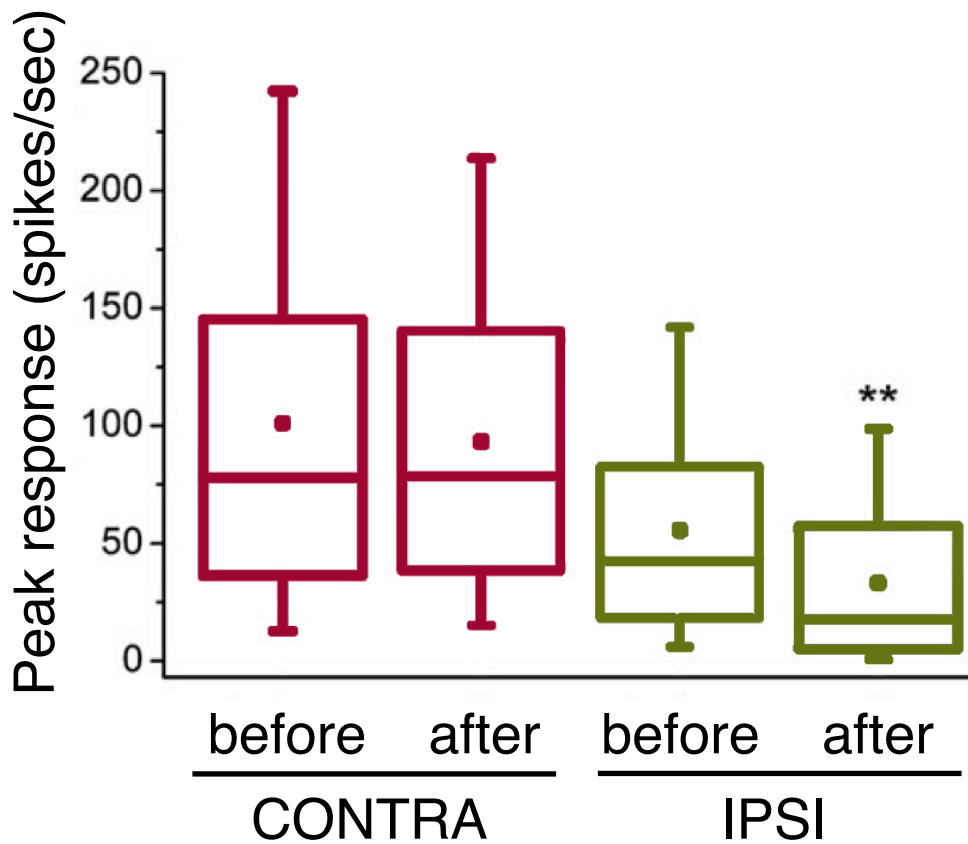
Fig.2 Box chart showing peak firing rates of visual cortical neurons in naïve rats injected with muscimol. There is no significant effect on contralateral (CONTRA) eye responses after muscimol injection in the cortex opposite to the recording site. There is a very significant reduction of the responses evoked by the ipsilateral (IPSI) eye following muscimol administration (n = 6; post ANOVA Dunn's test, ipsilateral eye, before versus after muscimol, $p < 0.01$). The horizontal lines in each box denote the 25th, 50th, and 75th percentile values. The error bars denote the 5th and 95th percentile values. The square symbols denote the mean of the column of data. Before muscimol, n = 133 cells; after muscimol, n = 134 cells, **$p < 0.01$.

IN VIVO INTRACELLULAR RECORDING OF ISOLATED CALLOSAL RESPONSES: PRELIMINARY DATA

To confirm the extracellular results obtained in rats with the silencing of callosal communication, and to extend them to another animal model, we performed a complementary experiment. We recorded intracellular in vivo patch clamp responses (whole cell, voltage follower) in naïve juvenile mice (Fig.3A) and in mice that received unilateral thalamic fluorescent muscimol injection in the dorsal lateral geniculate (dLGN, Fig.3B). By silencing the thalamic input to the cortex we were able to dissect the sole callosal contribution to V1 pyramidal neurons.

After patching the neuron, we recorded the postsynaptic potential (PSP) amplitude evoked by the visual stimulation of each eye (with a drifting grating) at different stimulus directions. We then calculated the sub threshold eye preference of the neuron at every tested stimulus direction, the OD index = (C-I)/(C+I) (Medini 2011).

For the animals that received the thalamic silencing, the accuracy and effectiveness of the muscimol injection were confirmed by anatomical (Fig.3B) and intrinsic imaging data (Fig. 3C). As expected, intrinsic imaging of V1 evoked activity shows a drop in responsiveness after the silencing of the dLGN, the major source of inputs to V1 (Fig.3C). Interestingly, OD index of neurons recorded in the injected animals indicated a tendency towards greater ipsilateral preference in three out of four stimulus conditions (0°, 90° and 180°) (Fig.4). Although not statistically significant (likely due to the small sample size; n = 4 - 10 cells), this ipsilateral shift of neurons driven solely by the callosum is complementary to the contralateral shift of neurons driven only through the thalamus.

Altogether, these data lead to a model for the establishment of binocularity in rodent visual cortex (Fig.5, see discussion). According to our model, cortical neurons receive a direct thalamic input that is predominantly driven by the contralateral eye and a callosal input carrying mainly ipsilateral eye responses. It is important to note that pyramidal excitatory neurons also receive a di-synaptic inhibition through both thalamic and callosal afferents impinging onto local GABAergic interneurons (Toyama et al. 1974) (Sugiyama et al. 2008) (Martin et al. 1983).
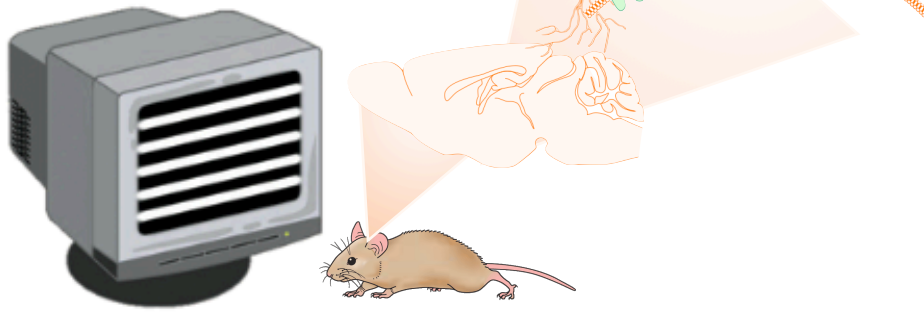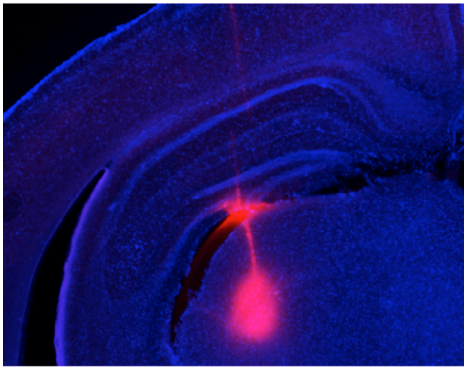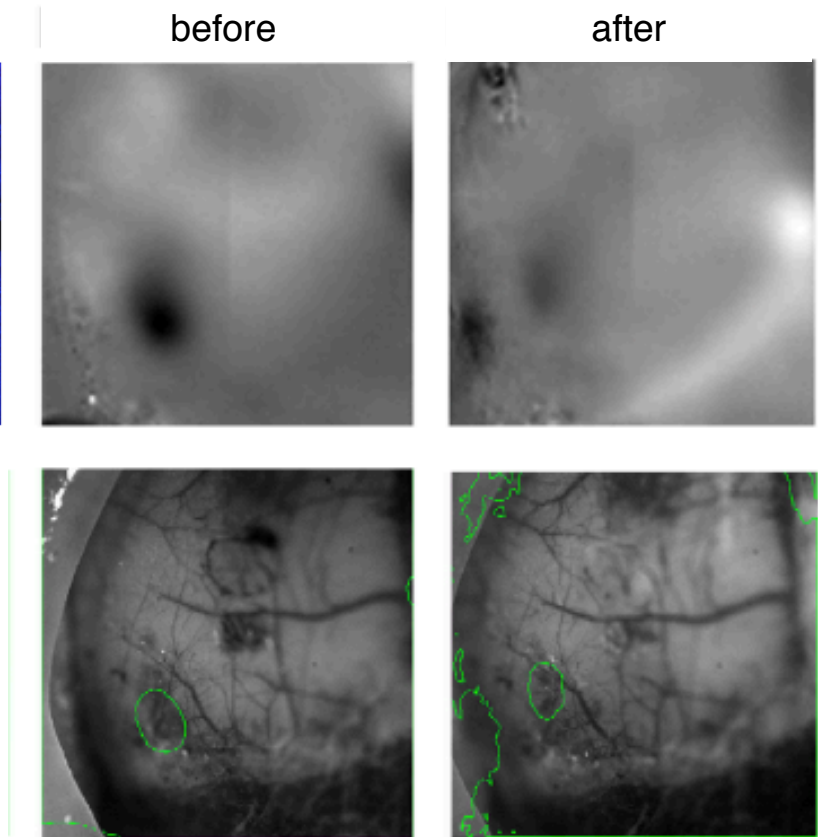
Fig.3 (**A**) Experimental setting for in vivo whole cell patch clamp in mouse V1 under visual stimulation. (**B**) Representative injection of fluorescent muscimol in the dLGN performed before the patch clamp experiment. (**C**) Representative intrinsic imaging responses to drifting gratings before and after muscimol injection in the dLGN, note the drop in the evoked activity level in V1 after muscimol delivery in the thalamic nucleus.
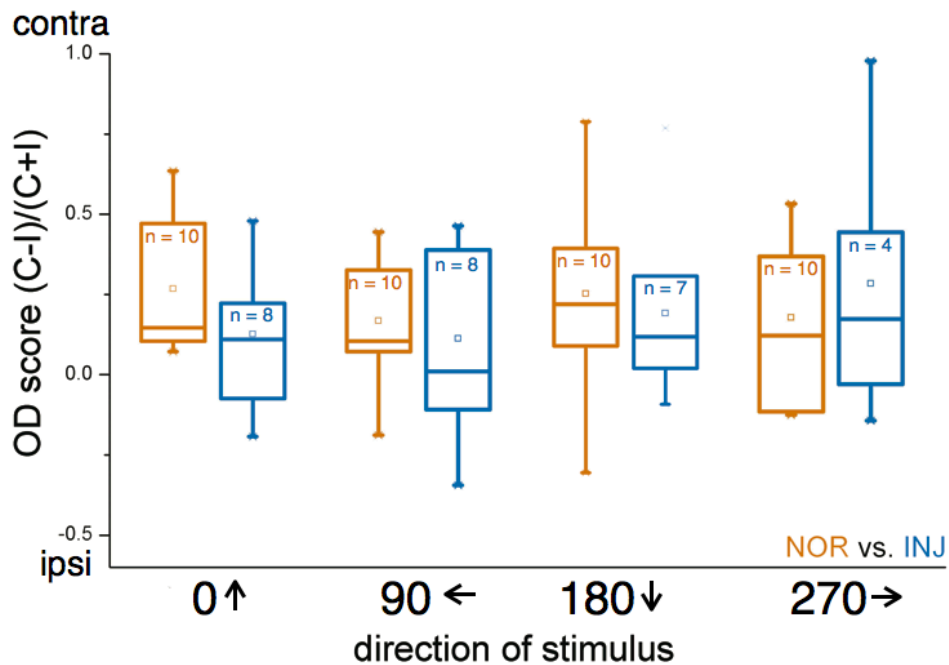
Fig.4 Box chart showing OD score values for V1 neurons in normal (NOR, orange plots) and animals that received unilateral muscimol injection in dLGN (INJ, light blue plots); OD score values were calculated from in vivo patch clamp measurements of membrane potential peaks evoked by visual stimulation of each eye. Data are shown for four different stimulus direction. In injected animals OD values indicate a more ipsilateral preference, significancy might not be reached because of the small sample size. Number of neurons as indicated for each column of data.

The horizontal lines in each box denote the 25th, 50th, and 75th percentile values. The error bars denote the 5th and 95th percentile values. The square symbols denote the mean of the column of data.
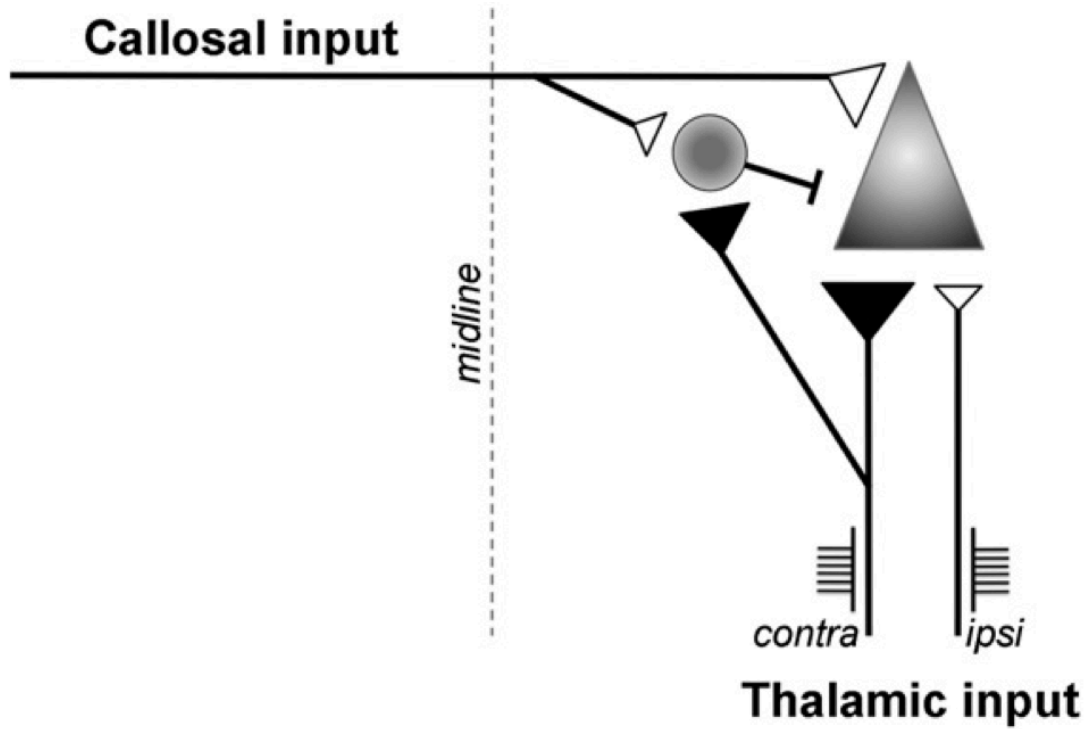
Fig.5 Thalamic and callosal inputs arrive to a principal neuron (triangle) and an inhibitory cell (circle). Contralateral eye- and ipsilateral eye-driven synaptic terminals are in black and white, respectively. Size of the terminals indicates relative synaptic strength.

ESTABLISHMENT OF BINOCULARITY IN THE VISUAL CORTEX

The model described above leads to three predictions: (i) latency of ipsilateral (IPSI) eye responses should be higher than those for the contralateral (CONTRA) eye, due to the substantial callosal component; (ii) responses of the two eyes should show no additivity, as late callosal inputs from the IPSI eye could be shunted by inhibition triggered via thalamic afferents; (iii) responses of the IPSI eye should be increased after suppression of CONTRA eye inputs due to removal of such inhibition.

We now test these predictions in turn and we show that they are fulfilled.

i) Longer latencies of ipsilateral eye responses

The first prediction of our model is that the latency of the responses of the ipsilateral eye should be higher than those of the contralateral eye, due to the substantial callosal component. To test this hypothesis we measured the peak latency of VEPs evoked through the contralateral and the ipsilateral eye, after adjusting the contrast of the stimuli presented to the two eyes as to get a C/I $\approx$ 1. Specifically, contrast presented was 15% for the contralateral eye and 30% for the ipsilateral eye. In this condition the population of neurons activated by the two eyes is roughly the same, and any possible confounding factor in the latency measurement is minimized.

As shown in Fig.6 the latency of the responses evoked through the ipsilateral eye is always greater than the one of the contralateral eye responses (n = 5; paired t-test p = 0,006; Fig.6). According to our model, ipsilateral eye input has a substantial callosal component, adding a delay that is reflected in longer response latencies.
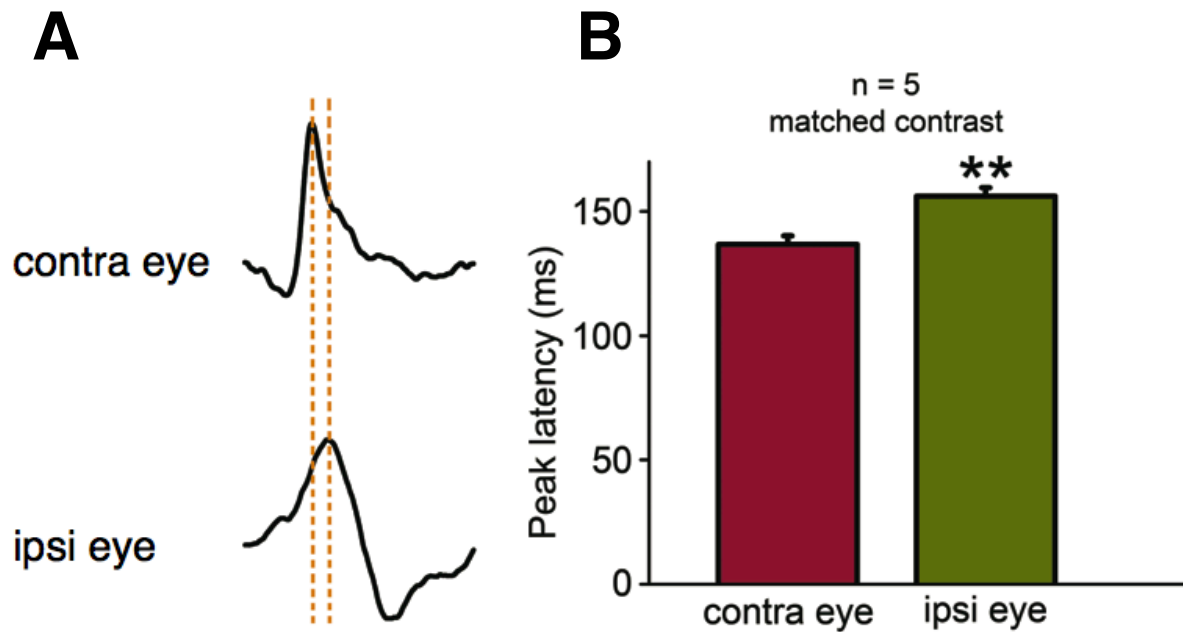
Fig.6 (**A**) Representative Visually Evoked Potentials through the ipsilateral (ipsi eye) and contralateral eye (contra eye) evoked adjusting the contrast to have C/I ≈ 1; the dotted line is in correspondence of the peak latency of each response. (**B**) Quantification of the peak latency for both eyes shows that the ipsilateral eye latency is significantly greater than the contralateral eye one (n = 5; paired t-test ** p = 0,006; mean ipsi: 0,156 ± 0,003 s.e.; mean contra: 0,137 ± 0,003 s.e.). Data are mean ± SE.

ii) Lack of additivity of single eye responses in naïve juvenile rats with VEPs and in vivo patch clamp

A. VEP measurements

To test the hypothesis of lack of additivity we recorded VEPs in three conditions: stimulation through both eyes (binocularly, BIN), evoked through the contralateral eye (CONTRA) or through the ipsilateral eye (IPSI).

We calculated the sum of the responses evoked independently by the two eyes (CONTRA + IPSI) and compared it to the response evoked with the two eyes open (BIN). Initial experiments were performed at near the maximal contrast (90%). we found that the sum of the two separate responses was always greater than the response evoked binocularly (n=7; paired t-test $p \leq 0.001$; Fig.7), indicating lack of additivity between the responses of the two eyes.

The lack of additivity suggests a process of inter-ocular suppression during binocular vision, that might prevent the two eyes from evoking their maximal response in the cortex when active at the same time.

To rule out the possibility that the lack of additivity was the result of contrast saturation we repeated the same experiment (n = 6) presenting the stimulus at different contrasts. The lack of additivity of the two eye responses persists when the stimulus was presented across a range of contrasts (10%, 30%, 90%), indicating that it is not the result of contrast saturation. In fact, the sum of the responses evoked independently by the two eyes was always greater than the responses evoked binocularly at contrasts 10%, 30%, 90% (n=6; two way repeated measures ANOVA, followed by Holm Sidak; test Fig.8).
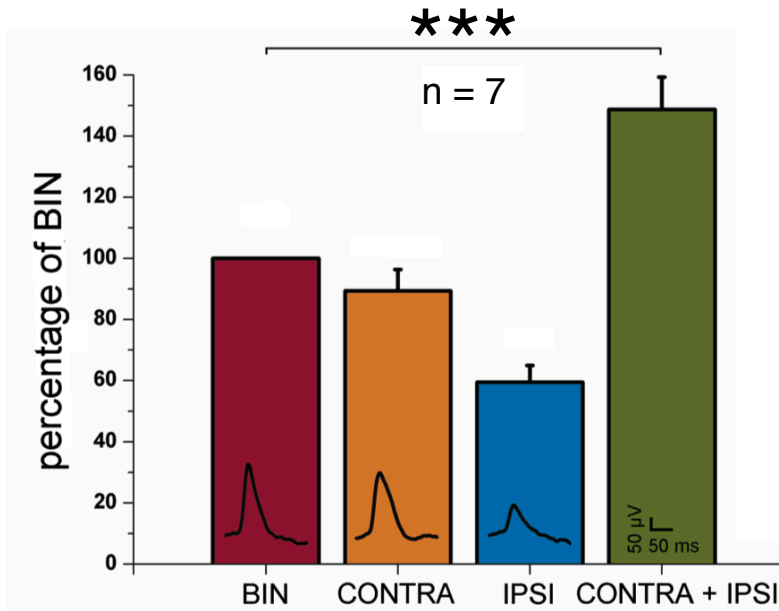
Fig.7 Visual Evoked Potentials of naïve juvenile rats under urethane anesthesia recorded in V1. VEPs are recorded when the stimulus is presented with the two eyes open (BIN), or presented to the contralateral eye alone (CONTRA) or the ipsilateral eye alone (IPSI). The sum of the responses evoked independently by the two eyes is shown in the CONTRA + IPSI column. Note that the CONTRA + IPSI sum is significantly higher than the response evoked with the two eyes open (BIN) (n = 7; paired t-test *** $p \leq 0.001$). Data are mean ± SE.
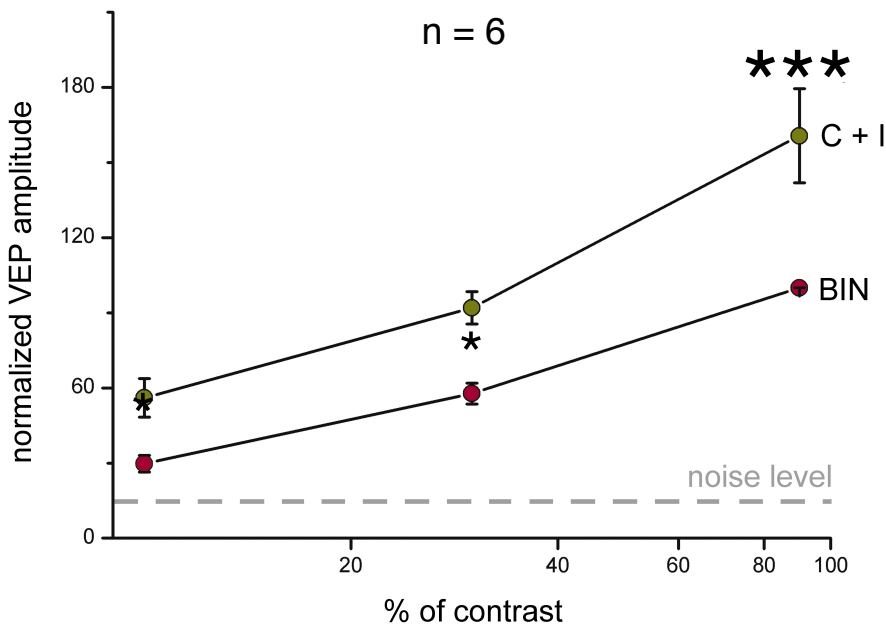

Fig.8 VEPs are recorded at different contrasts when the stimulus is presented with the two eyes open (BIN), or presented to the ipsi eye alone or the contra eye alone. Responses are normalized to the values obtained in the binocular condition (BIN) at 90% contrast. The sum of the responses evoked independently by the two eyes is shown in the CONTRA + IPSI curve. Note that the CONTRA + IPSI sum is always greater than the response evoked binocularly at every contrast, indicating that lack of additivity is not due to contrast saturation (n = 6; Two Way Repeated Measures ANOVA All Pairwise Multiple Comparison Procedures Holm-Sidak method; *** $p \leq 0.001$; * $p \leq 0.05$). Data are mean ± SE.
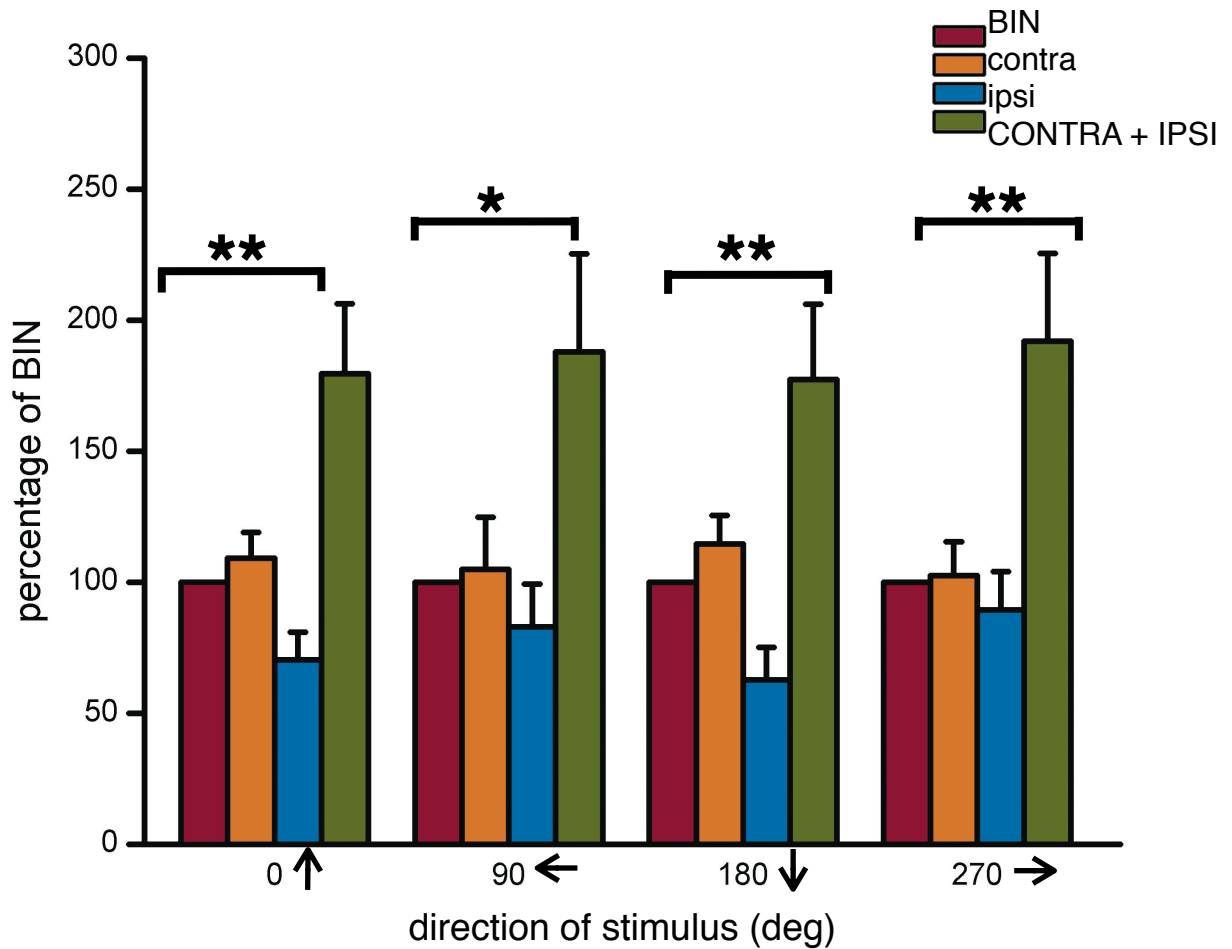
Fig.9 In vivo voltage follower patch clamp measurements of membrane potential peaks evoked by visual stimulation. Membrane potential of V1 pyramidal neurons were recorded during stimulation with drifting gratings at different directions, the stimulus is presented with the two eyes open (BIN), or presented to the contralateral eye alone (CONTRA) or the ipsilateral eye alone (IPSI). The sum of the responses evoked independently by the two eyes is shown in the CONTRA + IPSI column. Note that the CONTRA + IPSI sum is significantly higher than the response evoked with the two eyes open (BIN) at every tested direction; n = 8 cells, 6 mice; ** p≤ 0.01; * p≤ 0.05. Data are mean ± SE.è

B. In vivo Patch Clamp measurements

VEPs are extracellular population measurements of postsynaptic activity. To further explore the lack of additivity we performed intracellular recordings of single neurons in anesthetized mice (P27-33) with in vivo patch clamp in voltage follower mode. We measured the post synaptic potential (PSP) of pyramidal neurons in superficial layers during visual stimulation at four different stimulus directions (0°, 90°, 180°; 270°). As in the VEP experiment, we measured the peak of the response evoked through both eyes (binocularly, BIN), evoked through the contralateral eye (CONTRA) or through the ipsilateral eye (IPSI) for every stimulus direction. The sum of the responses evoked independently by the two eyes was always greater than the responses evoked binocularly at every direction (8 cells, n = 6 mice, Fig.9). These results confirm the lack of additivity previously shown with extracellular recordings.

Altogether, these data suggest that when late callosal inputs from the ipsilateral eye arrive to the cortex they could be shunted by inhibition triggered via thalamic afferents, thus validating our second hypothesis and suggesting a process of interocular suppression.

iii) TTX contra eye inactivation reveals inter-ocular suppression on ipsilateral eye responses

To test directly the hypothesis of inter-ocular suppression we measured the amplitude of VEPs evoked through each eye before and after TTX inactivation of the other eye in naïve juvenile rats (n = 23).

Contralateral eye responses were not affected by saline (n = 5) or TTX (n = 6) injection in the ipsilateral eye, and saline injection in the contra eye left ipsilateral eye responses unaffected. Interestingly, ipsilateral eye responses were greatly enhanced after injection of the contralateral eye with TTX (n = 7), as shown by the representative VEP traces in Fig. 10 and quantified in Fig.11 (One Way Anova p=0.005). These data demonstrate the presence of inter-ocular suppression onto ipsilateral eye responses due to the contralateral eye activity, confirming our third (iii) prediction.

A
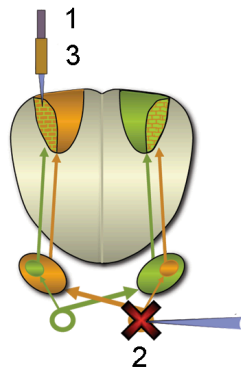
(1) measure VEP amplitude evoked through each eye

(2) Inactivation of one or the other eye by TTX intra-vitreal injection

(3) measure VEP amplitude evoked through the non-injected eye

B

PRE   POST   PRE   POST

○ CONTRA   ○ CONTRA

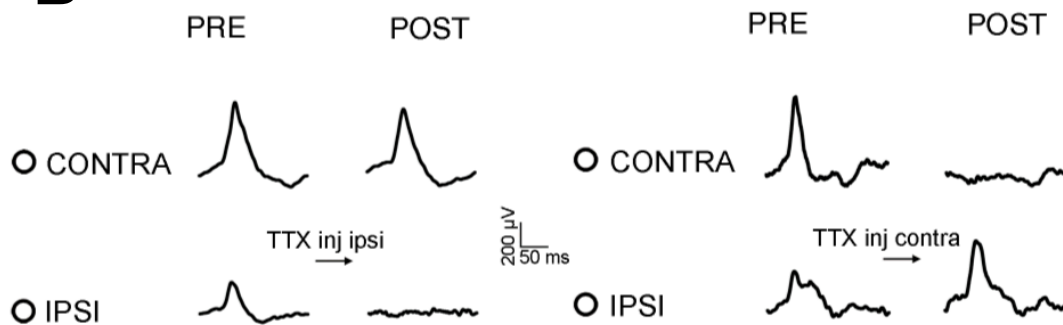TTX inj ipsi   200 µV   50 ms   TTX inj contra

○ IPSI   ○ IPSI

Fig.10 (**A**) Experimental scheme: to measure the effect of spontaneous activity in one eye on the evoked responses of the other eye we measured the amplitude of the responses evoked trough each eye before (**1**) and after (**3**) TTX inactivation of the other eye (**2**).

(**B**) Representative VEPs before and after TTX eye inactivation. Note that CONTRA eye VEPs are not affected by IPSI eye inactivation, while IPSI eye responses are greatly enhanced after CONTRA eye silencing, demonstrating an interocular suppression onto IPSI eye responses due to the CONTRA eye activity.
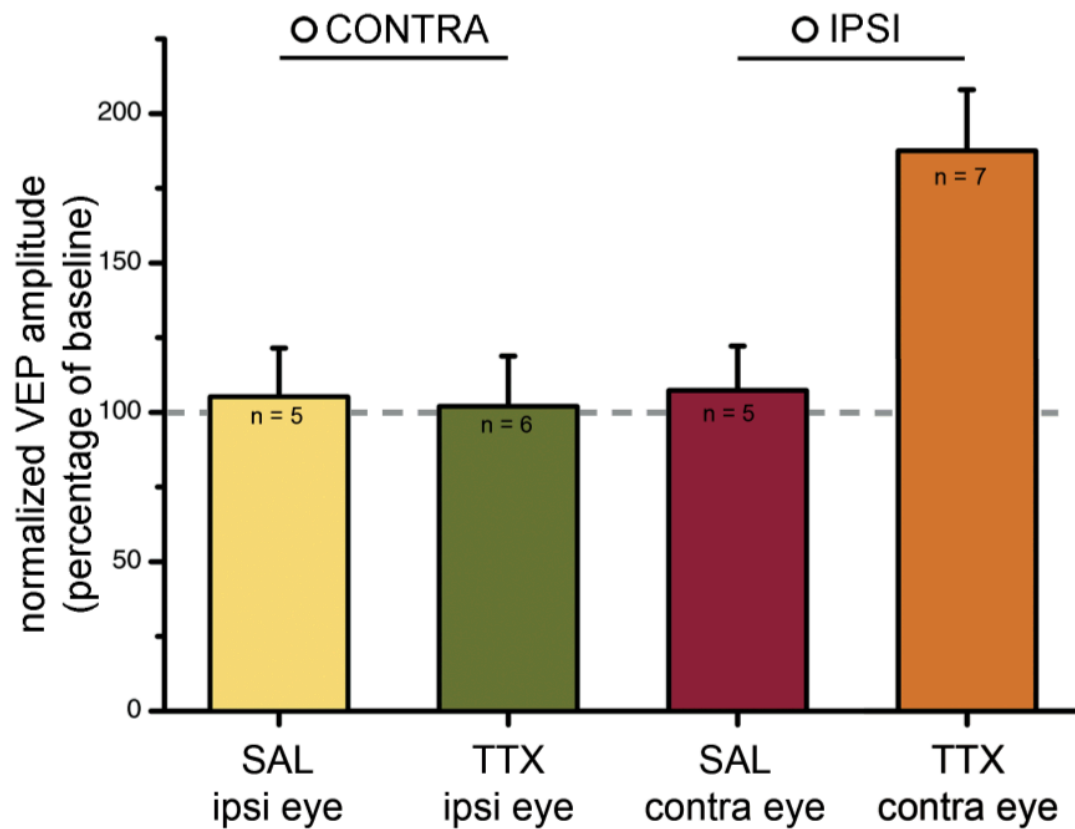
Fig.11 Quantification of VEP responses after TTX/saline eye injection. Responses are normalized to baseline pre-injection VEPs amplitude. Note that the amplitude of the CONTRA eye VEP responses is not affected by saline control injection (SAL ipsi eye) or TTX inactivation of the IPSI eye (TTX ipsi eye). Saline injection in the CONTRA eye has no effect on IPSI eye VEPs (SAL contra eye). Due to the interocular suppression phenomenon IPSI eye responses are significantly enhanced after CONTRA eye TTX silencing (TTX contra eye). One Way Anova ** p=0,005. Data are mean ± SE.

INTER-OCULAR SUPPRESSION OF IPSILATERAL EYE RESPONSES IS LOST AFTER 7 DAYS OF MONOCULAR DEPRIVATION

Cortical neurons in juvenile animals are highly plastic and capable of changing their properties to adapt to a changing environment. Visual neurons in particular can modify their responses if visual experience is altered. Notably, suturing one eyelid shut (Monocular Deprivation, MD) during a window of time where plasticity is maximal, (the "critical period") can shift the key property of visual pyramidal neurons, binocularity, in favor of the open eye.

In the previous sections we have demonstrated that in naïve rats the contralateral eye activity exerts a functional inhibition on the ipsilateral eye driven responses. During MD the contralateral eye is sutured shut and its ability in driving cortical neurons is reduced in favor of the ipsilateral, open eye. In this experiment we tested whether 7 days of MD at the peak of the critical period (P21-P28) could affect the inter-ocular suppression.

We deprived a group of rats (n = 25) at P21 for 7 days and measured the amplitude of VEPs evoked through the deprived contralateral eye and through the open ipsilateral eye before and after saline or TTX injection in the other eye.

After 7 days MD contralateral eye inactivation with TTX injection no longer unmasks ipsilateral eye responses (n = 10; paired t-test: n.s.; Fig.12,14;). These data demonstrate the loss of interocular suppression after 7 days of monocular deprivation in juvenile rats.

The next question was then to understand whether the loss of interocular suppression was paralleled with a shift in the control of cortical inhibition. Since we propose that in normal animals the contralateral eye shunts the ipsilateral eye responses through a fast thalamic control of the intracortical inhibitory system, it was tempting to speculate that in a deprived animal the control of the inhibition could switch from the contralateral deprived eye to the ipsilateral open eye. This was not the case, since TTX ipsilateral eye inactivation does not unmask contralateral eye responses (n = 15; paired t-test n.s.; Fig. 12,14).
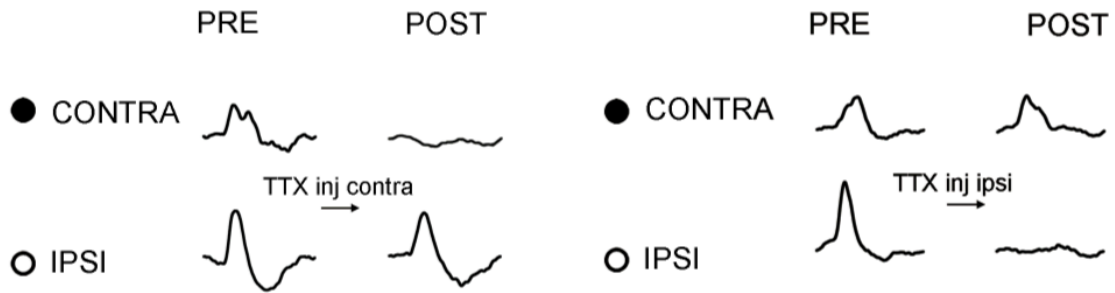
Fig.12 Representative VEPs of MD animals before and after TTX eye inactivation.

Closed symbol: deprived eye, open symbol: non deprived eye.

Note that CONTRA eye VEPs are not affected by IPSI eye inactivation, nor IPSI eye responses are enhanced after CONTRA eye silencing, demonstrating the loss of interocular suppression after MD.
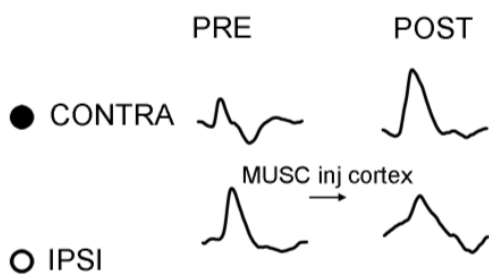

Fig.13 Representative contralateral eye VEPs of MD animals before and after muscimol (MUSC) inactivation of opposite V1. Closed symbol: deprived eye, open symbol: non deprived eye.

Note after muscimol injection in the IPSI eye controlled cortex CONTRA eye responses are greatly enhanced, indicating a role for the corpus callosum in the control of cortical inhibition after MD.
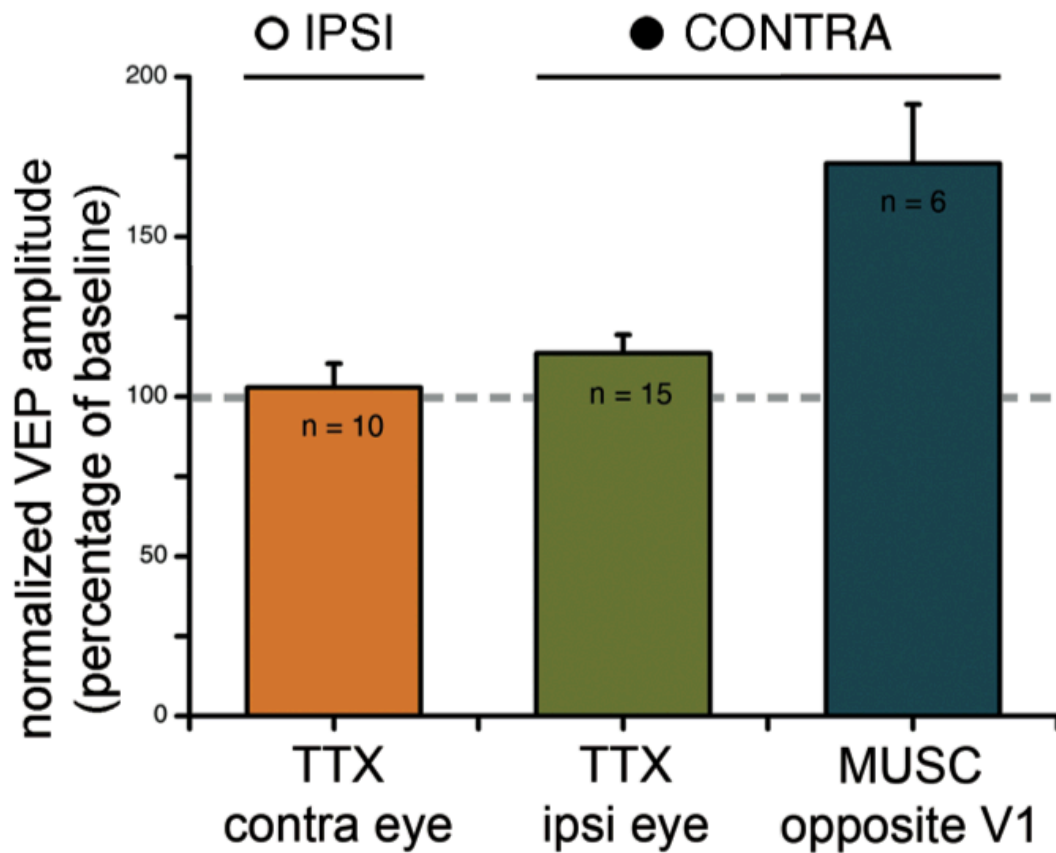
Fig.14 Quantification of VEP responses after TTX/MUSC injection in MD animals. Responses are normalized to baseline pre-injection VEPs amplitude. Open symbol: non deprived eye, closed symbol: deprived eye.

Note that the amplitude of the two eyes VEPs is not affected by TTX inactivation of the other eye (TTX contra eye, TTX ipsi eye). Muscimol inactivation of the IPSI open eye dominated cortex (MUSC opposite V1) results in a significant enhancement of CONTRA eye responses. (Paired t-test TTX contra eye pre vs post inj: n.s.; Paired t-Test TTX ipsi eye pre vs post inj: n.s.; Paired t-Test MUSC opposite V1 pre vs post inj: p=0.023). Data are mean ± SE.

CALLOSAL SILENCING ENHANCES CONTRALATERAL DEPRIVED EYE VEPS AFTER 7 DAYS OF MONOCULAR DEPRIVATION

We then tested the idea that during monocular deprivation the afferent control of cortical inhibition could be transferred to callosal inputs. We recorded the absolute amplitude of VEPs evoked through the contralateral eye before and after the injection of muscimol in the opposite visual cortex, i.e. before and after the inactivation of callosal communication with the ipsilateral eye driven hemisphere.

This lead to the unmasking of contralateral responses (n = 6; paired t-test p=0.023; Fig. 13,14), while there was no effect on VEP amplitudes for the ipsilateral eye (Fig. 13).

These findings suggesting that after MD the callosal pathway becomes selectively inhibitory onto contralateral closed eye responses.

CONTINUOUS SILENCING OF CALLOSAL INPUT DURING MD PREVENTS THE OD SHIFT

So far we have shown a role for callosal inputs in OD plasticity based on acute silencing of interhemispheric communication. To demonstrate that the callosal pathway is required for the shift of eye preference during MD, we performed experiments in which callosal input activity is blocked continuously throughout the period of sensory deprivation. This experiment silences callosal influences throughout the MD and allows competition to occur only via the thalamo-cortical route.

Young (P20–P23) rats were monocularly deprived for 7 days and muscimol (10–30 mM solution) or saline were concurrently infused by osmotic minipumps into the visual cortex ipsilateral to the closed eye. At the end of the deprivation period, minipumps were removed and the animals were placed in complete darkness for 26–48 hrs to allow washout of muscimol. In a series of experiments, we determined that minipump delivery of muscimol efficiently blocked activity in the infused side and that visual responses were restored by 26 hrs after minipump removal (data not shown).

OD was measured in the cortex contralateral to the occluded eye (i.e., contralateral to minipump infusion) and it was evaluated according to the methods of Hubel and Wiesel.

In animals treated with saline, OD histograms were strongly skewed in favor of the open, ipsilateral eye (n = 5; Fig.15). In contrast, we found a very significant attenuation of the OD shift in rats treated with muscimol (n = 12; Fig.15). Indeed, the OD distribution of muscimol-infused animals was significantly different from that of MD saline rats (c2 test p < 0.001).

A
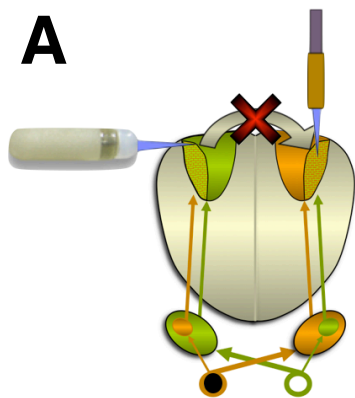
Fig.15 (**A**) Experimental scheme: to study the role of callosal communication in generating the OD shift induced by MD we blocked callosal communication continuously infusing muscimol with osmotic minipumps implanted in the visual cortex ipsilateral to the deprived eye.

(**B** and **C**) OD distributions of monocularly deprived rats that received minipump infusions of saline (**B**) or muscimol (MUSC; **C**) into the visual cortex ipsilateral to the deprivation. Recordings were performed contralateral to the occluded eye (filled circle). Note the substantial reduction of the OD shift in muscimol-treated rats (c2 test, MD saline versus MD muscimol, $p < 0.001$). Number of animals as indicated. SALINE, n = 188 cells; MUSC, n = 284 cells.

B

minipump
saline
n = 5

C
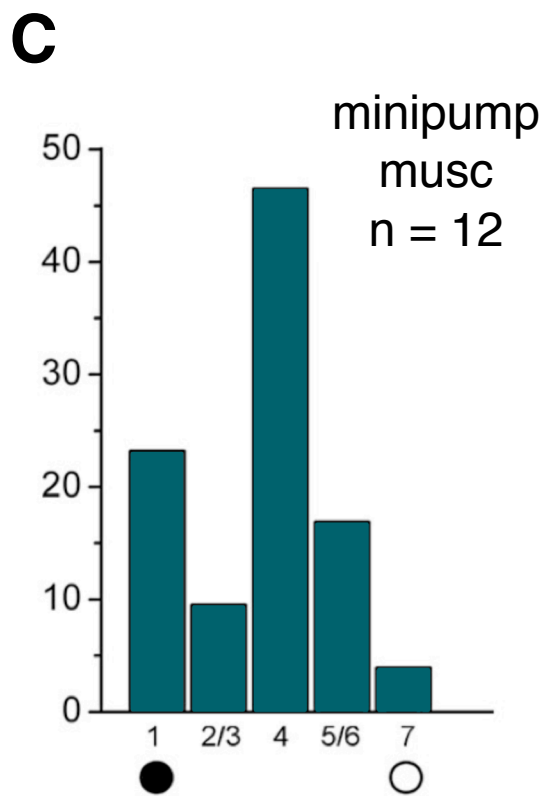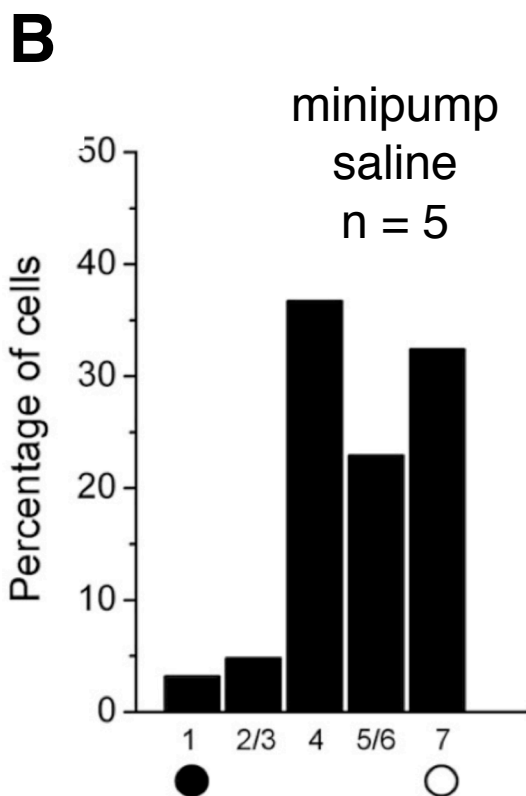
minipump
musc
n = 12

These data demonstrate that blockade of callosal input activity during MD consistently reduces the OD shift.

The prevention of the OD shift in the minipump animals is likely due to a lack of plastic rearrangements of the callosal pathway during MD. Altogether, these data indicate the requirement of callosal inputs in generating the monocular bias during MD.

CALLOSAL CELLS RETAIN A MAINLY EXCITATORY PHENOTYPE AFTER MD

The data presented so far are all consistent with the callosal pathway acting to inhibit the deprived eye responses during MD. It is worth noting that callosal inhibition of closed eye afferents differs from the naïve situation where the opposite hemisphere merely supplies ipsilateral eye input (see pag. 57). Theoretically, this excitatory/inhibitory switch following MD might be due either to a change in the neuro-chemical phenotype of callosal neurons or to an enhanced recruitment of inhibitory circuits in the other hemisphere. To examine whether MD produces alterations in the neurochemical phenotype of transcallosal cells, we performed retrograde labeling of callosal neurons [by cholera toxin B subunit (CTB) injection in the binocular portion of the visual cortex] combined with GABA immuno-staining in naïve (n = 4) and monocularly deprived (n = 4) rats (Fig.16). Co-localization of CTB and GABA was examined at the confocal microscope. We found that the percentage of callosal GABAergic neurons was extremely low in both naïve and deprived animals (naïve: 5 out of 497 cells; MD: 4 out of 325 cells; t test p = 0.89; Fig.16), ruling out that inhibition of closed eye input is exerted directly by callosal afferents.

We therefore favor the idea that callosal axons recruit inhibitory neurons in the opposite hemisphere during MD, as depicted in the model in Fig.17 (see discussion).

PRIOR EXPERIENCE ENHANCES PLASTICITY INDEPENDENTLY OF CALLOSAL INFLUENCES

Experiments by Hofer et al. (Hofer et al. 2006b) demonstrate that a transient shift in ocular dominance (OD), induced by monocular deprivation (MD) earlier in life, renders the adult visual cortex highly susceptible to subsequent MD many weeks later. Given the importance of the corpus callosum in generating the OD shift during MD in young animals we asked weather the callosum was involved also in MD plasticity triggered by previous experience.

Since these data were obtained with optical imaging and spiking measurements in mice, we tested whether we could observe the same effect with visually evoked potentials

(VEPs) in our model, the visual system of rats. Seven days of MD in adult animals have no effect on OD (Cerri et al. 2010), but a priming MD during the critical period (P21) can trigger subsequent MD plasticity in the adult (C/I=1; Fig.18B left bars). Our result confirmed the priming effect of a brief MD during the critical period.

We then addressed the role of the corpus callosum in adult MD plasticity induced by the memory trace left by a priming MD in young-hood. We deprived 10 young (P21) rats and reopened the sutured eye after 4-7 days. We then allowed normal visual experience through both eyes for 4-9 weeks and then we re-sutured the same eye for 4-7 days. During this second MD, the animals were also implanted with osmotic minipumps that released saline (n = 5) or muscimol (n = 5) in the cortex ipsilateral to the closed eye during the MD period, thus silencing callosal communication during the second MD (Fig. 18A).
After 4-7 days the minipump was removed, and the animals were placed in the dark for 2-3 days to allow the washout of the muscimol without interfering with the MD effects.
As shown in Fig. 18B (right bars) the C/I of the 5 animals undergoing blockade of callosal communication during the second MD does not differ from the C/I of the animals with the saline minipump, that experienced a normal callosal communication during the second MD (t-test, n.s.). These data suggest that the memory trace left by a priming MD during adolescence can facilitate adult plasticity independently of callosal communication.
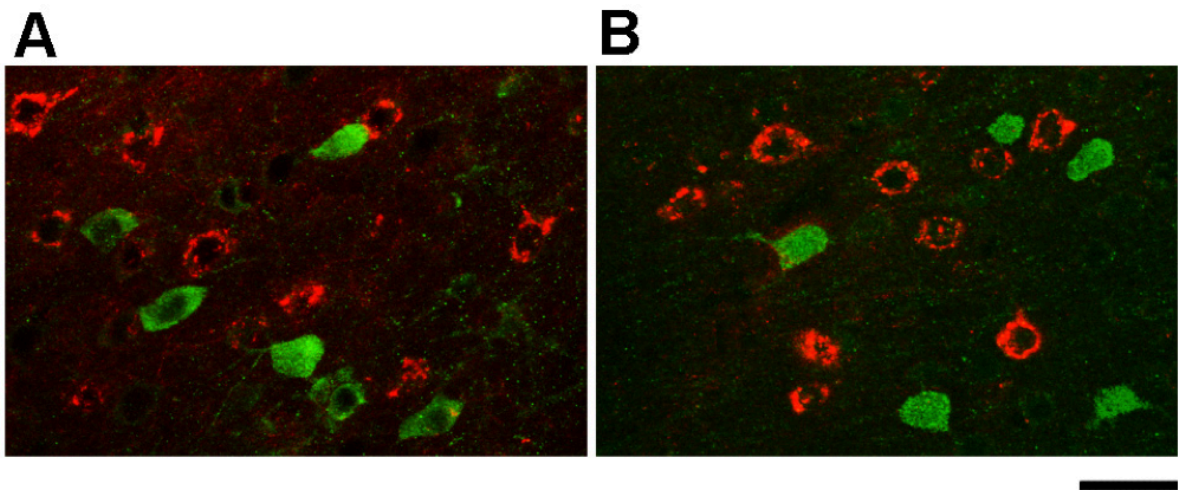
Fig.16 Neurochemical phenotype of callosal cells in naïve (n=4) and monocularly deprived (n=4) rats. Confocal images of coronal sections through the primary visual cortex of a naïve (**A**) and a monocularly deprived rat (**B**) that received CTB injections into the contralateral hemisphere 2 days before killing. Sections are immunostained for CTB (red) and GABA (green). The vast majority of callosal cells (labelled by CTB) do not show immunoreactivity for GABA. Scale bar = 25 μm.
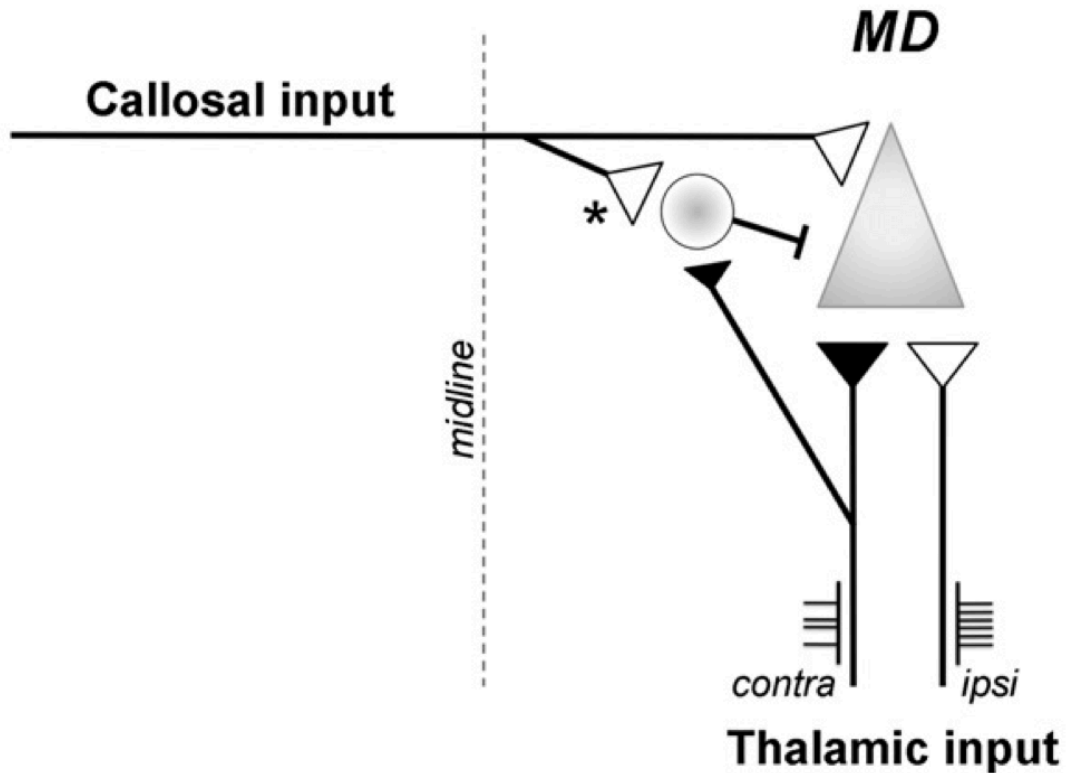
Fig.17 Thalamic and callosal inputs to a principal neuron (triangle) and an inhibitory cell (circle) are shown. Contralateral eye- and ipsilateral eye-driven synaptic terminals are in black and white, respectively. Size of the terminals indicates relative synaptic strength.

MD might result in strengthening of the synaptic connections (asterisk) between callosal afferents and inhibitory cells in the opposite hemisphere, thus masking weak inputs from the contralateral, deprived eye. Remodeling of callosal connections during MD would have no net effect on ipsilateral eye responses, since the increased inhibition via the callosum (asterisk) would be balanced by the normal trans-callosal excitatory drive. Thus, acute silencing of interhemispheric communication might selectively unmask contralateral, deprived eye inputs with no impact on ipsilateral, open eye responses.

A
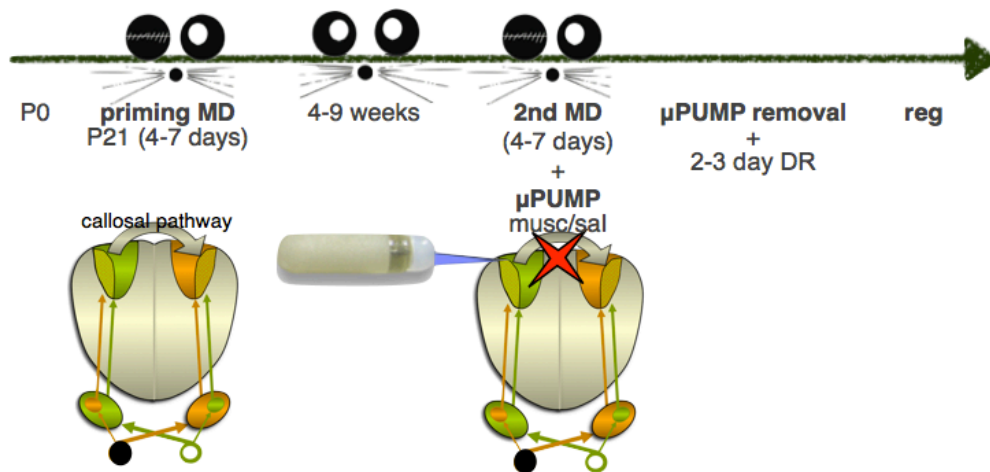
B
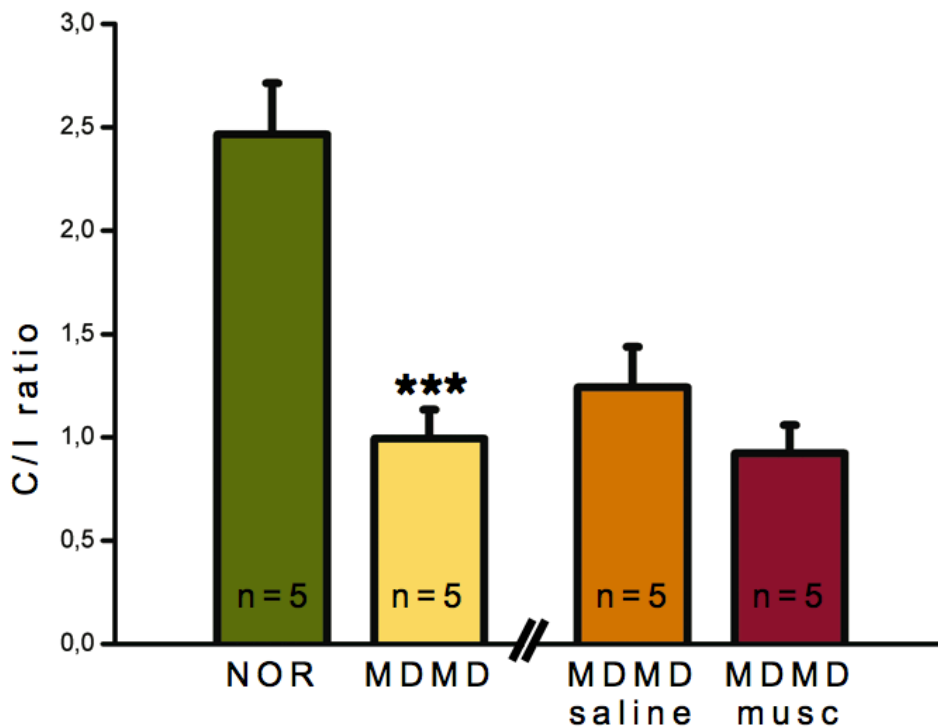
Fig.18 (**A**) Experimental scheme. (**B**) LEFT: C/I of normal (NOR) animals and animals that received a priming MD for 4-7 days at P21 and a subsequent MD for 4-7 days in adulthood (MDMD); NOR vs. MDMD t-test p≤0.001. RIGHT: C/I of animals that received a priming MD and were implanted with osmotic minipumps of saline (MDMD saline) or muscimol (MDMD musc) during the second MD in adulthood. No significant difference is present between animals infused with control saline solution or muscimol, indicating that the block of callosal communication does not impair the retrieval of the memory trace induced by the priming MD; (t-test, n.s.). Data are mean ± SE.

PART TWO: Tetanus neurotoxin-induced epilepsy in mouse visual cortex

Several decades of experiments aimed at characterising the physiology of the visual cortex make it a first choice model system for a detailed study of the functional alterations that occur in pathological conditions.

One of the most frequent and invalidating neurological disorders is epilepsy, but in spite of years of research, little is known about the pathological cortical rearrangements of an epileptic brain.

In the next set of experiments we exploited the properties of the clostridial Tetanus Neurotoxin (TeNT) to generate a chronic epilepsy model in mouse V1, a model for studying the basic mechanisms of hyper-excitability, starting with the biochemical and electrophysiological characterization of the toxin action in the brain parenchyma.

TIME COURSE OF VAMP2 DEGRADATION BY TeNT

Tetanus neurotoxin (TeNT) is a metalloprotease that is taken up by synaptic terminals and cleaves vesicle-associated membrane protein (VAMP), also known as synaptobrevin, and thus blocks neurotransmitter release (Schiavo et al. 2000). Although widely used to generate neuronal hyper-excitability, the time-course of TeNT proteolytic action in the brain has not been precisely determined.

To determine the emergence and duration of the proteolytic cleavage of the toxin in the mouse brain we performed TeNT injections in the mouse visual cortex.

We unilaterally infused TeNT into the left V1 of adult C57BL/6N mice, and dissected V1 samples at different times after injection (n = 22 mice). To analyze the time course of the proteolytic action of the toxin we performed immunoblotting on protein extracts from the visual cortices samples with either anti-VAMP2 mouse monoclonal antibody (Synaptic Systems) or anti-cleaved VAMP rabbit polyclonal antibody (produced by Ornella Rossetto, Padua University).

Immunoblotting shows that TeNT-truncated VAMP2 was first detectable 1–3 days after the injection and was maximal at 10 days (Fig.1A). Cleaved VAMP2 decreased at 21 days, was still detectable at 35 days and completely extinguished at 45–60 days (Fig.1A,C). Therefore, TeNT action peaks at 10 days, lasts for at least 35 days after injection, and is completely extinguished by 45 days. Immunoblotting for intact VAMP2 revealed a complementary pattern: in particular, a very robust decrease in the level of intact VAMP2

was observed between 10 and 21 days (Fig.1B,C). Interestingly, we observed no full recovery of VAMP-2 levels at the completion of toxin effects. In particular, VAMP-2 expression was about 70% of normal 45-60 days after TeNT (Fig.1C).
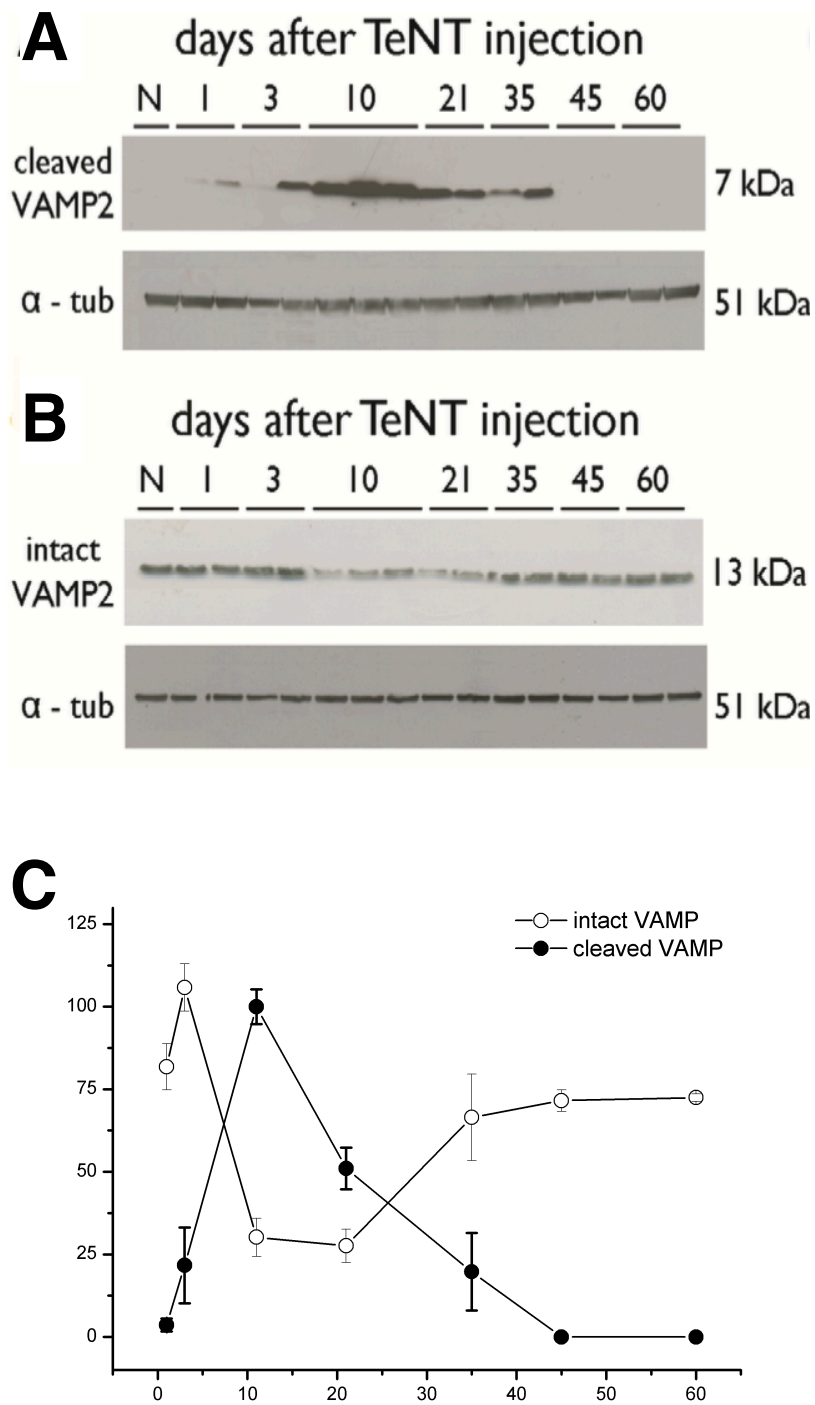
Fig.1 Western blot for cleaved (**A**) and intact (**B**) VAMP2 on protein extracts from visual cortices of TeNT-treated mice (α-Tubulin: internal standard). (**C**) Densitometric analysis of intact VAMP2 and of TeNT-cleaved VAMP2 at different time points after injection. TeNT effects are maximal between 10 and 21 days and decline thereafter, being completely off by 45 days. The decrease of intact VAMP2 follows a complementary pattern. n = 22.

REGIONAL SPECIFICITY OF VAMP2 DEGRADATION BY TeNT

We next investigated the regional specificity of TeNT action, conducting the analysis at the peak of TeNT action (10 days after injection).

At the immuno-histochemical level, a decrease in VAMP2 immuno-labeling was clearly found in the injected V1, as compared to more anterior, somatosensory areas (n = 7 mice; Fig.2).

The immunoblotting confirmed the regional specificity of TeNT action. VAMP2 cleavage was evident only in the injected visual cortex, whereas it was undetectable in the contralateral V1, and in the primary somatosensory cortex (S1) of both hemispheres (n = 8; Fig.3A). Similarly, a clear decrease in levels of intact VAMP2 was found only in the injected site (one-way ANOVA followed by Holm-Sidak test, p < 0.001; Fig.3B,C). Injection of vehicle solution as a control produced no alterations in VAMP2 levels (Fig. 3A,B).

In addition, we probed VAMP2 levels in the lateral geniculate nucleus (LGN, the primary afferent input to V1) of the thalamus ipsilateral to the toxin injection. Immunoblotting analysis showed the appearance of TeNT-cleaved VAMP2 (Fig.4A) and a consistent decrease in levels of intact VAMP2 (Fig.4B,C) in the ipsilateral LGN (n = 5 TeNT; n = 4 normal; t-test p = 0.016; Fig.4). Therefore, we conclude that TeNT action is restricted to the injected visual cortex and ipsilateral thalamus and that it does not spread to other cortical areas.

DEVELOPMENT OF ELECTROGRAPHIC EPILEPTIFORM ACTIVITY IN TeNT-TREATED ANIMALS

After the biochemical characterization of the model we analysed the electrophysiological phenotype induced by the TeNT injection. We implanted a bipolar Nichrome wire electrode to sample the local electrical activity originating in the TeNT-injected V1 of awake, freely moving mice [as described in (Di Garbo et al. 2011)]. Recordings of Local Field Potentials (LFP) began 3 days after surgery; the animal was habituated for 1 hour to the test cage before a 1 hour recording session, using a digital acquisition system.

A first subset of animals were implanted with chronic electrodes immediately after injection (n = 9 TeNT; n = 4 vehicle) and recorded 3–24 days later. A second subset of mice were recorded 48–60 days after TeNT/vehicle injection (i.e., when TeNT effects are off; n = 5 TeNT; n = 3 vehicle). Representative traces showing LFPs recorded in V1 of TeNT- and vehicle- treated animals are shown in Fig.5.
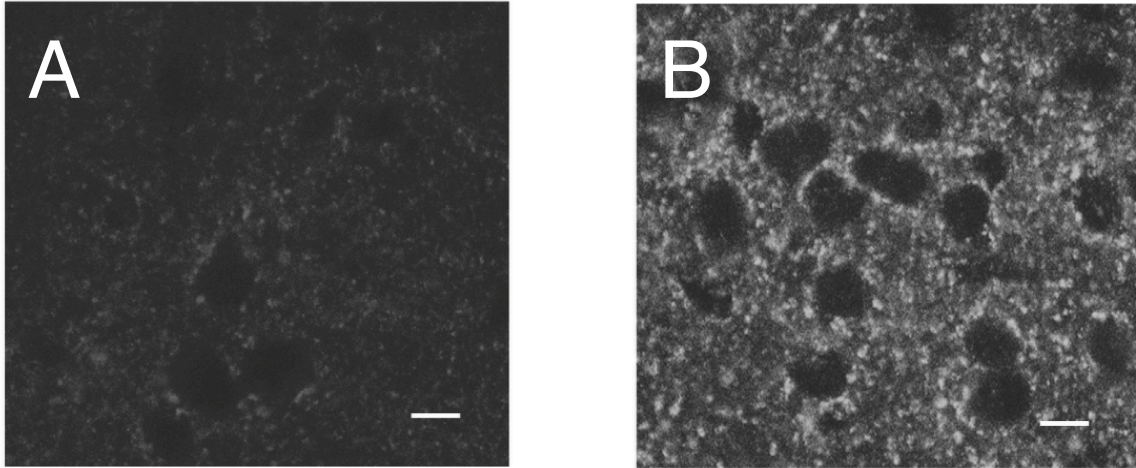
Fig.2 Representative confocal images of immunostaining for intact VAMP2 in a coronal section through layers II-III of the injected V1 (**A**) and ipsilateral primary somatosensory cortex (S1, **B**). While the somatosensory cortex displays the normal punctate staining characteristic of VAMP2, immunoreactivity is strongly decreased in the treated visual cortex. Scale bar = 10 µm.
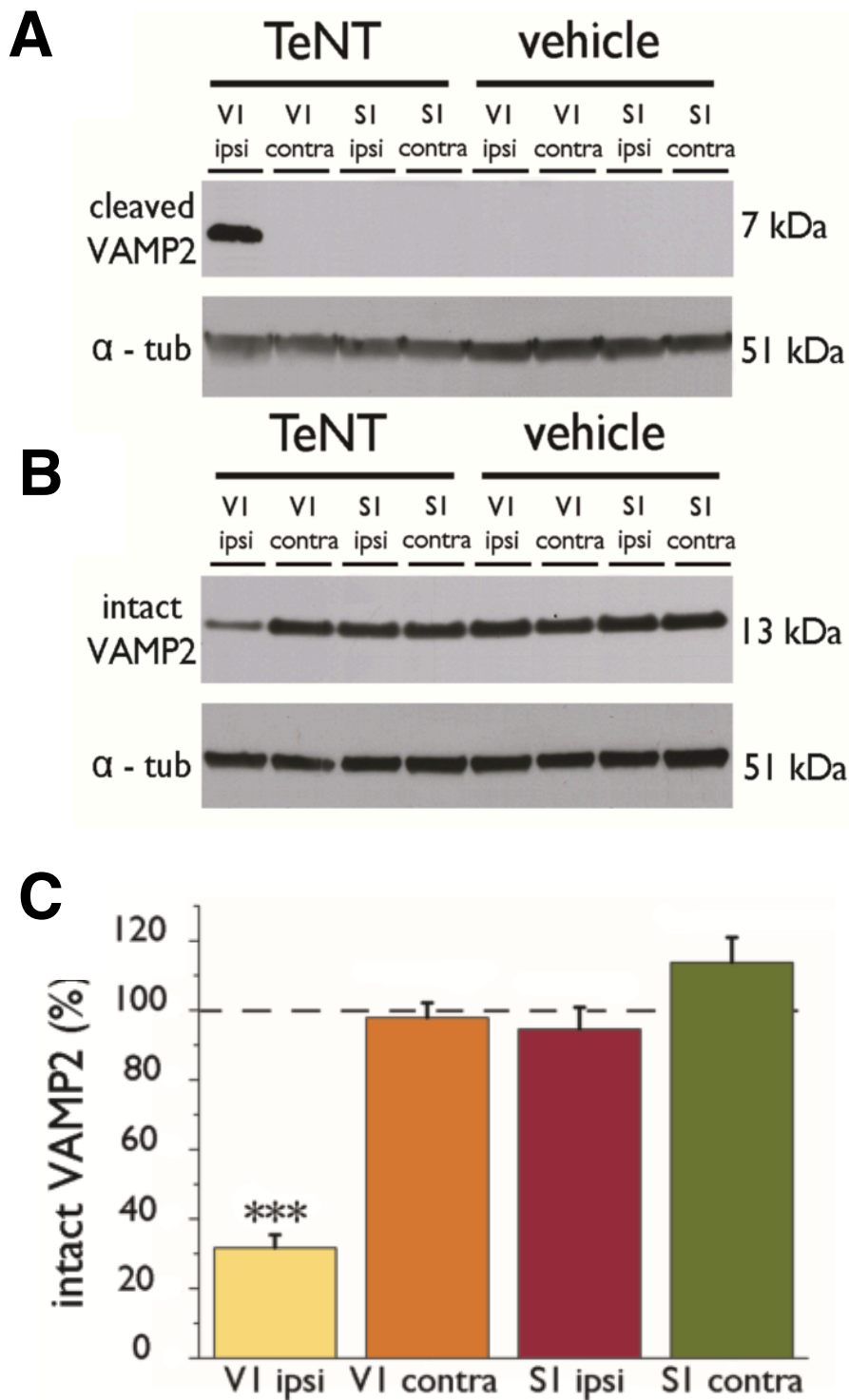
Fig.3 Western blot for cleaved (**A**) and intact (**B**) VAMP2 on protein extracts from the cortices of a TeNT- and a vehicle-treated mouse 10 days after injection. (**C**) Densitometric analysis of intact VAMP2 in different cortices.
Note appearance of TeNT-cleaved VAMP2 only in the toxin-injected V1 (**A**); similarly, levels of intact VAMP2 decrease only in the treated V1 (**B**,**C**). Data are mean ± SE.
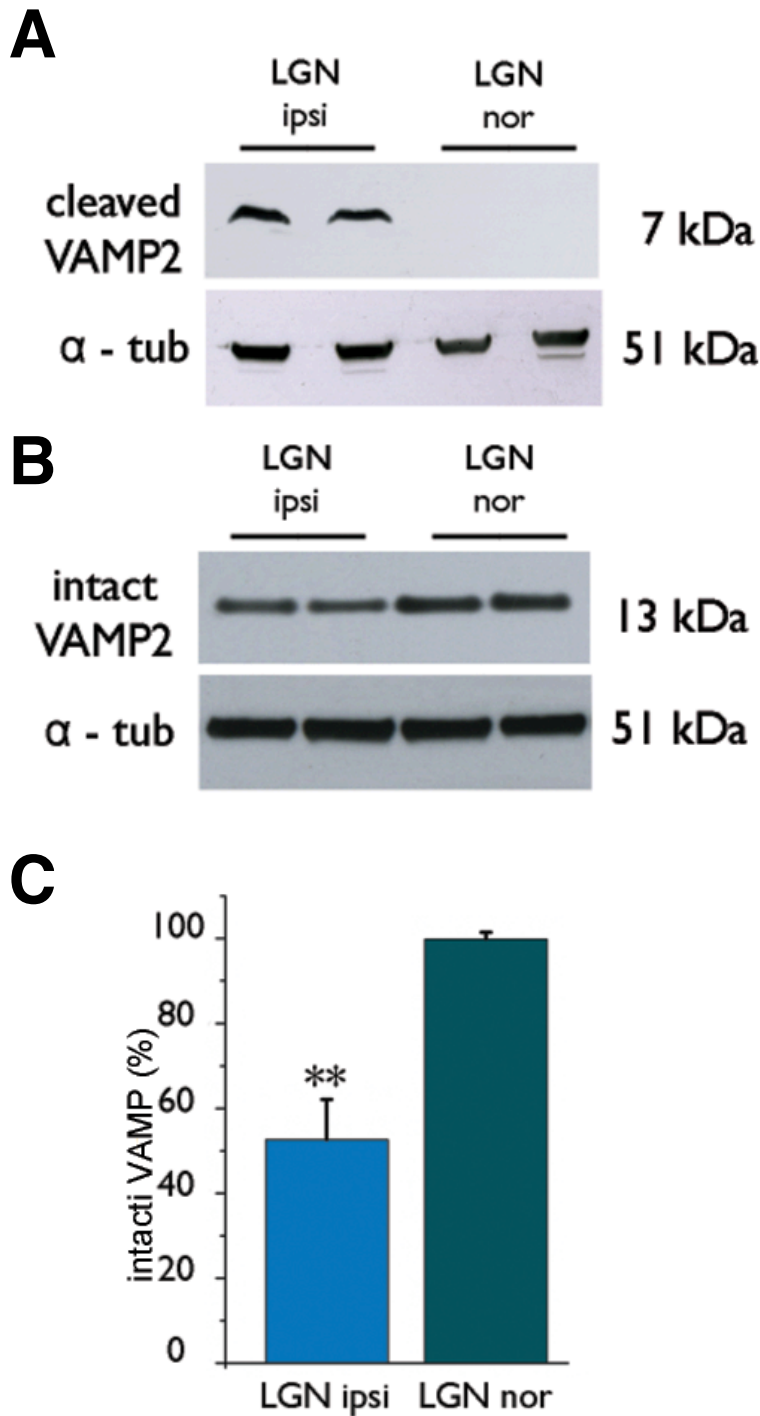
Fig.4 Western blot for cleaved (**A**) and intact (**B**) VAMP2 in the LGN ipsilateral to TeNT injection 10 days after injection (LGN ipsi) and in the LGN from naïve mice (LGN nor). (**C**) Quantification of the immunoblotting experiments experiments reveals a significant decrease of intact VAMP2 in the LGN ipsilateral to TeNT delivery (n = 5 TeNT; n = 4 naïve t-test, p = 0.016). Data are mean ± SE.

Note the appearance of cleaved VAMP2 and decrease of intact VAMP2 in the LGN of TeNT-injected animals.

We used an automatic quantification in which all large amplitude events (>4 times the standard deviation [SD] of the baseline) were considered epileptiform spikes (Antonucci et al. 2008a). Clusters of these high-amplitude spikes spaced less than 1 second and lasting for >4 s were classified as "ictal events". Trains of spikes lasting <4 s or isolated spikes were counted as "interictal events". Both ictal and interictal activity were clearly apparent in TeNT- but not vehicle-injected mice 10 days and 48 days after TeNT delivery, as depicted in the representative traces below (Fig.5), showing LFPs recorded in V1 of TeNT- and vehicle- treated animals. There were no detectable behavioral seizures, i.e. no behavioral correlate of the electrographic epileptiform activity.

For each animal, we calculated the number of electrographic ictal discharges as well as the total time spent in ictal activity. A longitudinal quantitative analysis demonstrated epileptiform discharges in TeNT animals both during and after the time window of toxin activity (Fig. 6A,B). No epileptic activity was evident in vehicle-injected mice (Fig. 6A,B). To characterize in more detail the electrographic features of this epilepsy model, we quantified interictal and ictal electrographic events. We found that interictal events outnumbered electrographic ictal discharges both during and after the time window of toxin activity (paired t-test, $p < 0.001$ Fig. 6C).
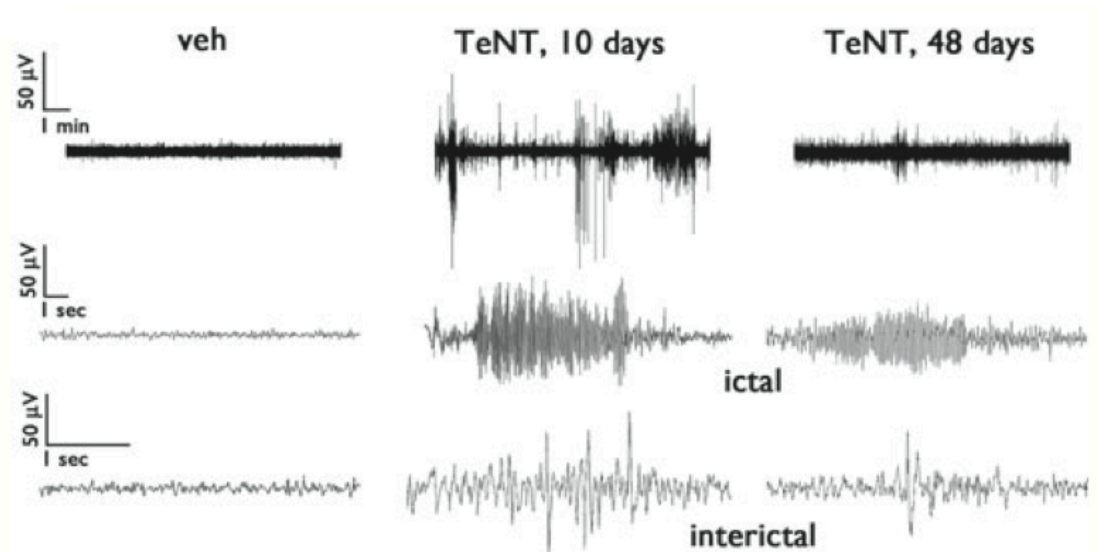
Fig.5 Representative LFP recordings displaying activity in the injected V1 following delivery of either vehicle (veh) or TeNT (10 and 48 days after injection). A 10-s and a 4-s epoch are shown on an enlarged scale to better discern ictal and interictal epileptiform events.

A
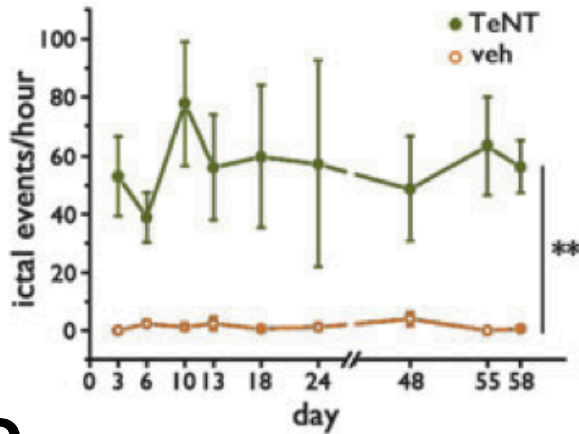
B
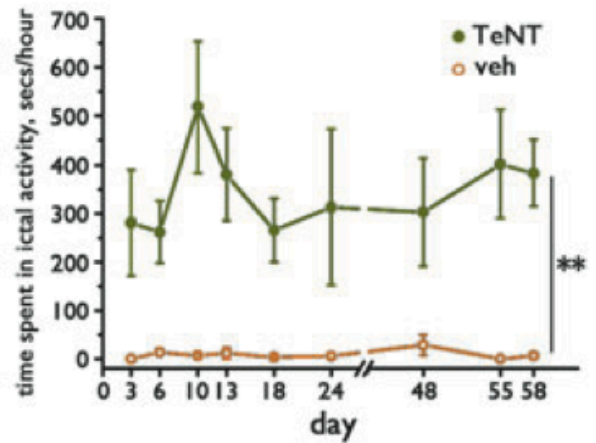
C
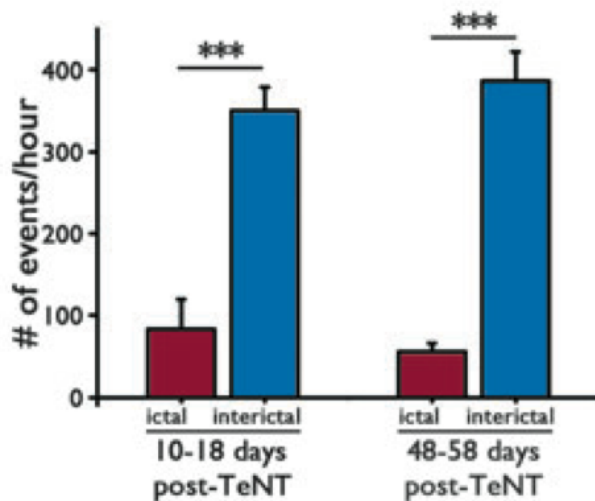
Fig.6 Quantification of electrographic epileptiform activity in V1: frequency of ictal events is in (**A**) while total time spent in ictal activity is in (**B**). Note that electrographic epileptic activity persists at days 48, 55, and 58 post-injection in TeNT mice. No ictal discharges are detectable in vehicle-injected mice (two-way repeated measures ANOVA, **, $p = 0.008$). Data are mean ± SE.

(**C**) Quantification of ictal and interictal electrographic epileptiform events at the peak of TeNT action (left, 10–18 days after injection) and after recovery from TeNT effects (right, 48–58 days after injection). Note the higher frequency of interictal discharges at both time points (paired t-test, $p < 0.001$). Data are mean ± SE.

ANATOMICAL EVIDENCE THAT TeNT-INDUCED HYPER-EXCITABILITY IS A NON LESIONAL MODEL FOR CORTICAL EPILEPSY IN V1

We also conducted an anatomical analysis in an independent subset of animals injected with TeNT (n = 4) or vehicle (n = 4) 55 days after injection. Immunostaining for NeuN and Iba-1 (markers for neuronal and microglial cells Fig.7A,C) show that neuronal and microglial densities were unaltered in TeNT-injected mice, as compared to either vehicle-injected or untreated mice (one-way ANOVA p > 0.18; Fig.7B,D). These findings show lack of significant histopathological changes in TeNT-treated cortices, indicating that TeNT-induced hyperexcitability can be used as a non lesional model for cortical epilepsy in V1.

vGLUT1 AND GAD65/67 EXPRESSION IN TeNT TREATED VISUAL CORTEX

Given the long lasting epileptic phenotype induced by TeNT, we measured two markers of inhibition (Gad 65/67; Fig. 8A) and excitation (vGlut1; Fig.8B) in search of any biochemical modification that could account for the observed hyperexcitability.

We performed immunoblotting on protein extracts from visual cortices of controls (3 naïve animals and 3 vehicle injected; n = 6) or TeNT injected mice (n = 3; 45 days post injection). We found no differences in the expression of vGlut1 and GAD65 or GAD67 between the control group and the TeNT group, (t-test n.s; Fig.8C,D).

Our experiments suggest that mechanisms other than the altered vGlut1/GAD65/67 expression might be responsible for the excitation/inhibition unbalance that we observe in TeNT treated cortices.
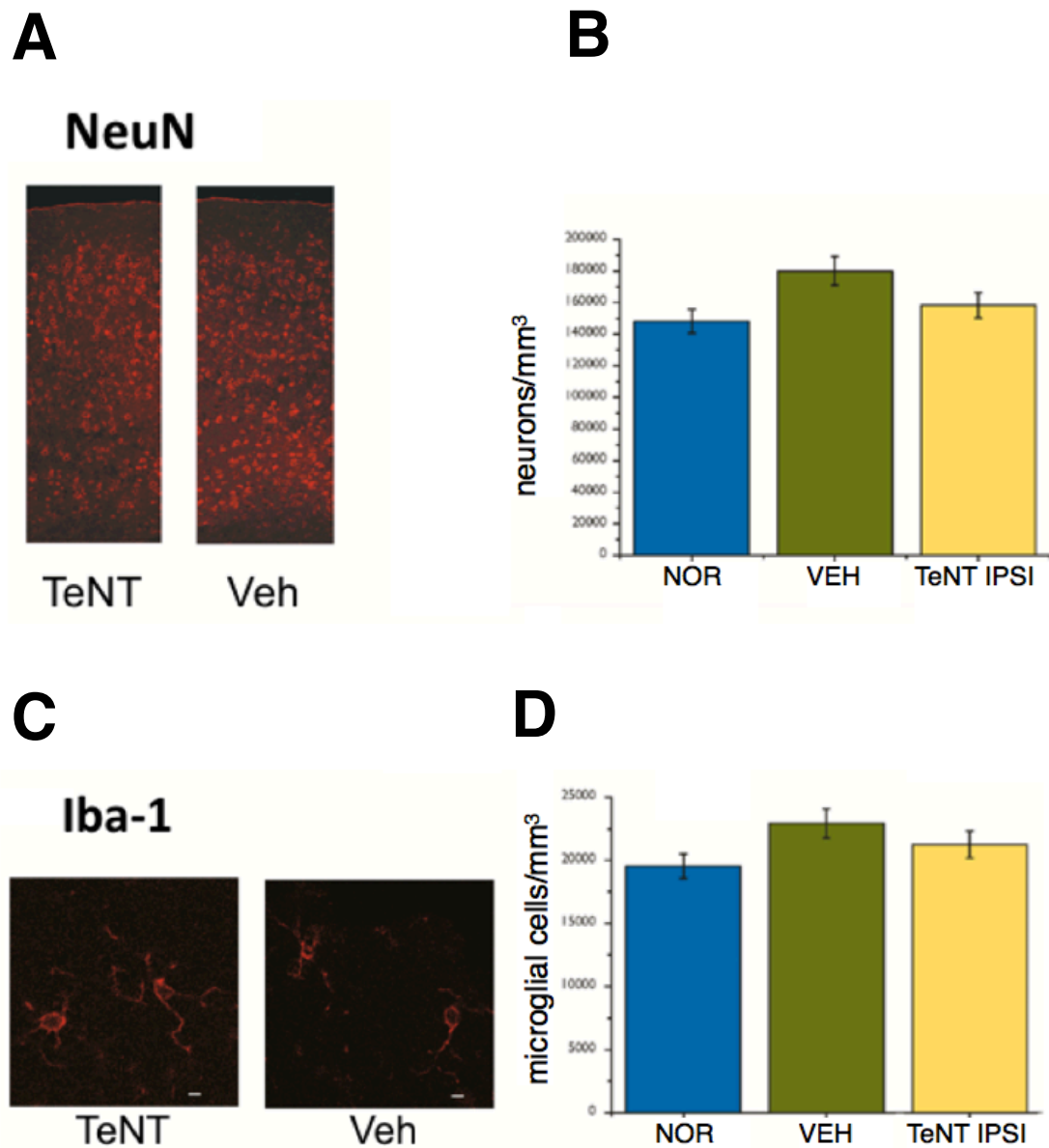
Fig. 7 (**A**) Representative immunostainings for the neuronal marker NeuN in the visual cortex in vehicle- (left) and TeNT-treated mice (right), 55 days after injection. (**B**) Quantification of neuronal densities reveals no differences among naïve animals and animals treated with vehicle or TeNT (one way ANOVA, p = 0.98). (**C**) Representative immunostainings for the microglial marker Iba-1 in vehicle- (left) and TeNT-treated mice (right). (**D**) Density of microglial cells is not affected by TeNT administration (one way ANOVA, p = 0.19).
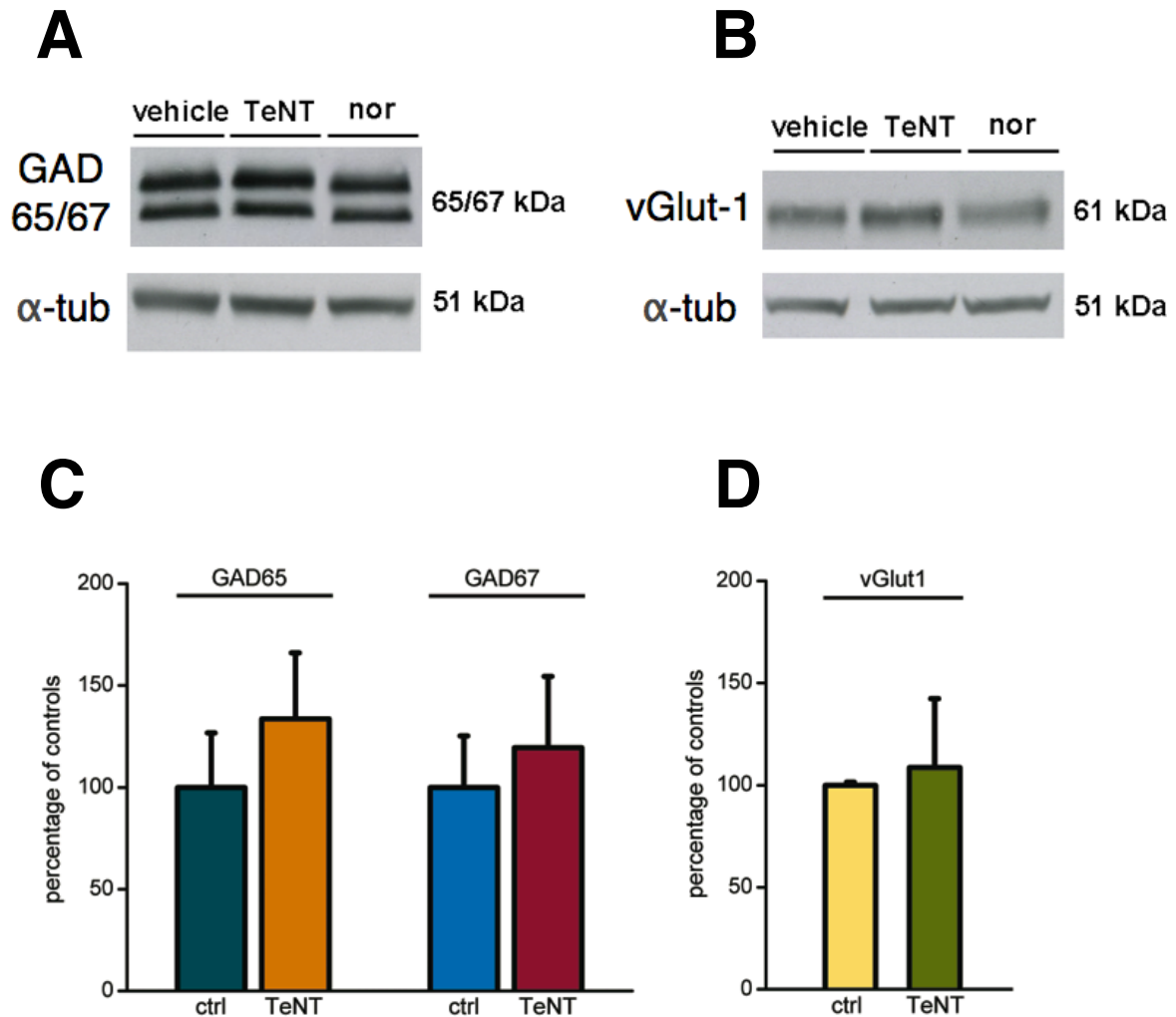
Fig. 8 Western blot for GAD 65/67 (**A**) and vGlut1 (**B**) on protein extracts from visual cortices of a vehicle injected mouse (veh), a TeNT injected mouse (TeNT) and an untreated animal (nor) after the completion of TeNT proteolytic action (45 days post injection). α-tubulin (α-tub) was used as internal standard.

There is no significant difference in the expression of the GABAergic GAD 65/67 markers (quantified in **C**) or the glutammatergic vGlut1 marker (quantified in **D**) between the controls (ctrl) and toxin treated animals (n = 6 controls, n = 3 TeNT injected; t-test TeNT treated vs. control; controls cumulate data from normal (nor) and vehicle-injected mice). Data are mean ± SE.

DISCUSSION

DISCUSSION

In the first part of this work, we have studied the role of the corpus callosum in juvenile rats and proved a role for interhemispheric connections in providing binocular inputs to cortical neurons. Our data demonstrate that the corpus callosum relays mainly ipsilateral eye input to the binocular portion of the primary visual cortex, and prompt a model of cortical circuitry that involves fast thalamic conveyed inputs and slower callosal inputs that converge on binocular neurons. This model is consistent with the presence of inter-ocular suppression, that we describe here for the first time in rat visual cortex. We then tried to elucidate the involvement of the corpus callosum and inter-ocular suppression in the plastic rearrangements of the circuit triggered by the monocular deprivation paradigm.

The physiology of the visual cortex is well known and can be exploited for a detailed definition of functional alterations within pathological conditions. Thus, we took advantage of the clostridial toxin Tetanus Neurotoxin to generate a model of focal epilepsy in mouse primary visual cortex. We provide here for the first time a detailed biochemical, physiologic, and histopathologic characterization of TeNT-induced epilepsy in mouse visual cortex.

PART ONE: Mechanisms of OD plasticity in primary visual cortex

ROLE OF THE CALLOSUM IN BINOCULARITY

Binocularity is a key property of visual neurons, not only because it is the physiological base of many perceptual visual features [like depth perception (Blakemore 1970; Blake and Wilson 2011)], but also because the ability to respond to a changing visual experience with modifications in binocularity reflects the ability of neuronal circuits to adapt to a changing environment, giving us a powerful tool to explore the plastic potential of the brain.

In rodents, visual cortex responsiveness is biased towards the contralateral eye (Fagiolini et al. 1994; Gordon and Stryker 1996; Hensch et al. 1998; Sawtell et al. 2003), due to the high percentage (over 95%) of retinal fibers crossing at the chiasm (Sefton 1995), as compared to about 50% of fibers (from the nasal hemiretina) in cats, monkeys, and humans. In rats and mice, the contralateral bias is progressively reduced getting closer to the highly binocular V1/V2 border (Gordon and Stryker 1996; Caleo et al. 1999b) that

maps the vertical meridian of the visual field and where callosal projections are particularly dense (Cusick and Lund 1981; Mizuno et al. 2007). The particularly high concentration of callosal terminals at this area prompts a role of the callosum in binocularity.

Throughout the years this question has been repeatedly addressed, measuring binocularity in callosotomized animals. However, experiments in cats have yielded contradictory results. Section of the callosum had no effect (Minciacchi and Antonini 1984) or lead to a dramatic reduction in binocularity (Payne et al. 1980; Blakemore et al. 1983; Elberger and Smith 1985; Yinon et al. 1992) in cat visual cortex. The discrepancies in these results may be consequence of technical aspects, including age at which the callosal section is performed and time elapsed between surgery and recording.

In rodents, the involvement of callosal connections in binocularity is also controversial (Drager 1975; Diao et al. 1983; Coleman et al. 2009). Using morphometric measures, Coleman et al. suggested that the contralateral ocular dominance (OD) in the primary visual cortex of the mouse can be accounted for solely by the relative density of feed-forward geniculo-cortical inputs from the two eyes. Indeed, the contralateral eye pathway exhibits a higher degree of convergence on geniculate neurons than the ipsilateral eye pathway (Coleman et al. 2009). Conversely, functional experiments by Diao et al. in albino rats suggest that the callosum could provide a relevant input to binocular neurons (Diao et al. 1983).

To clarify the role of the callosum in cortical binocularity in juvenile rats (P27-32), we measured binocularity by recording Visually Evoked field Potentials (VEPs) and extracellular spiking activity evoked through the eye contralateral and ipsilateral to the recording site before and after acute blockade of callosal input. This was achieved via delivery of muscimol, a $GABA_A$ receptor agonist, into the striate cortex contralateral to the recording site. As expected, both the CONTRA/IPSI (C/I) ratio and the ocular dominance distribution showed a contralateral preference in naïve animals, and saline control injections had no effect on binocularity. Interestingly, silencing callosal communication in naïve animals resulted in a robust shift in eye preference in favor of the contralateral eye, as showed by an increase in the C/I VEP ratio and in the proportion of class 1 cells (with a corresponding decrease of binocular units) in the ocular dominance distribution. These results indicate a role for the callosum in cortical binocularity.

The contralateral shift could be due both to an enhancement of the responses driven through the contralateral eye or to a decrease of the ipsilateral eye driven discharges. To clarify this point we measured the peak firing rates of cortical units following stimulation of the ipsilateral and contralateral eye before and after the removal of the callosal input. The contralateral eye driven peak response (spikes/second) displays no alteration after muscimol injection, but the ipsilateral eye driven response shows a robust decrease after

the removal of the callosal input. Our data demonstrate that after the inactivation of callosal communication there is a significant reduction of the ipsilateral eye driven responses, suggesting that ipsilateral eye inputs arrive to the cortex mainly through the callosal pathway.

These data clarify the callosal contribution to binocularity in rats, and are consistent with previous experiments by Diao et al. in albino rats that also show a dramatic shift of eye preference (due to loss of ipsilateral eye responses) after cooling of the opposite cortex (Diao et al. 1983).

We corroborated these data by a complementary experiment done by intracellular measurements of post synaptic potentials evoked by visual stimulation of each eye in naïve mice and in animals that received muscimol injection in the dLGN. Callosal silencing in rats resulted in a contralateral shift in binocularity. Geniculate silencing, on the other hand, showed a tendency for an eye preference shift towards the ipsilateral eye. Although preliminary, this tendency is in agreement with our model that propose that the callosum provides mainly ipsilateral eye inputs to the cortex, while the geniculate provides mainly the contralateral eye contribution. Moreover, to our knowledge this is the first attempt to record the isolated callosal input to binocular neurons in V1 of young mice with intracellular recordings. The lack of statistical significancy might be due to the small sample size (n = 4 - 10 cells).

A similar experiment was performed by our group with extracellular recordings in rats (Cerri et al. 2010). In this experiment, silencing of the ipsilateral geniculate was done by TTX injections. Geniculate inactivation produced a dramatic reduction in visual responses, with the C / I ratio dropping down, and a significant shift of OD towards the ipsilateral eye was apparent following inactivation of the ipsilateral dLGN. These data altogether indicate that, in addition to the geniculo-cortical pathway, a significant input from the ipsilateral eye to cortical cells is provided via callosal connections.

Our findings lead to a summarizing model depicted in Fig.1. In a pioneer work Toyama et al. (Toyama et al. 1974) demonstrated that pyramidal neurons in layers III-V of cat visual cortex can receive a direct monosynaptic input and an indirect dysinaptic inhibitory input both from the dorsal lateral geniculate body (the thalamic input to the cortex, dLGN) and from the commissural afferents originating from the contralateral visual cortex, as depicted in the scheme. These results were confirmed by Martin in 1983, that provided evidence of basket cells mono-synaptically contacted by callosal afferents in cat visual cortex (Martin et al. 1983).

In our model the contralateral eye input is relayed to the cortex mainly through the dLGN, since thalamic inactivation leads mainly to a decrease of contralateral responses. On the

other hand, the ipsilateral eye input is relayed mainly through the callosal pathway, since the inactivation of the callosal input causes a significant decrease in ipsilateral eye driven responses.
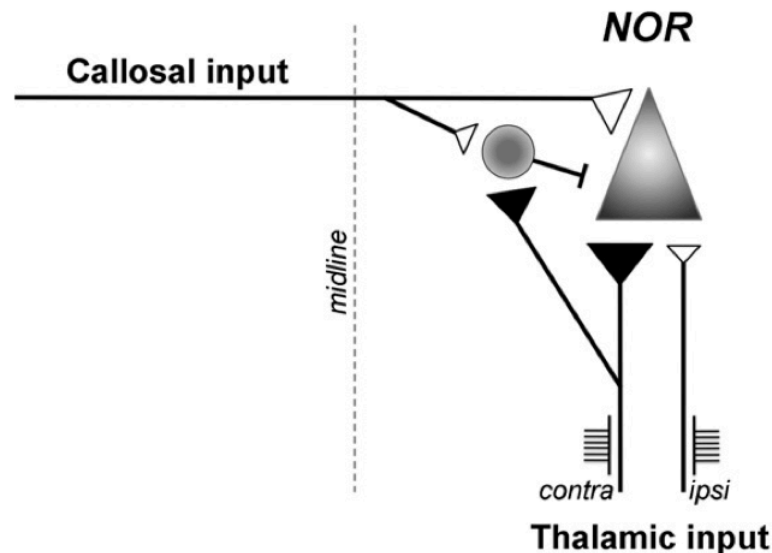
Figure 1: model of cortical circuitry in normal juvenile rats

This model leads to three predictions: (i) latency of ipsilateral eye responses should be higher than those for the contralateral eye, due to the substantial callosal component; (ii) responses of the two eyes should show no additivity, as late callosal inputs from the ipsilateral eye could be shunted by inhibition triggered via thalamic afferents; (iii) responses of the ipsilateral eye should be increased after suppression of contralateral eye inputs due to the removal of such inhibition. All these predictions were confirmed by our experiments.

To analyze VEP latency we adjusted the contrast of the visual stimulus to obtain responses with a similar amplitude from both eyes (C/I ≈ 1), so that the population of neurons activated by the two eyes was comparable, minimizing any possible confounding factor in the measurement. Visually Evoked Potentials recorded from the ipsilateral eye always had longer latency than contralateral eye VEPs. We measured a difference in the latencies of 19 milliseconds. This measurement is comparable with data obtained by other experiments in the lab: Cerri et al. found a difference in the latency of ipsilateral and contralateral VEPs of 22 milliseconds in naïve animals, stimulating both eyes with a saturating stimulus (in Cerri et al. VEP latency was increased after the inactivation of the

thalamic pathway, and the difference between IPSI and IPSI CONTRA VEP was abolished) (Cerri et al. 2010).

From recordings in naïve animals we also report lack of additivity between the responses evoked trough the IPSI and the CONTRA eye. This was not the result of contrast saturation, as it was true at 10%, 30% and 90% contrast. We showed lack of additivity both with extracellular VEP recordings in rats and with in vivo intracellular recordings in mice. Our observation are consistent with a recent paper that showed that binocular inputs to layer 2/3 pyramidal neurons are integrated sublinearly in an amplitude-dependent manner (Longordo et al. 2013).

Pre-cortical pathways in the retina and lateral geniculate nucleus (LGN) are separate and independent for stimuli presented to the two eyes, such that any interaction between inputs from the two eyes must primarily reflect cortical mechanisms. Stimulating two eyes instead of one should theoretically double the LGN input to primary visual cortex (V1), and therefore, if unopposed, should increase the overall neural activity in V1. Without a suppressive mechanism for balancing this larger excitatory drive, or other regulatory mechanisms, binocular stimuli would evoke considerably more synaptic and neural activity than monocular stimuli (Moradi and Heeger 2009). The lack of additivity that emerged from our data is consistent with fMRI data obtained in humans by Moradi and Heeger. The authors showed that V1 responses to binocular stimulation can be explained by a normalization model that assumes mutual suppression between the eyes. This mutual suppression could be due to inhibiting interactions between the two stimuli.

Our proposed model of the visual cortex circuitry lead us to speculate that during binocular vision, i.e. when the two eyes are stimulated at the same time, the two inputs interaction in the cortical circuitry somehow shunts the responses evoked through one or the other eye. This inter-ocular suppression could inhibit late callosal ipsilateral eye responses via a fast thalamic recruitment of intracortical interneurons excited mainly by the contralateral eye.

In the cat visual system, pioneering electrophysiological experiments investigating binocular interactions were performed during studies of depth perception. Results were mixed, with some neurons showing additivity or inhibitory interactions between the stimuli presented to the two eyes (Pettigrew et al. 1968; Bishop et al. 1971; Ferster 1981).

To directly test the hypothesis of inter-ocular suppression in the visual cortex we measured VEPs amplitude evoked by one or the other eye before and after tetrodotoxin inactivation of the other eye. TTX is a sodium channel blocker commonly used to abolish

cell firing in the retina (Caleo et al. 1999b; Iurilli et al. 2012). Pupil dilation and lack of VEP responses evoked through the injected eye were checked during the whole recording to make sure that TTX persistent eye inactivation was maintained. Our data demonstrate that the contralateral eye responses are not affected by TTX injection in the ipsilateral eye. Interestingly, TTX injection in the contralateral eye lead to a significant increase in ipsilateral eye driven responses. Saline control injections had no effect on VEP amplitude of both eyes. These data demonstrate that contralateral eye activity exerts a functional inhibition onto IPSI eye evoked responses.

It is interesting to report data in the literature that demonstrate inhibiting interactions between sensory stimuli relayed to the two hemispheres. An elegant study by the group of Matthew Larkum (Palmer et al. 2012) demonstrate that the ipsilateral hindpaw stimulation can inhibit responses evoked by the contralateral hindpaw in layer 5 neurons of the somatosensory cortex. Interestingly, this interhemispheric inhibition is mediated by the activation of layer1 interneurons by the corpus callosum, and is present only if the ipsilateral hindpaw is stimulated 200 or 400 ms before the contralateral one. The long time course of this inhibition is compatible with the involvement of $GABA_B$ receptors, as demonstrated by the authors.

Another nice example of inhibition between sensory afferents is shown by Daniel Simons and collaborators (Kelly et al. 1999). The authors report that in rats there is a strong inhibition exerted by adjacent whiskers onto the principal whisker in the barrel cortex. In behaving rats, the removal of whiskers surrounding the principal whisker was associated with a mean 20% increase in cortical activity. These findings are consistent with the idea that, in the behaving animal, each barrel uses multi-whisker thalamic inputs and local inhibitory circuitry to sharpen the receptive field properties of its constituent neurons.

Contralateral eye driven inhibition could have a "phasic" and a "tonic" component. Phasic inhibition onto ipsilateral eye could occur during binocular stimulation mediated by active vision through the CONTRA eye. Tonic inhibition could instead be persistently activated by the CONTRA eye spontaneous activity. Our experimental paradigm can only prove the existence of this latter type of inhibition. The "phasic" component of the inter-ocular suppression can only be inferred by the lack of additivity during binocular vision showed previously.

A beautiful example of phasic inhibition is described in Palmer et al. (Palmer et al. 2012). L1 interneurons in somatosensory cortex are callosally activated by the stimulation of the ipsilateral hindpaw, and exert a $GABA_B$ mediated inhibition on L5 pyramids dendrites, in turn activated by contralateral sensory stimulation. Interhemispheric inhibition can

significantly reduce the firing output of L5 pyramidal neurons through a dendritically located mechanism that manifests only when the neuron is spiking.

In our model, the type of interneurons that mediate the interaction between stimuli conveyed by the two eyes is still under investigation. The two largest and best studied subclasses of interneurons that could mediate this effect are the fast spiking parvalbumin basket cells (Martin et al. 1983), and somatostatin-expressing interneurons. Interestingly, in mouse barrel cortex stimulation frequencies similar to those evoked by natural whisking robustly and persistently activate somatostatin interneurons rather than parvalbumin, that are activated only transiently (Tan et al. 2008). This suggest an active role for this subpopulation of neurons in shaping sensory responses, and make them an ideal candidate in mediating sensory integration also in the visual cortex. The combination of in-vivo electrophysiology and the availability of mouse strains with genetically marked subpopulations of interneurons will undoubtedly be useful in clarifying the role of different interneurons in in sensory integration.

ROLE OF THE CALLOSUM IN OCULAR DOMINANCE PLASTICITY

Due to the massive fiber crossing at the chiasm, the visual cortex has a natural contralateral bias. In juvenile animals this bias can be reverted with the classic paradigm of monocular deprivation (MD). Suturing one eyelid shut for seven days shifts the ocular dominance (OD) in favor of the open eye. We then asked whether the CONTRA eye mediated inter-ocular suppression that we observed was retained after modifying the relative eye strength with MD. We sutured the contralateral eye shut for 7 days, and after reopening we measured ipsilateral eye VEP amplitude before and after TTX injection in the contralateral deprived eye. We found that interocular suppression onto IPSI eye responses was lost after 7 days of MD. CONTRA VEP amplitude is highly reduced after 3 days of MD, and a subsequent IPSI VEP amplitude increase is reported after 7 days, a phenomenon described as "potentiation" (Frenkel and Bear 2004). Silencing CONTRA eye spontaneous activity with TTX during MD leads to a faster IPSI eye potentiation (Frenkel and Bear 2004), again suggesting a role both for phasic and tonic inhibition onto IPSI eye responses in naïve animals. CONTRA eye reduced ability in driving cortical responses could account for the loss of the fast thalamic control of the inhibitory interneurons, thus leading to a loss of inter-ocular suppression. It was interesting to investigate whether after MD the intracortical inhibition was under control of the now stronger ipsilateral open eye, leading to a suppression of contralateral eye responses.

This was not the case, as TTX inactivation of the ipsilateral eye had no effect on the contralateral deprived eye responses.

We then tested the idea that during monocular deprivation cortical rather than thalamic rearrangements could occur in the circuits that control cortical inhibition. We recorded VEPs evoked through the contralateral eye before and after the injection of muscimol (a GABA$_A$ agonist) in the opposite visual cortex, that means before and after the inactivation of the callosal communication with the ipsilateral eye driven hemisphere. This lead to the unmasking of contralateral eye responses, proving the emergence of interhemispheric suppression of CONTRA eye responses after 7 days of MD.
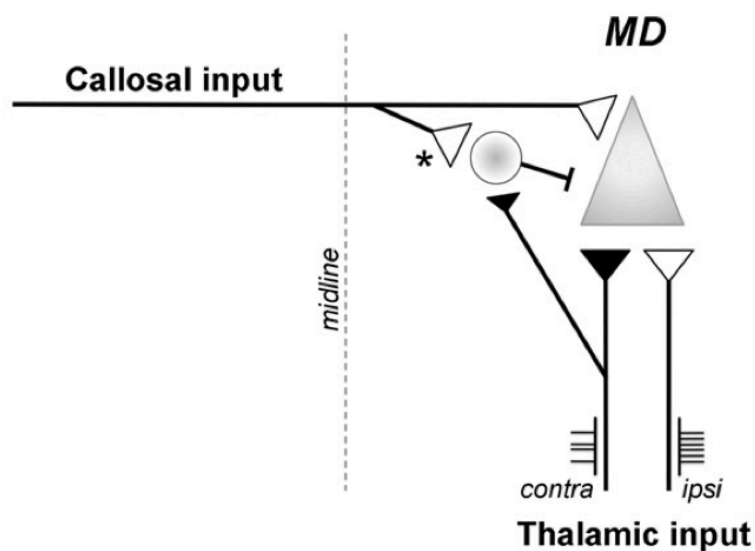
Figure 2: model of cortical circuitry in juvenile rats after 7 days MD

The loss of inter-ocular suppression after MD and the callosal shift in the control of the inhibition are coherent with the model depicted in the Fig.2. This model proposes that after MD there is mainly a potentiation of the ipsilateral eye callosal synapses onto inhibitory interneurons, and of the ipsilateral eye thalamic synapses onto pyramidal neurons. The weakening of contralateral eye thalamic afferents is also taken into account. The potentiation of the callosal ipsilateral eye input onto intracortical interneurons could explain the loss of the CONTRA eye ability to shunt IPSI eye responses and the callosal suppression of the CONTRA eye responses in the deprived animal. Under these conditions, responses from the contralateral deprived eye could be effectively suppressed by callosal afferents.

To further demonstrate that interhemispheric connections contribute to the competition process itself, we continuously blocked callosal input activity by contralateral muscimol

infusion throughout the MD and then examined the ocular dominance (OD) properties in the absence of muscimol. This experiment silences callosal influences throughout the MD and allows competition to occur only via the thalamo-cortical route. The data were clear in indicating a substantial reduction of the OD shift, due to a blockade of the weakening of deprived eye responses. The prevention of the OD shift observed in the animals with minipump infusion of muscimol is likely due to a lack of plastic rearrangements of the callosal pathway during MD. These data indicate the requirement of callosal inputs in generating the monocular bias during MD.

These experiments uncover a novel role for callosal inputs in OD plasticity. Specifically, we have shown that after monocular visual deprivation the transcallosal pathway changes from a mainly excitatory action (supplying ipsilateral eye input) to a predominantly inhibitory function (providing selective suppression of deprived eye afferents). Theoretically, this excitatory/inhibitory switch following MD might be due either to a change in the neurochemical phenotype of callosal neurons or to the recruitment of inhibitory circuits in the other hemisphere. The neurochemical switch is described in the kainic acid induced hippocampal hyperexcitability. After kainate administration, interneurons in the hilus start to degenerate leading to a lack of inhibition onto dentate gyrus granule cells. To compensate this reduction in inhibition, granule cells start to express the inhibitory marker NPY along their axons (Makiura et al. 1999; Antonucci et al. 2008a).

We ruled out this first hypothesis by performing retrograde labeling of callosally projecting neurons combined with GABA immunostaining in normal and monocularly deprived rats. The percentage of commissural GABAergic cells remained extremely low (about 1%) in both cases, excluding the possibility of a neurochemical switch. We therefore favor the hypothesis of the recruitment of inhibitory circuits in the other hemisphere. A very interesting point that remains to be clarified is the type of interneurons recruited by the thalamus in normal conditions, and by the callosum after MD.

It is worth pointing out that the emergence of transcallosal inhibition has been demonstrated to participate in plastic events occurring during several pathological conditions of the human brain. For example, it has been shown in neglect patients that some of the behavioral symptoms are attributable to a pathological state of increased inhibition exerted onto the damaged parietal cortex by the contralateral, intact hemisphere (Fecteau et al. 2006; Fierro et al. 2006). Indeed, inactivation of the unaffected hemisphere by transcranial magnetic stimulation ameliorates visuospatial neglect (Fecteau et al. 2006; Fierro et al. 2006). It has also been reported that changes in transcallosal inhibition

contribute to the occurrence of mirror movements in Parkinson's disease and ischemic patients (Cincotta et al. 2006) (Li et al. 2007; Nair et al. 2007).

In a brilliant paper Hofer et al al. (Hofer et al. 2006b) found that a transient shift in OD, induced by monocular deprivation (MD) earlier in life, renders the adult visual cortex highly susceptible to subsequent MD many weeks later. Thus, a specific trace is left in visual cortex, which maintains a memory of its deprivation history. Given the importance of interhemispheric connections in normal conditions and during ocular dominance plasticity we reasoned whether the callosum was also important in retrieving the memory trace induced by prior experience. Our results confirmed the facilitating effect of a priming monocular deprivation on a subsequent MD, but blocking the callosal communication during the second MD did not prevent plasticity to occur. This demonstrated that the priming MD leaves a long lasting memory trace in the cortex able to facilitate subsequent MD plasticity independently of the callosal pathway. Mechanisms other than callosal plasticity might be involved in the retaining of the memory trace, such as spine density and size. Indeed, the same authors showed in adult mice that the priming MD promotes the formation of new spines that shrink after eye re-opening and increase in size again after the second MD (Hofer et al. 2009).

PART TWO: Tetanus neurotoxin-induced epilepsy in mouse visual cortex

The physiology of the visual cortex is well known and can be exploited for a detailed definition of functional alterations within pathologies. Epilepsy is one of the most common serious disorders of the brain. It affects at least 50 million people worldwide; 80% of them are in the developing world, where 80–90% of people are believed to receive inadequate or no treatment at all (Meinardi et al. 2001), also because there are many open questions about the development and the specific features of the pathology.

Here we exploited the properties of a bacterial toxin, Tetanus Neurotoxin (TeNT) to generate a model of cortical epilepsy in mouse visual cortex. This chronic epilepsy model in mouse V1 could be very useful for studying the basic mechanisms of hyperexcitability in such a common and complex disease.

Tetanus Neurotoxin is a clostridial metalloprotease that that cleaves the synaptic protein VAMP/synaptobrevin, acting on nerve terminals to prevent exocytosis. The peripheral administration of TeNT leads to tetanus, a spastic paralysis due the toxin-mediated blockade of inhibitory circuits in the spinal cord. TeNT does not normally cross an intact blood brain barrier, however, several investigators have deliberately introduces this toxin in the brains of experimental animals to interfere with neural transmission (Caleo and Schiavo 2009). TeNT delivery in the brain has long been used to produce epileptic foci. Although this model is widely used in rats, the time course and spatial specificity of TeNT proteolytic action have not been precisely defined. We provide here for the first time a detailed biochemical, physiological, and histopathologic al characterization of TeNT-induced epilepsy in mouse visual cortex.

We conducted the biochemical analysis on protein extracts from brains of mice that received a single unilateral injection of TeNT in the visual cortex. To analyzed the time course of the TeNT proteolytic action, we probed by western blot and immunochemistry the level of intact and TeNT-cleaved VAMP in mouse visual cortex at different times after the toxin injection in the brain parenchyma. The decrease of the intact form of the protein and the concurrent appearance of the cleaved product of the toxin shows that TeNT effects peak at 10 days, decrease by 21–35 days, and are completely extinguished by 45 days post injection. This time course fits well with previous data examining inhibitory postsynaptic currents in slices from TeNT-injected rat hippocampi (Whittington and Jefferys 1994). By 45 days the TeNT cleaved form of VAMP is no longer detectable, and intact VAMP levels goes back almost to 75% of controls. Interestingly, VAMP2 levels do not recover completely even 60 days after TeNT injection. The lower level of this synaptic protein might be implicated in the long lasting epileptic phenotype in TeNT injected mice, that persists after the TeNT mediated cleavage is over.

Most previous reports in the literature have emphasized a loss of inhibition following TeNT that likely plays a key role in triggering hyperexcitability (Empson et al. 1993) (Whittington and Jefferys 1994). In the peripheral system, TeNT undergoes transcytosis to inhibitory interneurons, where it exerts its toxic effects. On the other hand, in the brain, our quantitative analysis of intact VAMP2 indicated a loss of about 70% of normal expression in the injected V1. Because GABAergic inhibitory cells constitute only about 20–30% of cortical neurons, and VAMP2 is expressed in both excitatory and inhibitory neurons (Yeh et al. 2010a), a selective action of TeNT in interneurons would not account for the observed conspicuous decrease of VAMP2 levels. Entry of TeNT into cortical excitatory neurons and proteolytic cleavage of VAMP2 in these cells has been shown recently (Yeh et al. 2010a). Therefore, TeNT-induced epilepsy is likely to involve a contribution from excitatory neurons.

We detected a consistent reduction of VAMP2 level was also in the thalamus ipsilateral to the TeNT injection. The spreading of TeNT action to subcortical, monosynaptically linked nuclei is reported here for the first time and should be taken into account as an important feature of this model, since subcortical disinhibition may contribute - via altered patterns of afferent activity - to the establishment of an epileptic focus in the cortex. There was no detectable propagation of TeNT effects to the contralateral hemisphere or distant areas via cortico-cortical connections. One explanation is that TeNT injection was performed within the core of V1, a site where callosal and cortico-cortical connections are quite sparse (Mizuno et al. 2007; Di Garbo et al. 2011).

The detection, characterization and time course of epileptic events were analyzed by electrophysiological recordings of chronic local field potential (LFP) in awake, freely moving mice. We clearly detected both ictal and interictal electrographic epileptiform discharges in TeNT animals, but no behavioral correlates of this activity. The lack of the behavioural correlated does not invalidate our model, since electrographic epileptiform events by themselves are sufficient to define an epileptic condition. Nevertheless, behavioral aspects of this model have not been investigated so far, and given the reported visual cortex hyperexcitability, behavioral tests aimed at probing visual performance in TeNT treated animals would be of great usefulness.

Interestingly, epileptiform discharges persisted after the completion of TeNT mediated VAMP cleavage, proving the establishment of a long lasting epileptic focus induced by the toxin injection. Also, anatomic analyses on brain slices found no evidence for long term tissue damage, such as neuronal loss or microglia activation.

The biochemical mechanisms that give rises to the epileptiform activity are not known. The TeNT-impaired neurotransmission could allow some kind of maladaptive plasticity that leads to a persistent unbalance in the inhibition/excitation ratio in the cortex. To clarify whether this unbalance was due to an increase or decrease in the inhibitory or excitatory transmission we measured a marker of inhibition (Gad 65/67) and excitation (vGlut1). Western blot on protein extracts displayed no differences in the probed proteins between TeNT injected and controls mice 45 days after TeNT delivery, leaving this question open.

We conclude that a single cortical TeNT injection can reliably generate epileptiform discharges in mouse visual cortex that persist after the toxin proteolytic effect is over. This is a non-lesional model of cortical epilepsy that could also take great advantage of the increasingly available genetic mouse strains to answer the many open questions in this complex pathology, including the role of interhemispheric communication in a brain with asymmetric inhibition/excitation balance.

BIBLIOGRAFIA

BIBLIOGRAFIA

Abbott, L. F. and S. B. Nelson (2000). "Synaptic plasticity: taming the beast." Nat Neurosci 3 Suppl: 1178-1183.

Alarcon, J. M., R. Hodgman, et al. (2004). "Selective modulation of some forms of schaffer collateral-CA1 synaptic plasticity in mice with a disruption of the CPEB-1 gene." Learn Mem 11(3): 318-327.

Antonini, A., M. Fagiolini, et al. (1999). "Anatomical correlates of functional plasticity in mouse visual cortex." J Neurosci 19(11): 4388-4406.

Antonini, A., D. C. Gillespie, et al. (1998). "Morphology of single geniculocortical afferents and functional recovery of the visual cortex after reverse monocular deprivation in the kitten." J Neurosci 18(23): 9896-9909.

Antonini, A. and M. P. Stryker (1993). "Rapid remodeling of axonal arbors in the visual cortex." Science 260(5115): 1819-1821.

Antonini, A. and M. P. Stryker (1996). "Plasticity of geniculocortical afferents following brief or prolonged monocular occlusion in the cat." J Comp Neurol 369(1): 64-82.

Antonucci, F., C. Cerri, et al. (2010). "Acute neuroprotection by the synaptic blocker botulinum neurotoxin E in a rat model of focal cerebral ischaemia." Neuroscience 169(1): 395-401.

Antonucci, F., A. Di Garbo, et al. (2007). "Botulinum neurotoxin E (BoNT/E) reduces CA1 neuron loss and granule cell dispersion, with no effects on chronic seizures, in a mouse model of temporal lobe epilepsy." Exp Neurol.

Antonucci, F., A. Di Garbo, et al. (2008a). "Botulinum neurotoxin E (BoNT/E) reduces CA1 neuron loss and granule cell dispersion, with no effects on chronic seizures, in a mouse model of temporal lobe epilepsy." Exp Neurol 210(2): 388-401.

Antonucci, F., C. Rossi, et al. (2008b). "Long-distance retrograde effects of botulinum neurotoxin A." J Neurosci 28(14): 3689-3696.

Atwal, J. K., J. Pinkston-Gosse, et al. (2008). "PirB is a functional receptor for myelin inhibitors of axonal regeneration." Science 322(5903): 967-970.

Baker, D. H., T. S. Meese, et al. (2007). "Binocular interaction: contrast matching and contrast discrimination are predicted by the same model." Spatial vision 20(5): 397-413.

Baldwin, M. R. and J. T. Barbieri (2007). "Association of botulinum neurotoxin serotypes a and B with synaptic vesicle protein complexes." Biochemistry 46(11): 3200-3210.

Baroncelli, L., A. Sale, et al. (2010). "Experience-dependent reactivation of ocular dominance plasticity in the adult visual cortex." Experimental neurology 226(1): 100-109.

Bartoletti, A., P. Medini, et al. (2004). "Environmental enrichment prevents effects of dark-rearing in the rat visual cortex." Nat Neurosci 7(3): 215-216.

Bear, M. F. (2003). "Bidirectional synaptic plasticity: from theory to reality." Philos Trans R Soc Lond B Biol Sci 358(1432): 649-655.

Bear, M. F., A. Kleinschmidt, et al. (1990). "Disruption of experience-dependent synaptic modifications in striate cortex by infusion of an NMDA receptor antagonist." J Neurosci 10(3): 909-925.

Bear, M. F. and C. D. Rittenhouse (1999). "Molecular basis for induction of ocular dominance plasticity." J Neurobiol 41(1): 83-91.

Bearse, M. A., Jr. and R. D. Freeman (1994). "Binocular summation in orientation discrimination depends on stimulus contrast and duration." Vision research 34(1): 19-29.

Beaver, C. J., Q. Ji, et al. (2001). "Cyclic AMP-dependent protein kinase mediates ocular dominance shifts in cat visual cortex." Nat Neurosci 4(2): 159-163.

Benevento, L. A., B. W. Bakkum, et al. (1995). "gamma-Aminobutyric acid and somatostatin immunoreactivity in the visual cortex of normal and dark-reared rats." Brain Res 689(2): 172-182.

Benevento, L. A., B. W. Bakkum, et al. (1992). "The effects of dark-rearing on the electrophysiology of the rat visual cortex." Brain Res 572(1-2): 198-207.

Berardi, N., S. Bisti, et al. (1988). "The transfer of visual information across the corpus callosum in cats, monkeys and humans: spatial and temporal properties." Prog Brain Res 75: 181-185.

Berardi, N., A. Cellerino, et al. (1994). "Monoclonal antibodies to nerve growth factor affect the postnatal development of the visual system." Proc Natl Acad Sci U S A 91(2): 684-688.

Berardi, N., T. Pizzorusso, et al. (2000). "Critical periods during sensory development." Curr Opin Neurobiol 10(1): 138-145.

Berardi, N., T. Pizzorusso, et al. (2003). "Molecular basis of plasticity in the visual cortex." Trends Neurosci 26(7): 369-378.

Berlucchi, G. (2011). "What is callosal plasticity? in *Cortical plasticity,* Chalupa L.M. et al." MIT press.

Berlucchi, G., S. Aglioti, et al. (1995). "Corpus callosum and simple visuomotor integration." Neuropsychologia 33(8): 923-936.

Berlucchi, G. and G. Rizzolatti (1968). "Binocularly driven neurons in visual cortex of split-chiasm cats." Science 159(812): 308-310.

Berman, N. E. and B. R. Payne (1983). "Alterations in connections of the corpus callosum following convergent and divergent strabismus." Brain Res 274(2): 201-212.

Bishop, P. O., G. H. Henry, et al. (1971). "Binocular interaction fields of single units in the cat striate cortex." J Physiol 216(1): 39-68.

Blais, B. S., H. Z. Shouval, et al. (1999). "The role of presynaptic activity in monocular deprivation: comparison of homosynaptic and heterosynaptic mechanisms." Proc Natl Acad Sci U S A 96(3): 1083-1087.

Blake, R. and H. Wilson (2011). "Binocular vision." Vision Res 51(7): 754-770.

Blakemore, C. (1970). "Binocular depth perception and the optic chiasm." Vision Res 10(1): 43-47.

Blakemore, C., Y. C. Diao, et al. (1983). "Possible functions of the interhemispheric connexions between visual cortical areas in the cat." J Physiol 337: 331-349.

Bloom, J. S. and G. W. Hynd (2005). "The role of the corpus callosum in interhemispheric transfer of information: excitation or inhibition?" Neuropsychol Rev 15(2): 59-71.

Bocci, T., M. Caleo, et al. (2011). "Transcallosal inhibition dampens neural responses to high contrast stimuli in human visual cortex." Neuroscience 187: 43-51.

Bohnert, S. and G. Schiavo (2005). "Tetanus toxin is transported in a novel neuronal compartment characterized by a specialized pH regulation." The Journal of biological chemistry 280(51): 42336-42344.

Bonhoeffer, T. (1996). "Neurotrophins and activity-dependent development of the neocortex." Curr Opin Neurobiol 6(1): 119-126.

Bozzi, Y., L. Costantin, et al. (2006). "Action of botulinum neurotoxins in the central nervous system: antiepileptic effects." Neurotox Res 9(2-3): 197-203.

Bozzi, Y., T. Pizzorusso, et al. (1995). "Monocular deprivation decreases the expression of messenger RNA for brain-derived neurotrophic factor in the rat visual cortex." Neuroscience 69(4): 1133-1144.

Brace, H. M., J. G. Jefferys, et al. (1985). "Long-term changes in hippocampal physiology and learning ability of rats after intrahippocampal tetanus toxin." The Journal of physiology 368: 343-357.

Bradbury, E. J., L. D. Moon, et al. (2002). "Chondroitinase ABC promotes functional recovery after spinal cord injury." Nature 416(6881): 636-640.

Brener, K., Y. Amitai, et al. (1991). "Chronic epileptic foci in neocortex: in vivo and in vitro effects of tetanus toxin." The European journal of neuroscience 3(1): 47-54.

Buhl, E. H. and W. Singer (1989). "The callosal projection in cat visual cortex as revealed by a combination of retrograde tracing and intracellular injection." Exp Brain Res 75(3): 470-476.

Burrone, J. and V. N. Murthy (2003). "Synaptic gain control and homeostasis." Curr Opin Neurobiol 13(5): 560-567.

Cabelli, R. J., D. L. Shelton, et al. (1997). "Blockade of endogenous ligands of trkB inhibits formation of ocular dominance columns." Neuron 19(1): 63-76.

Cagenello, R., A. Arditi, et al. (1993). "Binocular enhancement of visual acuity." Journal of the Optical Society of America. A, Optics, image science, and vision 10(8): 1841-1848.

Caleo, M., C. Lodovichi, et al. (1999a). "Effects of nerve growth factor on visual cortical plasticity require afferent electrical activity." Eur J Neurosci 11(8): 2979-2984.

Caleo, M., C. Lodovichi, et al. (1999b). "Expression of the transcription factor Zif268 in the visual cortex of monocularly deprived rats: effects of nerve growth factor." Neuroscience 91(3): 1017-1026.

Caleo, M., L. Restani, et al. (2007). "Transient synaptic silencing of developing striate cortex has persistent effects on visual function and plasticity." J Neurosci 27(17): 4530-4540.

Caleo, M. and G. Schiavo (2009). "Central effects of tetanus and botulinum neurotoxins." Toxicon 54(5): 593-599.

Caminiti, R., H. Ghaziri, et al. (2009). "Evolution amplified processing with temporally dispersed slow neuronal connectivity in primates." Proceedings of the National Academy of Sciences of the United States of America 106(46): 19551-19556.

Cancedda, L., E. Putignano, et al. (2003). "Patterned vision causes CRE-mediated gene expression in the visual cortex through PKA and ERK." J Neurosci 23(18): 7012-7020.

Cancedda, L., E. Putignano, et al. (2004). "Acceleration of visual system development by environmental enrichment." J Neurosci 24(20): 4840-4848.

Carmeli, C., L. Lopez-Aguado, et al. (2007). "A novel interhemispheric interaction: modulation of neuronal cooperativity in the visual areas." PLoS ONE 2(12): e1287.

Carron, C., A. Bourdelas, et al. (2005). "Antagonistic interaction between IGF and Wnt/JNK signaling in convergent extension in Xenopus embryo." Mechanisms of development 122(11): 1234-1247.

Castren, E., F. Zafra, et al. (1992). "Light regulates expression of brain-derived neurotrophic factor mRNA in rat visual cortex." Proc Natl Acad Sci U S A 89(20): 9444-9448.

Cerri, C., A. Fabbri, et al. (2011). "Activation of Rho GTPases triggers structural remodeling and functional plasticity in the adult rat visual cortex." The Journal of neuroscience : the official journal of the Society for Neuroscience 31(42): 15163-15172.

Cerri, C., L. Restani, et al. (2010). "Callosal contribution to ocular dominance in rat primary visual cortex." Eur J Neurosci 32(7): 1163-1169.

Chapman, B., M. D. Jacobson, et al. (1986). "Ocular dominance shift in kitten visual cortex caused by imbalance in retinal electrical activity." Nature 324(6093): 154-156.

Chapman, B. and M. P. Stryker (1993). "Development of orientation selectivity in ferret visual cortex and effects of deprivation." J Neurosci 13(12): 5251-5262.

Chen, B., S. S. Wang, et al. (2008a). "The Fezf2-Ctip2 genetic pathway regulates the fate choice of subcortical projection neurons in the developing cerebral cortex." Proc Natl Acad Sci U S A 105(32): 11382-11387.

Chen, C., M. R. Baldwin, et al. (2008b). "Molecular basis for tetanus toxin coreceptor interactions." Biochemistry 47(27): 7179-7186.

Cincotta, M., A. Borgheresi, et al. (2006). "Mechanisms underlying mirror movements in Parkinson's disease: a transcranial magnetic stimulation study." Mov Disord 21(7): 1019-1025.

Clarke, S. and J. Miklossy (1990). "Occipital cortex in man: organization of callosal connections, related myelo- and cytoarchitecture, and putative boundaries of functional visual areas." J Comp Neurol 298(2): 188-214.

Coleman, J. E., K. Law, et al. (2009). "Anatomical origins of ocular dominance in mouse primary visual cortex." Neuroscience 161(2): 561-571.

Cotrufo, T., A. Viegi, et al. (2003). "Effects of neurotrophins on synaptic protein expression in the visual cortex of dark-reared rats." J Neurosci 23(9): 3566-3571.

Cowey, A. and V. H. Perry (1979). "The projection of the temporal retina in rats, studied by retrograde transport of horseradish peroxidase." Exp Brain Res 35(3): 457-464.

Curtis, D. R. and W. C. De Groat (1968). "Tetanus toxin and spinal inhibition." Brain research 10(2): 208-212.

Cusick, C. G. and R. D. Lund (1981). "The distribution of the callosal projection to the occipital visual cortex in rats and mice." Brain Res 214(2): 239-259.

Cynader, M. and D. E. Mitchell (1980). "Prolonged sensitivity to monocular deprivation in dark-reared cats." J Neurophysiol 43(4): 1026-1040.

Davis, G. W. (2006). "Homeostatic control of neural activity: from phenomenology to molecular design." Annu Rev Neurosci 29: 307-323.

Davletov, B., M. Bajohrs, et al. (2005). "Beyond BOTOX: advantages and limitations of individual botulinum neurotoxins." Trends Neurosci 28(8): 446-452.

Deinhardt, K., O. Berninghausen, et al. (2006a). "Tetanus toxin is internalized by a sequential clathrin-dependent mechanism initiated within lipid microdomains and independent of epsin1." The Journal of cell biology 174(3): 459-471.

Deinhardt, K., S. Salinas, et al. (2006b). "Rab5 and Rab7 control endocytic sorting along the axonal retrograde transport pathway." Neuron 52(2): 293-305.

Desai, N. S. (2003). "Homeostatic plasticity in the CNS: synaptic and intrinsic forms." J Physiol Paris 97(4-6): 391-402.

Di Cristo, G., N. Berardi, et al. (2001). "Requirement of ERK activation for visual cortical plasticity." Science 292(5525): 2337-2340.

Di Garbo, A., M. Mainardi, et al. (2011). "Environmental enrichment modulates cortico-cortical interactions in the mouse." PLoS One 6(9): e25285.

Diamond, M. E., W. Huang, et al. (1994). "Laminar comparison of somatosensory cortical plasticity." Science 265(5180): 1885-1888.

Diao, Y. C., Y. K. Wang, et al. (1983). "Binocular responses of cortical cells and the callosal projection in the albino rat." Exp Brain Res 49(3): 410-418.

Dong, M., D. A. Richards, et al. (2003). "Synaptotagmins I and II mediate entry of botulinum neurotoxin B into cells." The Journal of cell biology 162(7): 1293-1303.

Dong, M., W. H. Tepp, et al. (2007). "Mechanism of botulinum neurotoxin B and G entry into hippocampal neurons." The Journal of cell biology 179(7): 1511-1522.

Dong, M., F. Yeh, et al. (2006). "SV2 is the protein receptor for botulinum neurotoxin A." Science 312(5773): 592-596.

Dougherty, R. F., M. Ben-Shachar, et al. (2005). "Functional organization of human occipital-callosal fiber tracts." Proc Natl Acad Sci U S A 102(20): 7350-7355.

Drager, U. C. (1975). "Receptive fields of single cells and topography in mouse visual cortex." J Comp Neurol 160(3): 269-290.

Duffy, F. H., J. L. Burchfiel, et al. (1976). "Bicuculline reversal of deprivation amblyopia in the cat." Nature 260(5548): 256-257.

Duncan, J. S., J. W. Sander, et al. (2006). "Adult epilepsy." Lancet 367(9516): 1087-1100.

Elberger, A. J. (1984). "The existence of a separate, brief critical period for the corpus callosum to affect visual development." Behav Brain Res 11(3): 223-231.

Elberger, A. J. (1989). "Selective labeling of visual corpus callosum connections with aspartate in cat and rat." Vis Neurosci 2(1): 81-85.

Elberger, A. J. and E. L. Smith, 3rd (1985). "The critical period for corpus callosum section to affect cortical binocularity." Exp Brain Res 57(2): 213-223.

Empson, R. M., Y. Amitai, et al. (1993). "Injection of tetanus toxin into the neocortex elicits persistent epileptiform activity but only transient impairment of GABA release." Neuroscience 57(2): 235-239.

Endo, A., N. Nagai, et al. (1999). "Proteolysis of neuronal cell adhesion molecule by the tissue plasminogen activator-plasmin system after kainate injection in the mouse hippocampus." Neurosci Res 33(1): 1-8.

Engel, A. K., P. Konig, et al. (1991). "Interhemispheric synchronization of oscillatory neuronal responses in cat visual cortex." Science 252(5010): 1177-1179.

Fagiolini, M., J. M. Fritschy, et al. (2004). "Specific GABAA circuits for visual cortical plasticity." Science 303(5664): 1681-1683.

Fagiolini, M. and T. K. Hensch (2000). "Inhibitory threshold for critical-period activation in primary visual cortex." Nature 404(6774): 183-186.

Fagiolini, M., H. Katagiri, et al. (2003). "Separable features of visual cortical plasticity revealed by N-methyl-D-aspartate receptor 2A signaling." Proc Natl Acad Sci U S A 100(5): 2854-2859.

Fagiolini, M., T. Pizzorusso, et al. (1994). "Functional postnatal development of the rat primary visual cortex and the role of visual experience: dark rearing and monocular deprivation." Vision Res 34(6): 709-720.

Fagiolini, M., T. Pizzorusso, et al. (1997). "Transplant of Schwann cells allows normal development of the visual cortex of dark-reared rats." Eur J Neurosci 9(1): 102-112.

Fecteau, S., A. Pascual-Leone, et al. (2006). "Paradoxical facilitation of attention in healthy humans." Behav Neurol 17(3-4): 159-162.

Ferster, D. (1981). "A comparison of binocular depth mechanisms in areas 17 and 18 of the cat visual cortex." J Physiol 311: 623-655.

Fierro, B., F. Brighina, et al. (2006). "Improving neglect by TMS." Behav Neurol 17(3-4): 169-176.

Frenkel, M. Y. and M. F. Bear (2004). "How monocular deprivation shifts ocular dominance in visual cortex of young mice." Neuron 44(6): 917-923.

Frost, D. O. and Y. P. Moy (1989). "Effects of dark rearing on the development of visual callosal connections." Exp Brain Res 78(1): 203-213.

Gandhi, S. P., Y. Yanagawa, et al. (2008). "Delayed plasticity of inhibitory neurons in developing visual cortex." Proceedings of the National Academy of Sciences of the United States of America 105(43): 16797-16802.

Gazzaniga, M. S. (2000). "Cerebral specialization and interhemispheric communication: does the corpus callosum enable the human condition?" Brain 123 (Pt 7): 1293-1326.

George, G. and J. Mellanby (1982). "Memory deficits in an experimental hippocampal epileptiform syndrome in rats." Experimental neurology 75(3): 678-689.

Gianfranceschi, L., R. Siciliano, et al. (2003). "Visual cortex is rescued from the effects of dark rearing by overexpression of BDNF." Proc Natl Acad Sci U S A 100(21): 12486-12491.

Gilbert, C. D. (1992). "Horizontal integration and cortical dynamics." Neuron 9(1): 1-13.

Gillespie, D. C., M. C. Crair, et al. (2000). "Neurotrophin-4/5 alters responses and blocks the effect of monocular deprivation in cat visual cortex during the critical period." J Neurosci 20(24): 9174-9186.

Godement, P., J. Salaun, et al. (1984). "Prenatal and postnatal development of retinogeniculate and retinocollicular projections in the mouse." J Comp Neurol 230(4): 552-575.

Goldberg, E. M. and D. A. Coulter (2013). "Mechanisms of epileptogenesis: a convergence on neural circuit dysfunction." Nature reviews. Neuroscience 14(5): 337-349.

Gonchar, Y., Q. Wang, et al. (2007). "Multiple distinct subtypes of GABAergic neurons in mouse visual cortex identified by triple immunostaining." Frontiers in neuroanatomy 1: 3.

Gordon, J. A. and M. P. Stryker (1996). "Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse." J Neurosci 16(10): 3274-3286.

Greifzu, F., S. Schmidt, et al. (2011). "Global impairment and therapeutic restoration of visual plasticity mechanisms after a localized cortical stroke." Proceedings of the National Academy of Sciences of the United States of America 108(37): 15450-15455.

Guire, E. S., M. E. Lickey, et al. (1999). "Critical period for the monocular deprivation effect in rats: assessment with sweep visually evoked potentials." J Neurophysiol 81(1): 121-128.

Harris, A. E., G. B. Ermentrout, et al. (1997). "A model of ocular dominance column development by competition for trophic factor." Proc Natl Acad Sci U S A 94(18): 9944-9949.

Hattox, A. M. and S. B. Nelson (2007). "Layer V neurons in mouse cortex projecting to different targets have distinct physiological properties." J Neurophysiol 98(6): 3330-3340.

He, H. Y., W. Hodos, et al. (2006). "Visual deprivation reactivates rapid ocular dominance plasticity in adult visual cortex." J Neurosci 26(11): 2951-2955.

He, H. Y., B. Ray, et al. (2007). "Experience-dependent recovery of vision following chronic deprivation amblyopia." Nat Neurosci 10(9): 1134-1136.

Heimel, J. A., R. J. Hartman, et al. (2007). "Screening mouse vision with intrinsic signal optical imaging." Eur J Neurosci 25(3): 795-804.

Hellige, J. B. (1993). "Hemispheric asymmetry: what's right and what's left." Harvard University Press, Cambridge MA.

Hensch, T. K. (2004). "Critical period regulation." Annu Rev Neurosci 27: 549-579.

Hensch, T. K. (2005). "Critical period plasticity in local cortical circuits." Nat Rev Neurosci 6(11): 877-888.

Hensch, T. K., M. Fagiolini, et al. (1998). "Local GABA circuit control of experience-dependent plasticity in developing visual cortex." Science 282(5393): 1504-1508.

Hensch, T. K. and M. P. Stryker (2004). "Columnar architecture sculpted by GABA circuits in developing cat visual cortex." Science 303(5664): 1678-1681.

Herreros, J., T. Ng, et al. (2001). "Lipid rafts act as specialized domains for tetanus toxin binding and internalization into neurons." Molecular biology of the cell 12(10): 2947-2960.

Hess, R. F. and J. S. Pointer (1989). "Spatial and temporal contrast sensitivity in hemianopia. A comparative study of the sighted and blind hemifields." Brain 112 (Pt 4): 871-894.

Heynen, A. J., B. J. Yoon, et al. (2003). "Molecular mechanism for loss of visual cortical responsiveness following brief monocular deprivation." Nat Neurosci 6(8): 854-862.

Hickey, T. L. and R. W. Guillery (1974). "An autoradiographic study of retinogeniculate pathways in the cat and the fox." The Journal of comparative neurology 156(2): 239-253.

Hockfield, S., R. G. Kalb, et al. (1990). "Expression of neural proteoglycans correlates with the acquisition of mature neuronal properties in the mammalian brain." Cold Spring Harb Symp Quant Biol 55: 505-514.

Hofer, S. B., H. Ko, et al. (2011). "Differential connectivity and response dynamics of excitatory and inhibitory neurons in visual cortex." Nature neuroscience 14(8): 1045-1052.

Hofer, S. B., T. D. Mrsic-Flogel, et al. (2006a). "Lifelong learning: ocular dominance plasticity in mouse visual cortex." Curr Opin Neurobiol 16(4): 451-459.

Hofer, S. B., T. D. Mrsic-Flogel, et al. (2006b). "Prior experience enhances plasticity in adult visual cortex." Nat Neurosci 9(1): 127-132.

Hofer, S. B., T. D. Mrsic-Flogel, et al. (2009). "Experience leaves a lasting structural trace in cortical circuits." Nature 457(7227): 313-317.

Holtmaat, A. J., J. T. Trachtenberg, et al. (2005). "Transient and persistent dendritic spines in the neocortex in vivo." Neuron 45(2): 279-291.

Houzel, J. C. and C. Milleret (1999). "Visual inter-hemispheric processing: constraints and potentialities set by axonal morphology." J Physiol Paris 93(4): 271-284.

Huang, Z. J., A. Kirkwood, et al. (1999). "BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex." Cell 98(6): 739-755.

Hubel, D. H. and T. N. Wiesel (1962). "Receptive fields, binocular interaction and functional architecture in the cat's visual cortex." J Physiol 160: 106-154.

Hubel, D. H. and T. N. Wiesel (1963). "Shape and arrangement of columns in cat's striate cortex." J Physiol 165: 559-568.

Hubel, D. H. and T. N. Wiesel (1967). "Cortical and callosal connections concerned with the vertical meridian of visual fields in the cat." J Neurophysiol 30(6): 1561-1573.

Hubel, D. H. and T. N. Wiesel (1970). "The period of susceptibility to the physiological effects of unilateral eye closure in kittens." J Physiol 206(2): 419-436.

Hughes, R. and B. C. Whaler (1962). "Influence of nerve-ending activity and of drugs on the rate of paralysis of rat diaphragm preparations by Cl. botulinum type A toxin." The Journal of physiology 160: 221-233.

Innocenti, G. M. (1980). "The primary visual pathway through the corpus callosum: morphological and functional aspects in the cat." Arch Ital Biol 118(2): 124-188.

Innocenti, G. M. (1981). "Growth and reshaping of axons in the establishment of visual callosal connections." Science 212(4496): 824-827.

Innocenti, G. M. (2009). "Dynamic interactions between the cerebral hemispheres." Experimental brain research. Experimentelle Hirnforschung. Experimentation cerebrale 192(3): 417-423.

Innocenti, G. M., D. Aggoun-Zouaoui, et al. (1995). "Cellular aspects of callosal connections and their development." Neuropsychologia 33(8): 961-987.

Innocenti, G. M. and R. Caminiti (1980). "Postnatal shaping of callosal connections from sensory areas." Exp Brain Res 38(4): 381-394.

Innocenti, G. M., S. Clarke, et al. (1986). "Interchange of callosal and association projections in the developing visual cortex." J Neurosci 6(5): 1384-1409.

Innocenti, G. M. and D. O. Frost (1979). "Effects of visual experience on the maturation of the efferent system to the corpus callosum." Nature 280(5719): 231-234.

Innocenti, G. M., D. O. Frost, et al. (1985). "Maturation of visual callosal connections in visually deprived kittens: a challenging critical period." J Neurosci 5(2): 255-267.

Innocenti, G. M., P. Lehmann, et al. (1994). "Computational structure of visual callosal axons." The European journal of neuroscience 6(6): 918-935.

Innocenti, G. M. and D. J. Price (2005). "Exuberance in the development of cortical networks." Nat Rev Neurosci 6(12): 955-965.

Iurilli, G., F. Benfenati, et al. (2012). "Loss of visually driven synaptic responses in layer 4 regular-spiking neurons of rat visual cortex in absence of competing inputs." Cereb Cortex 22(9): 2171-2181.

Jacky, B. P., P. E. Garay, et al. (2013). "Identification of fibroblast growth factor receptor 3 (FGFR3) as a protein receptor for botulinum neurotoxin serotype A (BoNT/A)." PLoS pathogens 9(5): e1003369.

Jacobson, S. (1970). "Distribution of commissural axon terminals in the rat neocortex." Exp Neurol 28(2): 193-205.

Jacobson, S. and J. Q. Trojanowski (1974). "The cells of origin of the corpus callosum in rat, cat and rhesus monkey." Brain Res 74(1): 149-155.

Jahn, R., T. Lang, et al. (2003). "Membrane fusion." Cell 112(4): 519-533.

Jalili, M., S. Lavoie, et al. (2007). "Dysconnection topography in schizophrenia revealed with state-space analysis of EEG." PloS one 2(10): e1059.

Jefferys, G. R. J. a. W., M.C. (2006). "Tetanus Toxin Model of Focal Epilepsy." Models of Seizures and Epilepsy.

Jefferys, J. G. (1989). "Chronic epileptic foci in vitro in hippocampal slices from rats with the tetanus toxin epileptic syndrome." Journal of neurophysiology 62(2): 458-468.

Jefferys, J. G. and S. F. Williams (1987). "Physiological and behavioural consequences of seizures induced in the rat by intrahippocampal tetanus toxin." Brain : a journal of neurology 110 (Pt 2): 517-532.

Jiang, B., Y. Akaneya, et al. (2003). "Long-term depression is not induced by low-frequency stimulation in rat visual cortex in vivo: a possible preventing role of endogenous brain-derived neurotrophic factor." J Neurosci 23(9): 3761-3770.

Jiruska, P., G. T. Finnerty, et al. (2010). "Epileptic high-frequency network activity in a model of non-lesional temporal lobe epilepsy." Brain : a journal of neurology 133(Pt 5): 1380-1390.

Kafitz, K. W., C. R. Rose, et al. (1999). "Neurotrophin-evoked rapid excitation through TrkB receptors." Nature 401(6756): 918-921.

Kameyama, K., K. Sohya, et al. (2010). "Difference in binocularity and ocular dominance plasticity between GABAergic and excitatory cortical neurons." The Journal of neuroscience : the official journal of the Society for Neuroscience 30(4): 1551-1559.

Katz, L. C. and C. J. Shatz (1996). "Synaptic activity and the construction of cortical circuits." Science 274(5290): 1133-1138.

Keller, J. E., F. Cai, et al. (2004). "Uptake of botulinum neurotoxin into cultured neurons." Biochemistry 43(2): 526-532.

Kelly, M. K., G. E. Carvell, et al. (1999). "Sensory loss by selected whisker removal produces immediate disinhibition in the somatosensory cortex of behaving rats." J Neurosci 19(20): 9117-9125.

Kennedy, H. and C. Dehay (1988). "Functional implications of the anatomical organization of the callosal projections of visual areas V1 and V2 in the macaque monkey." Behav Brain Res 29(3): 225-236.

Khibnik, L. A., K. K. Cho, et al. (2010). "Relative contribution of feedforward excitatory connections to expression of ocular dominance plasticity in layer 4 of visual cortex." Neuron 66(4): 493-500.

Kimura, F. and R. W. Baughman (1997). "GABAergic transcallosal neurons in developing rat neocortex." Eur J Neurosci 9(6): 1137-1143.

Kiper, D. C., M. G. Knyazeva, et al. (1999). "Visual stimulus-dependent changes in interhemispheric EEG coherence in ferrets." Journal of neurophysiology 82(6): 3082-3094.

Kirkwood, A., M. C. Rioult, et al. (1996). "Experience-dependent modification of synaptic plasticity in visual cortex." Nature 381(6582): 526-528.

Kirkwood, A., A. Silva, et al. (1997). "Age-dependent decrease of synaptic plasticity in the neocortex of alphaCaMKII mutant mice." Proc Natl Acad Sci U S A 94(7): 3380-3383.

Knyazeva, M. G., E. Fornari, et al. (2006). "Imaging of a synchronous neuronal assembly in the human visual brain." NeuroImage 29(2): 593-604.

Knyazeva, M. G. and G. M. Innocenti (2001). "EEG coherence studies in the normal brain and after early-onset cortical pathologies." Brain research. Brain research reviews 36(2-3): 119-128.

Knyazeva, M. G., D. C. Kiper, et al. (1999). "Visual stimulus-dependent changes in interhemispheric EEG coherence in humans." Journal of neurophysiology 82(6): 3095-3107.

Koeda, T., M. Knyazeva, et al. (1995). "The EEG in acallosal children. Coherence values in the resting state: left hemisphere compensatory mechanism?" Electroencephalography and clinical neurophysiology 95(6): 397-407.

Koppe, G., G. Bruckner, et al. (1997). "Developmental patterns of proteoglycan-containing extracellular matrix in perineuronal nets and neuropil of the postnatal rat brain." Cell Tissue Res 288(1): 33-41.

Korzus, E., M. G. Rosenfeld, et al. (2004). "CBP histone acetyltransferase activity is a critical component of memory consolidation." Neuron 42(6): 961-972.

Kovalchuk, Y., E. Hanse, et al. (2002). "Postsynaptic Induction of BDNF-Mediated Long-Term Potentiation." Science 295(5560): 1729-1734.

Lee, C. L., J. Hannay, et al. (2001). "Spatial learning deficits without hippocampal neuronal loss in a model of early-onset epilepsy." Neuroscience 107(1): 71-84.

Lee, C. L., R. A. Hrachovy, et al. (1995). "Tetanus toxin-induced seizures in infant rats and their effects on hippocampal excitability in adulthood." Brain research 677(1): 97-109.

Legge, G. E. (1984). "Binocular contrast summation--I. Detection and discrimination." Vision research 24(4): 373-383.

Lehmann, K., K. F. Schmidt, et al. (2012). "Vision and visual plasticity in ageing mice." Restorative neurology and neuroscience 30(2): 161-178.

Lewis, J. W. and J. F. Olavarria (1995). "Two rules for callosal connectivity in striate cortex of the rat." J Comp Neurol 361(1): 119-137.

Li, J. Y., A. J. Espay, et al. (2007). "Interhemispheric and ipsilateral connections in Parkinson's disease: relation to mirror movements." Mov Disord 22(6): 813-821.

Lickey, M. E., T. A. Pham, et al. (2004). "Swept contrast visual evoked potentials and their plasticity following monocular deprivation in mice." Vision Res 44(28): 3381-3387.

Lindqvist, S., J. Skranes, et al. (2011). "Visual function and white matter microstructure in very-low-birth-weight (VLBW) adolescents--a DTI study." Vision Res 51(18): 2063-2070.

Lodato, S., C. Rouaux, et al. (2011). "Excitatory projection neuron subtypes control the distribution of local inhibitory interneurons in the cerebral cortex." Neuron 69(4): 763-779.

Lodovichi, C., N. Berardi, et al. (2000). "Effects of neurotrophins on cortical plasticity: same or different?" J Neurosci 20(6): 2155-2165.

Longordo, F., M. S. To, et al. (2013). "Sublinear integration underlies binocular processing in primary visual cortex." Nat Neurosci 16(6): 714-723.

Louis, E. D., P. D. Williamson, et al. (1990). "Chronic focal epilepsy induced by microinjection of tetanus toxin into the cat motor cortex." Electroencephalography and clinical neurophysiology 75(6): 548-557.

Lund, R. D. (1965). "Uncrossed Visual Pathways of Hooded and Albino Rats." Science 149(3691): 1506-1507.

Lund, R. D. and D. E. Mitchell (1979). "The effects of dark-rearing on visual callosal connections of cats." Brain research 167(1): 172-175.

Ma, W. P., Y. T. Li, et al. (2013). "Downregulation of Cortical Inhibition Mediates Ocular Dominance Plasticity during the Critical Period." The Journal of neuroscience : the official journal of the Society for Neuroscience 33(27): 11276-11280.

Ma, W. P., B. H. Liu, et al. (2010). "Visual representations by cortical somatostatin inhibitory neurons--selective but with weak and delayed responses." The Journal of neuroscience : the official journal of the Society for Neuroscience 30(43): 14371-14379.

Maffei, A., K. Nataraj, et al. (2006). "Potentiation of cortical inhibition by visual deprivation." Nature 443(7107): 81-84.

Maffei, A., S. B. Nelson, et al. (2004). "Selective reconfiguration of layer 4 visual cortical circuitry by visual deprivation." Nat Neurosci 7(12): 1353-1359.

Maffei, L., N. Berardi, et al. (1986). "Interocular transfer of adaptation after effect in neurons of area 17 and 18 of split chiasm cats." J Neurophysiol 55(5): 966-976.

Maffei, L., N. Berardi, et al. (1992). "Nerve growth factor (NGF) prevents the shift in ocular dominance distribution of visual cortical neurons in monocularly deprived rats." J Neurosci 12(12): 4651-4662.

Mahrhold, S., A. Rummel, et al. (2006). "The synaptic vesicle protein 2C mediates the uptake of botulinum neurotoxin A into phrenic nerves." FEBS letters 580(8): 2011-2014.

Mainardi, M., S. Landi, et al. (2010). "Environmental enrichment potentiates thalamocortical transmission and plasticity in the adult rat visual cortex." J Neurosci Res 88(14): 3048-3059.

Makarov, V. A., K. E. Schmidt, et al. (2008). "Stimulus-dependent interaction between the visual areas 17 and 18 of the 2 hemispheres of the ferret (Mustela putorius)." Cerebral cortex 18(8): 1951-1960.

Makiura, Y., F. Suzuki, et al. (1999). "Excitatory granule cells of the dentate gyrus exhibit a double inhibitory neurochemical content after intrahippocampal administration of kainate in adult mice." Exp Neurol 159(1): 73-83.

Marder, E. and A. A. Prinz (2003). "Current compensation in neuronal homeostasis." Neuron 37(1): 2-4.

Margrie, T. W., M. Brecht, et al. (2002). "In vivo, low-resistance, whole-cell recordings from neurons in the anaesthetized and awake mammalian brain." Pflugers Arch 444(4): 491-498.

Martin, K. A., P. Somogyi, et al. (1983). "Physiological and morphological properties of identified basket cells in the cat's visual cortex." Exp Brain Res 50(2-3): 193-200.

Mataga, N., S. Fujishima, et al. (2001). "Experience-dependent plasticity of mouse visual cortex in the absence of the neuronal activity-dependent marker egr1/zif268." J Neurosci 21(24): 9724-9732.

Mataga, N., Y. Mizuguchi, et al. (2004). "Experience-dependent pruning of dendritic spines in visual cortex by tissue plasminogen activator." Neuron 44(6): 1031-1041.

Mataga, N., N. Nagai, et al. (2002). "Permissive proteolytic activity for visual cortical plasticity." Proc Natl Acad Sci U S A 99(11): 7717-7721.

Matteoli, M., C. Verderio, et al. (1996). "Synaptic vesicle endocytosis mediates the entry of tetanus neurotoxin into hippocampal neurons." Proceedings of the National Academy of Sciences of the United States of America 93(23): 13310-13315.

Maya Vetencourt, J. F., A. Sale, et al. (2008). "The antidepressant fluoxetine restores plasticity in the adult visual cortex." Science 320(5874): 385-388.

McAllister, A. K., L. C. Katz, et al. (1999). "Neurotrophins and synaptic plasticity." Annu Rev Neurosci 22: 295-318.

McGee, A. W., Y. Yang, et al. (2005). "Experience-driven plasticity of visual cortex limited by myelin and Nogo receptor." Science 309(5744): 2222-2226.

Medini, P. (2011). "Layer- and cell-type-specific subthreshold and suprathreshold effects of long-term monocular deprivation in rat visual cortex." J Neurosci 31(47): 17134-17148.

Medini, P. and T. Pizzorusso (2008). "Visual experience and plasticity of the visual cortex: a role for epigenetic mechanisms." Front Biosci 13: 3000-3007.

Meinardi, H., R. A. Scott, et al. (2001). "The treatment gap in epilepsy: the current situation and ways forward." Epilepsia 42(1): 136-149.

Mellanby, J., G. George, et al. (1977). "Epileptiform syndrome in rats produced by injecting tetanus toxin into the hippocampus." Journal of neurology, neurosurgery, and psychiatry 40(4): 404-414.

Mellanby, J., H. Johansen-Berg, et al. (1999). "The effects of tetanus toxin-induced limbic epilepsy on the exploratory response to novelty in the rat." Epilepsia 40(8): 1058-1061.

Mellios, N., H. Sugihara, et al. (2011). "miR-132, an experience-dependent microRNA, is essential for visual cortex plasticity." Nature neuroscience 14(10): 1240-1242.

Miller, K. D., J. B. Keller, et al. (1989). "Ocular dominance column development: analysis and simulation." Science 245(4918): 605-615.

Milleret, C. (1994). "Visual callosal connections and strabismus." Behav Brain Res 64(1-2): 85-95.

Minciacchi, D. and A. Antonini (1984). "Binocularity in the visual cortex of the adult cat does not depend on the integrity of the corpus callosum." Behav Brain Res 13(2): 183-192.

Mitchell, D. E. and C. Blakemore (1970). "Binocular depth perception and the corpus callosum." Vision Res 10(1): 49-54.

Mizuno, H., T. Hirano, et al. (2007). "Evidence for activity-dependent cortical wiring: formation of interhemispheric connections in neonatal mouse visual cortex requires projection neuron activity." J Neurosci 27(25): 6760-6770.

Montecucco, C. and J. Molgo (2005). "Botulinal neurotoxins: revival of an old killer." Curr Opin Pharmacol 5(3): 274-279.

Moore, T., H. R. Rodman, et al. (1996). "Greater residual vision in monkeys after striate cortex damage in infancy." J Neurophysiol 76(6): 3928-3933.

Moradi, F. and D. J. Heeger (2009). "Inter-ocular contrast normalization in human visual cortex." J Vis 9(3): 13 11-22.

Morales, B., S. Y. Choi, et al. (2002). "Dark rearing alters the development of GABAergic transmission in visual cortex." J Neurosci 22(18): 8084-8090.

Mower, A. F., D. S. Liao, et al. (2002). "cAMP/Ca2+ response element-binding protein function is essential for ocular dominance plasticity." J Neurosci 22(6): 2237-2245.

Mower, G. D. (1991). "The effect of dark rearing on the time course of the critical period in cat visual cortex." Brain Res Dev Brain Res 58(2): 151-158.

Mower, G. D. and W. G. Christen (1989). "Evidence for an enhanced role of GABA inhibition in visual cortical ocular dominance of cats reared with abnormal monocular experience." Brain Res Dev Brain Res 45(2): 211-218.

Mrsic-Flogel, T. D., S. B. Hofer, et al. (2007). "Homeostatic regulation of eye-specific responses in visual cortex during ocular dominance plasticity." Neuron 54(6): 961-972.

Muller, C. M. and C. B. Griesinger (1998). "Tissue plasminogen activator mediates reverse occlusion plasticity in visual cortex." Nat Neurosci 1(1): 47-53.

Munk, M. H., L. G. Nowak, et al. (1995). "Structural basis of cortical synchronization. II. Effects of cortical lesions." Journal of neurophysiology 74(6): 2401-2414.

Nair, D. G., S. Hutchinson, et al. (2007). "Imaging correlates of motor recovery from cerebral infarction and their physiological significance in well-recovered patients." Neuroimage 34(1): 253-263.

Nedivi, E., D. Hevroni, et al. (1993). "Numerous candidate plasticity-related genes revealed by differential cDNA cloning." Nature 363(6431): 718-722.

Nicole, O., F. Docagne, et al. (2001). "The proteolytic activity of tissue-plasminogen activator enhances NMDA receptor-mediated signaling." Nat Med 7(1): 59-64.

Nilsen, K. E., M. C. Walker, et al. (2005). "Characterization of the tetanus toxin model of refractory focal neocortical epilepsy in the rat." Epilepsia 46(2): 179-187.

Noebels, J. L. (2003). "The biology of epilepsy genes." Annual review of neuroscience 26: 599-625.

O'Leary, D. D., B. B. Stanfield, et al. (1981). "Evidence that the early postnatal restriction of the cells of origin of the callosal projection is due to the elimination of axonal collaterals rather than to the death of neurons." Brain research 227(4): 607-617.

Olavarria, J. F. and R. Hiroi (2003). "Retinal influences specify cortico-cortical maps by postnatal day six in rats and mice." The Journal of comparative neurology 459(2): 156-172.

Olavarria, J. F., R. Laing, et al. (2008a). "Topography and axon arbor architecture in the visual callosal pathway: effects of deafferentation and blockade of N-methyl-D-aspartate receptors." Biological research 41(4): 413-424.

Olavarria, J. F., R. Laing, et al. (2008b). "Topography and axon arbor architecture in the visual callosal pathway: effects of deafferentation and blockade of N-methyl-D-aspartate receptors." Biol Res 41(4): 413-424.

Olavarria, J. F. and R. C. Van Sluyters (1995). "Overall pattern of callosal connections in visual cortex of normal and enucleated cats." J Comp Neurol 363(2): 161-176.

Oray, S., A. Majewska, et al. (2004). "Dendritic spine dynamics are regulated by monocular deprivation and extracellular matrix degradation." Neuron 44(6): 1021-1030.

Palmer, L. M., J. M. Schulz, et al. (2012). "The cellular basis of GABA(B)-mediated interhemispheric inhibition." Science 335(6071): 989-993.

Paramei, G. V. and B. A. Sabel (2008). "Contour-integration deficits on the intact side of the visual field in hemianopia patients." Behavioural brain research 188(1): 109-124.

Paul, L. K., W. S. Brown, et al. (2007). "Agenesis of the corpus callosum: genetic, developmental and functional aspects of connectivity." Nat Rev Neurosci 8(4): 287-299.

Payne, B. R. (1990). "Function of the corpus callosum in the representation of the visual field in cat visual cortex." Vis Neurosci 5(2): 205-211.

Payne, B. R., A. J. Elberger, et al. (1980). "Binocularity in the cat visual cortex is reduced by sectioning the corpus callosum." Science 207(4435): 1097-1099.

Payne, B. R. and D. F. Siwek (1991). "Visual-field map in the callosal recipient zone at the border between areas 17 and 18 in the cat." Vis Neurosci 7(3): 221-236.

Payne, B. R., D. F. Siwek, et al. (1991). "Complex transcallosal interactions in visual cortex." Vis Neurosci 6(3): 283-289.

Peters, A., B. R. Payne, et al. (1990). "Transcallosal non-pyramidal cell projections from visual cortex in the cat." J Comp Neurol 302(1): 124-142.

Pettigrew, J. D., T. Nikara, et al. (1968). "Binocular interaction on single units in cat striate cortex: simultaneous stimulation by single moving slit with receptive fields in correspondence." Exp Brain Res 6(4): 391-410.

Pham, T. A., S. J. Graham, et al. (2004). "A semi-persistent adult ocular dominance plasticity in visual cortex is stabilized by activated CREB." Learn Mem 11(6): 738-747.

Pham, T. A., S. Impey, et al. (1999). "CRE-mediated gene transcription in neocortical neuronal plasticity during the developmental critical period." Neuron 22(1): 63-72.

Pinto, L., D. Drechsel, et al. (2009). "AP2gamma regulates basal progenitor fate in a region- and layer-specific manner in the developing cortex." Nat Neurosci 12(10): 1229-1237.

Pizzorusso, T., M. Fagiolini, et al. (1997a). "Temporal aspects of contrast visual evoked potentials in the pigmented rat: effect of dark rearing." Vision Res 37(4): 389-395.

Pizzorusso, T., P. Medini, et al. (2002). "Reactivation of ocular dominance plasticity in the adult visual cortex." Science 298(5596): 1248-1251.

Pizzorusso, T., P. Medini, et al. (2006). "Structural and functional recovery from early monocular deprivation in adult rats." Proc Natl Acad Sci U S A 103(22): 8517-8522.

Pizzorusso, T., V. Porciatti, et al. (1997b). "Transplant of polymer-encapsulated cells genetically engineered to release nerve growth factor allows a normal functional development of the visual cortex in dark-reared rats." Neuroscience 80(2): 307-311.

Poo, M. M. (2001). "Neurotrophins as synaptic modulators." Nat Rev Neurosci 2(1): 24-32.

Porciatti, V., T. Pizzorusso, et al. (1999). "The visual physiology of the wild type mouse determined with pattern VEPs." Vision Res 39(18): 3071-3081.

Prusky, G. T., N. M. Alam, et al. (2006). "Enhancement of vision by monocular deprivation in adult mice." The Journal of neuroscience : the official journal of the Society for Neuroscience 26(45): 11554-11561.

Putignano, E., G. Lonetti, et al. (2007). "Developmental downregulation of histone posttranslational modifications regulates visual cortical plasticity." Neuron 53(5): 747-759.

Putnam, M. C., M. S. Steven, et al. (2010). "Cortical projection topography of the human splenium: hemispheric asymmetry and individual differences." J Cogn Neurosci 22(8): 1662-1669.

Qian, Z., M. E. Gilbert, et al. (1993). "Tissue-plasminogen activator is induced as an immediate-early gene during seizure, kindling and long-term potentiation." Nature 361(6411): 453-457.

Resta, V., E. Novelli, et al. (2007). "Acute retinal ganglion cell injury caused by intraocular pressure spikes is mediated by endogenous extracellular ATP." Eur J Neurosci 25(9): 2741-2754.

Restani, L., F. Antonucci, et al. (2011a). "Evidence for anterograde transport and transcytosis of botulinum neurotoxin A (BoNT/A)." The Journal of neuroscience : the official journal of the Society for Neuroscience 31(44): 15650-15659.

Restani, L., F. Antonucci, et al. (2011b). "Evidence for anterograde transport and transcytosis of botulinum neurotoxin A (BoNT/A)." J Neurosci 31(44): 15650-15659.

Restani, L., C. Cerri, et al. (2009). "Functional masking of deprived eye responses by callosal input during ocular dominance plasticity." Neuron 64(5): 707-718.

Restani, L., E. Novelli, et al. (2012). "Botulinum neurotoxin A impairs neurotransmission following retrograde transynaptic transport." Traffic 13(8): 1083-1089.

Rittenhouse, C. D., H. Z. Shouval, et al. (1999). "Monocular deprivation induces homosynaptic long-term depression in visual cortex." Nature 397(6717): 347-350.

Rizzo, M. and D. A. Robin (1996). "Bilateral effects of unilateral visual cortex lesions in human." Brain 119 (Pt 3): 951-963.

Roberts, E. B., M. A. Meredith, et al. (1998). "Suppression of NMDA receptor function using antisense DNA block ocular dominance plasticity while preserving visual responses." J Neurophysiol 80(3): 1021-1032.

Rochefort, N. L., P. Buzas, et al. (2009). "Functional selectivity of interhemispheric connections in cat visual cortex." Cereb Cortex 19(10): 2451-2465.

Rossetto, O., L. Morbiato, et al. (2006). "Presynaptic enzymatic neurotoxins." Journal of neurochemistry 97(6): 1534-1545.

Rummel, A., T. Eichner, et al. (2007). "Identification of the protein receptor binding site of botulinum neurotoxins B and G proves the double-receptor concept." Proceedings of the National Academy of Sciences of the United States of America 104(1): 359-364.

Rummel, A., T. Karnath, et al. (2004). "Synaptotagmins I and II act as nerve cell receptors for botulinum neurotoxin G." The Journal of biological chemistry 279(29): 30865-30870.

Saenz, M. and I. Fine (2010). "Topographic organization of V1 projections through the corpus callosum in humans." Neuroimage 52(4): 1224-1229.

Saghatelyan, A. K., A. Dityatev, et al. (2001). "Reduced perisomatic inhibition, increased excitatory transmission, and impaired long-term potentiation in mice deficient for the extracellular matrix glycoprotein tenascin-R." Mol Cell Neurosci 17(1): 226-240.

Sala, R., A. Viegi, et al. (1998). "Nerve growth factor and brain-derived neurotrophic factor increase neurotransmitter release in the rat visual cortex." Eur J Neurosci 10(6): 2185-2191.

Sale, A., J. F. Maya Vetencourt, et al. (2007). "Environmental enrichment in adulthood promotes amblyopia recovery through a reduction of intracortical inhibition." Nat Neurosci 10(6): 679-681.

Salinas, S., G. Schiavo, et al. (2010). "A hitchhiker's guide to the nervous system: the complex journey of viruses and toxins." Nature reviews. Microbiology 8(9): 645-655.

Sawtell, N. B., M. Y. Frenkel, et al. (2003). "NMDA receptor-dependent ocular dominance plasticity in adult visual cortex." Neuron 38(6): 977-985.

Scali, M., L. Baroncelli, et al. (2012). "A rich environmental experience reactivates visual cortex plasticity in aged rats." Experimental gerontology 47(4): 337-341.

Schiavo, G. (2006). "Structural biology: dangerous liaisons on neurons." Nature 444(7122): 1019-1020.

Schiavo, G., M. Matteoli, et al. (2000). "Neurotoxins affecting neuroexocytosis." Physiol Rev 80(2): 717-766.

Schmidt, K. E., S. G. Lomber, et al. (2010). "Specificity of neuronal responses in primary visual cortex is modulated by interhemispheric corticocortical input." Cerebral cortex 20(12): 2776-2786.

Schwab, M. E. (2004). "Nogo and axon regeneration." Curr Opin Neurobiol 14(1): 118-124.

Schwab, M. E., K. Suda, et al. (1979). "Selective retrograde transsynaptic transfer of a protein, tetanus toxin, subsequent to its retrograde axonal transport." The Journal of cell biology 82(3): 798-810.

Sefton, A. J. D., B. (1995). Visual System. The Rat Nervous System. G. Paxinos, Academic Peress: 833-989.

Sengpiel, F. (2005). "Visual cortex: overcoming a no-go for plasticity." Curr Biol 15(24): R1000-1002.

Sengpiel, F. and P. C. Kind (2002). "The role of activity in development of the visual system." Curr Biol 12(23): R818-826.

Sherman, S. M. and P. D. Spear (1982). "Organization of visual pathways in normal and visually deprived cats." Physiol Rev 62(2): 738-855.

Sillito, A. M., J. A. Kemp, et al. (1981). "The role of GABAergic inhibition in the cortical effects of monocular deprivation." Nature 291(5813): 318-320.

Sorensen, A. T. and M. Kokaia (2013). "Novel approaches to epilepsy treatment." Epilepsia 54(1): 1-10.

Sorensen, S. A., T. A. Jones, et al. (2003). "Neonatal enucleation reduces the proportion of callosal boutons forming multiple synaptic contacts in rat striate cortex." Neurosci Lett 351(1): 17-20.

Sugiyama, S., A. A. Di Nardo, et al. (2008). "Experience-dependent transfer of Otx2 homeoprotein into the visual cortex activates postnatal plasticity." Cell 134(3): 508-520.

Sugiyama, S., A. Prochiantz, et al. (2009). "From brain formation to plasticity: insights on Otx2 homeoprotein." Development, growth & differentiation 51(3): 369-377.

Sun, J. S., B. Li, et al. (1994). "Transcallosal circuitry revealed by blocking and disinhibiting callosal input in the cat." Vis Neurosci 11(2): 189-197.

Sutton, M. A., H. T. Ito, et al. (2006). "Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis." Cell 125(4): 785-799.

Syken, J., T. Grandpre, et al. (2006). "PirB restricts ocular-dominance plasticity in visual cortex." Science 313(5794): 1795-1800.

Tagawa, Y., P. O. Kanold, et al. (2005). "Multiple periods of functional ocular dominance plasticity in mouse visual cortex." Nat Neurosci 8(3): 380-388.

Taha, S., J. L. Hanover, et al. (2002). "Autophosphorylation of alphaCaMKII is required for ocular dominance plasticity." Neuron 36(3): 483-491.

Taha, S. and M. P. Stryker (2002). "Rapid ocular dominance plasticity requires cortical but not geniculate protein synthesis." Neuron 34(3): 425-436.

Tan, Z., H. Hu, et al. (2008). "Robust but delayed thalamocortical activation of dendritic-targeting inhibitory interneurons." Proc Natl Acad Sci U S A 105(6): 2187-2192.

Tettoni, L., F. Gheorghita-Baechler, et al. (1998). "Constant and variable aspects of axonal phenotype in cerebral cortex." Cerebral cortex 8(6): 543-552.

Tognini, P., E. Putignano, et al. (2011). "Experience-dependent expression of miR-132 regulates ocular dominance plasticity." Nature neuroscience 14(10): 1237-1239.

Tohmi, M., H. Kitaura, et al. (2006). "Enduring critical period plasticity visualized by transcranial flavoprotein imaging in mouse primary visual cortex." J Neurosci 26(45): 11775-11785.

Tomasch, J. (1954). "Size, distribution, and number of fibres in the human corpus callosum." Anat Rec 119(1): 119-135.

Tomasi, S., R. Caminiti, et al. (2012). "Areal differences in diameter and length of corticofugal projections." Cerebral cortex 22(6): 1463-1472.

Toyama, K., K. Matsunami, et al. (1974). "An intracellular study of neuronal organization in the visual cortex." Exp Brain Res 21(1): 45-66.

Tropea, D., G. Kreiman, et al. (2006). "Gene expression changes and molecular pathways mediating activity-dependent plasticity in visual cortex." Nat Neurosci 9(5): 660-668.

Tsukamoto, K., T. Kohda, et al. (2005). "Binding of Clostridium botulinum type C and D neurotoxins to ganglioside and phospholipid. Novel insights into the receptor for clostridial neurotoxins." The Journal of biological chemistry 280(42): 35164-35171.

Turrigiano, G. (2007). "Homeostatic signaling: the positive side of negative feedback." Curr Opin Neurobiol 17(3): 318-324.

Turrigiano, G. G. and S. B. Nelson (2004). "Homeostatic plasticity in the developing nervous system." Nat Rev Neurosci 5(2): 97-107.

Van Essen, D. C., W. T. Newsome, et al. (1982). "The pattern of interhemispheric connections and its relationship to extrastriate visual areas in the macaque monkey." J Neurosci 2(3): 265-283.

van Versendaal, D., R. Rajendran, et al. (2012). "Elimination of inhibitory synapses is a major component of adult ocular dominance plasticity." Neuron 74(2): 374-383.

Verderio, C., O. Rossetto, et al. (2006). "Entering neurons: botulinum toxins and synaptic vesicle recycling." EMBO reports 7(10): 995-999.

Viegi, A., T. Cotrufo, et al. (2002). "Effects of dark rearing on phosphorylation of neurotrophin Trk receptors." Eur J Neurosci 16(10): 1925-1930.

Voigt, T., S. LeVay, et al. (1988). "Morphological and immunocytochemical observations on the visual callosal projections in the cat." The Journal of comparative neurology 272(3): 450-460.

Vreugdenhil, M., S. P. Hack, et al. (2002). "Tetanus toxin induces long-term changes in excitation and inhibition in the rat hippocampal CA1 area." Neuroscience 114(4): 983-994.

Watroba, L., P. Buser, et al. (2001). "Impairment of binocular vision in the adult cat induces plastic changes in the callosal cortical map." Eur J Neurosci 14(6): 1021-1029.

Whittington, M. A. and J. G. Jefferys (1994). "Epileptic activity outlasts disinhibition after intrahippocampal tetanus toxin in the rat." J Physiol 481 (Pt 3): 593-604.

Wierenga, C. J., M. F. Walsh, et al. (2006). "Temporal regulation of the expression locus of homeostatic plasticity." J Neurophysiol 96(4): 2127-2133.

Wiesel, T. N. and D. H. Hubel (1963). "Single-Cell Responses in Striate Cortex of Kittens Deprived of Vision in One Eye." J Neurophysiol 26: 1003-1017.

Wiesel, T. N. and D. H. Hubel (1965). "Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens." J Neurophysiol 28(6): 1029-1040.

Wu, Y. P., C. J. Siao, et al. (2000). "The tissue plasminogen activator (tPA)/plasmin extracellular proteolytic system regulates seizure-induced hippocampal mossy fiber outgrowth through a proteoglycan substrate." J Cell Biol 148(6): 1295-1304.

Xu, X., K. D. Roby, et al. (2010). "Immunochemical characterization of inhibitory mouse cortical neurons: three chemically distinct classes of inhibitory cells." The Journal of comparative neurology 518(3): 389-404.

Yates, M. A. and J. M. Juraska (2007). "Increases in size and myelination of the rat corpus callosum during adulthood are maintained into old age." Brain Res 1142: 13-18.

Yazaki-Sugiyama, Y., S. Kang, et al. (2009). "Bidirectional plasticity in fast-spiking GABA circuits by visual experience." Nature 462(7270): 218-221.

Yeh, F. L., M. Dong, et al. (2010a). "SV2 mediates entry of tetanus neurotoxin into central neurons." PLoS Pathog 6(11): e1001207.

Yeh, F. L., M. Dong, et al. (2010b). "SV2 mediates entry of tetanus neurotoxin into central neurons." PLoS pathogens 6(11): e1001207.

Yinon, U., M. Chen, et al. (1992). "Binocularity and excitability loss in visual cortex cells of corpus callosum transected kittens and cats." Brain Res Bull 29(5): 541-552.

Zhang, L. I. and M. M. Poo (2001). "Electrical activity and development of neural circuits." Nat Neurosci 4 Suppl: 1207-1214.

Zhang, W. and D. J. Linden (2003). "The other side of the engram: experience-driven changes in neuronal intrinsic excitability." Nat Rev Neurosci 4(11): 885-900.

Zlatkova, M. B., R. S. Anderson, et al. (2001). "Binocular summation for grating detection and resolution in foveal and peripheral vision." Vision research 41(24): 3093-3100.