Influence of Binocular Competition on the Expression Profiles of CRMP2, CRMP4, Dyn I, and Syt I in Developing Cat Visual Cortex

The visual cortex is vulnerable to changes in visual input, especially during the critical period when numerous molecules drive the refinement of the circuitry. From a list of potential actors identified in a recent proteomics study, we selected 2 collapsin response mediator proteins (CRMP2/CRMP4) and 2 synaptic proteins, Dynamin I (Dyn I) and Synaptotagmin I (Syt I), for in-depth analysis of their developmental expression profile in cat visual cortex. CRMP2 and CRMP4 levels were high early in life and clearly declined toward adulthood. In contrast, Dyn I expression levels progressively augmented during maturation. Syt I showed low levels at eye opening and in adults, high levels around the peak of the critical period, and maximal levels at juvenile age. We further determined a role for each molecule in ocular dominance plasticity. CRMP2 and Syt I levels decreased in area 17 upon monocular deprivation, whereas CRMP4 and Dyn I levels remained unaffected. In contrast, binocular removal of pattern vision had no influence on CRMP2 and Syt I expression in kitten area 17. This study illustrates that not the loss of quality of vision through visual deprivation, but disruption of normal binocular visual experience is crucial to induce the observed molecular changes.

Keywords: area 17, binocular deprivation, critical period, kitten, monocular deprivation, postnatal development

Introduction

The primary visual cortex is the first stage along the retino-thalamo-cortical pathway where information of the 2 eyes merges onto binocularly driven neurons (Hubel and Wiesel 1962, 1970; Sherman and Spear 1982). However, alternating parallel bands of ~500 μm, called ocular dominance columns, are preferentially activated by either the left or right eye (LeVay et al. 1978; Shatz and Stryker 1978; Daw 1995; Payne and Peters 2002). One of the seminal discoveries in developmental neuroscience is that altering visual experience in kittens through monocular deprivation can modify both the typical physiology of the visual cortex and the anatomical representation of the 2 eyes in the cortex. The closure or damage of one eye (but not both) yields an expansion of the columns serving the open eye at the expense of those responding to the deprived eye, which become reduced in size and afferent complexity (Hubel and Wiesel 1963; Wiesel and Hubel 1965; LeVay et al. 1978; Shatz and Stryker 1978). This results in poor visual acuity (amblyopia) and contrast sensitivity and loss of depth perception (Wiesel and Hubel 1963; Daw 1995; Berardi et al. 2003). Amblyopia occurs despite the absence of damage to the retina or its target in the visual thalamus and is determined in the neocortex. The cortical rearrangements are restricted to a certain developmental stage, the critical period, that starts around eye opening and ends well before adulthood (Hubel and Wiesel 1970; Shatz and Luskin 1986; Daw 1995). Despite the progress in our understanding of visual cortex development, many issues at the molecular level and the underlying regulated cascades of gene expression remain to be elucidated.

Molecules hypothesized to play a role in visual cortex plasticity are generally expected to either show high levels during early postnatal development versus low in mature visual cortex, or else the opposite, in order to assist the refinement of cortical synaptic connectivity (Hubel and Wiesel 1970; Daw 1995). A recent large-scale screening proteome analysis has provided a list of proteins that show such a development-related expression profile in cat visual cortex (Van den Bergh, Clerens, Cnops, et al. 2003; Van den Bergh, Clerens, Vandesande, et al. 2003). From this list we have selected 4 molecules for which existing literature further supported a potential role in structural and cellular processes of cortical plasticity (Byk et al. 1998; Napolitano et al. 1999; Quinn et al. 1999; Schwab et al. 2001; Charrier et al. 2003; Kennedy and Ehlers 2006). We considered 2 members of the collapsin response mediator protein family (CRMP2 and CRMP4) and 2 molecules that are involved in the exocytosis–endocytosis machinery at the neuronal synapse, namely Dynamin I (Dyn I) and Synaptotagmin I (Syt I). CRMPs are phosphoproteins implicated in neuronal differentiation, axon guidance, and growth cone collapse through the signal cascade pathway of Sema3A/Collapsin-1 (Min tung et al. 1995; Wang and Strittmatter 1996; Goshima et al. 2000). Dyn I plays a role in synaptic vesicle recycling by clathrin-mediated endocytosis (Liu and Robinson 1995), whereas, Syt I functions as a calcium sensor that triggers an essential step in exocytosis, namely the fusion of vesicle and plasma membrane during neurotransmission (Sudhof and Rizo 1996). In addition, in a parallel study on those 4 molecules, we could show that binocular retinal lesions in adult cats affect the expression of CRMP2 and CRMP4 (but not of Dyn I and Syt I) in the primary visual cortex, implicating these molecules in adult cortical plasticity (Cnops et al. 2007). In this study, we focus on their role in developmental plasticity.

Therefore, the first goal was to determine the expression profiles of CRMP2, CRMP4, Dyn I, and Syt I at 4 time points that are critical for visual cortex maturation. We analyzed area 17 of kittens of postnatal day 10 (P10), the time of eye opening, and of P30, the peak of the critical period when the visual cortex is most sensitive to changes in visual input such as induced by monocular deprivation. Furthermore, we studied cats of juvenile age (5 months), when the vulnerability to experience-dependent alterations is already decreased in layer IV but less so in supra- and infragranular layers (Daw et al. 1992; Daw
1994; Katz and Shatz 1996; Beaver et al. 2001), and adult cats that demonstrate fully matured cortex anatomy and visual properties. The second objective was to determine the involvement of these proteins in ocular dominance plasticity. Their expression was therefore monitored in the visual cortex of kittens in which one eye was deprived from pattern vision. To control whether the observed molecular changes were a consequence of eye competition or resulted from the removal of pattern vision per se, parallel analysis of binocularly deprived kitten was performed.

Materials and Methods

Animal Care and Tissue Preparation

All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/ 609/EEC) and were approved by the Institutional Ethical Committee of the Katholieke Universiteit Leuven, Belgium. All efforts were made to minimize animals’ discomfort and to use the minimum number of animals necessary to obtain adequate scientific data. All animals were raised under a daily photoperiod of 10 h light and 14 h darkness with water and food available ad libitum (Animal Facilities, Katholieke Universiteit Leuven). All kittens were derived from 3 distinct litters.

To investigate development-related expression profiles, cats from 4 different ages were analyzed: kittens of P10 (n = 3) and of P30 (n = 6), juvenile cats of 5 months (Juv; n = 3), and adult cats of minimal 1 year old (Ad; n = 3).

Ocular dominance plasticity was studied in monocularly deprived (MD) kittens (n = 3). In this animal model, the left eye was deprived from pattern vision by eye opening (~P10) until P30 by a double-thickness linen mask that was adapted daily to the size of the growing head (Fig. 2B). Both the ipsilateral (left) and contralateral (right) hemispheres of MD kittens were analyzed because it is known that the effect of monocular deprivation is more pronounced in the hemisphere ipsilateral to the deprived eye (Hubel and Wiesel 1962; Shatz and Stryker 1978; Daw et al. 1992; Tychsen and Burkhalter 1997; Crair et al. 1998). For normal kittens (P30; n = 3) that received no visual manipulations (Fig. 2A), the visual cortex of the left or right hemisphere was randomly selected for analysis. As a control, the effect of elimination of pattern vision alone was also examined using kittens binocularly deprived (BD; n = 3) from pattern vision from P10 until P30 by a linen eye patch covering both eyes (Fig. 2C). All visual deprivation experiments have been carried out by K.B. as described before (Zernicki 1991; Burnat et al. 2002, 2005). The effect of mask rearing is comparable to lid suturing but less traumatic (Kossut et al. 1978; Zernicki 1991; Burnat et al. 2002, 2005). In both experimental conditions cats are deprived from pattern vision. In contrast to dark rearing where animals are kept in total darkness, binocular deprivation reduces retinal illumination but still allows diffuse light to reach the retina either through the eyelids or masks (Sherman and Spear 1982; Zernicki 1991).

Prior to the administration of an overdose of sodium pentobarbital (Nembutal, 60 mg/kg, intravenously) under deep ketamine anesthesia (Ketalar, 10 mg/kg, intramuscularly), all animals were maintained overnight in total darkness followed by 1-h daylight stimulation. During this short period of light stimulation that induced the rapid and transient expression of immediate early genes (IEG) in the visual cortex, deprived kittens were still wearing their masks. Immediately thereafter brains were dissected, snap frozen by immersion in dry ice-cooled 2-methylbutane (Merck Eurolab, Leuven, Belgium), and stored until use at ~70 °C. Coronal sections of 200 μm (for protein and RNA isolation) alternated with 25-μm sections (for in situ hybridization) were cut on a cryostat and stored at ~70 °C or ~20 °C, respectively.

Western Blotting

Area 17 was cut out manually from 2 or 3 cryosections of 3 cats of each condition (P10, P30, Juv, Ad, MD, and BD) and homogenized in 200 μl lysis buffer (2% sodium dodecyl sulfate, 50 mM Tris–HCl, 10% glycerol [pH 6.8]) containing 8 μl Complete Protease Inhibitor (Roche Diagnostics, Brussels, Belgium). After 5 times sonication for 10 s, 5 min heating at 70 °C, and 20-min centrifugation at 10,000 r.p.m., protein concentration of the supernatant was determined with the Micro BCA Protein Assay Reagent kit (Perbio, Erembodegem, Belgium). Protein samples of 2.5 μg for CRMP2 or Syt I, 20 μg for CRMP4, and 7.5 μg for Dyn I were loaded onto a 4–12% bis-tris NuPage gel (Invitrogen, Groningen, The Netherlands). Electrophoresis and blotting were carried out using the Xcell II module (Invitrogen) according to the manufacturer’s instructions. After blotting, the polyvinylidene difluoride membrane was rinsed for 1 h with 5% blocking solution (GE Healthcare, Roosendaal, The Netherlands) and incubated with antibodies against CRMP2 (1/12 000, mouse antibody, C4g, Dr Y. Ibara, Japan), CRMP4 (1/10 000, rabbit AB5145, Chemicon, Wevelgem, Belgium), Dyn I (1/1000, goat antibody sc-6402, Santa Cruz Biotechnology, Heidelberg, Germany), Syt I (1/4000, mouse MAB5202, Chemicon), or glyceraldehyde 3-phosphate dehydrogenase (GAPDH, 1/100 000, mouse MAB374, Chemicon). Immunoreactivity was visualized using chemiluminescence detection (ECL-plus kit, GE Healthcare) on ECL hyperfilm (GE Healthcare).

Data Analysis

The protein bands were semi-quantitatively evaluated by densitometry (ImageMaster 1D prime, GE Healthcare). Optical density values of protein bands were normalized to the corresponding GAPDH bands to avoid interlane variability in protein loading. In preliminary control experiments, we determined the optimal protein amount to be loaded for each antibody by serial dilutions. Standard curves of the optical density in function of the protein concentration were generated, and only protein concentrations in the linear range were used for further experiments to avoid saturation of the ECL film (Cnops et al. 2004). Each experiment on the same cat was repeated at least 2 times. We report here the mean value from all cats of the same condition (± standard deviation). Statistical comparisons for multiple ages or conditions were analyzed with 1-way analysis of variance with post hoc tests, whereas comparison between 2 groups was done by the unpaired ttest. Statistical differences were indicated by ‘*’ P < 0.05, ‘**’ P < 0.01, or ‘***’ P < 0.001.

Antisera Specificity

The specificity of CRMP2, CRMP4, and GAPDH antibodies in cat visual cortex was already demonstrated before (Cnops et al. 2004). For CRMP2 blots in which all 4 ages were simultaneously detected (Fig. 1), the film exposure time onto the blot was optimized so that especially the major 64-kDa CRMP2 band was optimally detected for all ages under investigation. From those blots, we measured only this major band because the lower 62-kDa band is age independent and the upper 66-kDa band of CRMP2 is hardly detectable in adult cat visual cortex (Cnops et al. 2004). In blots where kittens of the same age (P30, MD, and BD) were analyzed (Figs 3 and 5), we could simultaneously detect and measure all posttranslationally modified CRMP forms (66, 64, and 62 kDa) at the used protein concentration.

For Dyn I and Syt I, we detected one specific immunoreactive band in cat visual cortex. The size of 65 kDa for Syt I and 100 kDa for Dyn I was comparable to what has been described for rodent, chicken, and human (SCaife and Margolis 1990; Bergmann et al. 1999; Noakes et al. 1999; Yao et al. 2003). In addition, control experiments in which we omitted primary or secondary antibodies resulted in a blank lane (data not shown).

In Situ Hybridization

The hybridization experiments on kitten sections containing primary visual area 17 were performed exactly the same as described before (Cnops et al. 2004). The zif268 oligonucleotide was complementary to nucleotides 3–47 of the rat zif268 sequence (Acc. no.: AY551092, Wisden et al. 1990) and radioactive labeled with 35P-DATP. The specificity of the oligonucleotide probe in cat visual system and control experiments were fully described elsewhere (Zhang et al. 1994,
1995; Arckens et al. 2000). All experiments were carried out on 3 kittens per condition. The P30, MD, and BD kittens were simultaneously analyzed in one experiment and detected onto the same autoradiographic film to exclude interfilm or interexperimental variability.

Real-time Polymerase Chain Reaction

RNA extraction was performed with the Quick Prep Micro mRNA kit (GE Healthcare). After biophotometric analysis (Eppendorf, VWR International, Leuven, Belgium), identical quantities of mRNA samples from P30 and MD kittens were reverse transcribed. Primers and probes were designed with Primer Express program (Applied Biosystems, Foster City, CA), based on the partial cat sequence of crmp2 (Acc. no.: AY785162), syt I (Acc. no.: DQ186664), or gapdh (Acc. no.: AB038241) (Table 1). Primers were produced by Eurogentec (Seraing, Belgium), and FAM-TAMRA TaqMan probes were purchased from Applied Biosystems. cDNAs were subjected to polymerase chain reaction (PCR) utilizing the ABI Prism 7000 Sequence Detection System in a 25-µL reaction volume of 1× Abgene Absolute QPCR Mix (Westburg, Leusden, The Netherlands) with primers at final concentration of 300 nM and probes of 200 nM. Serial dilutions of control cDNA for generating standard curves were run in duplicate for each gene, whereas target samples were run in triplicate on the same well-plate under standard amplification settings (1 cycle of 50 °C for 2 min, 1 cycle of 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 1 min). Analysis of the results was executed with ABI Prism 7000 SDS software (version 1.1). Quantities were normalized to the endogenous control (gapdh) to account for variability in the initial concentration of mRNA samples and to compensate differences in conversion efficiency of the reverse transcriptase reaction. The relative amount of transcript was quantified by the relative standard method. To confirm reproducibility, we performed real-time PCR analysis on each cat (n = 3) at least 2 times. Statistical differences of experimental data were measured with the unpaired t-test.

Results

In-depth investigation of the developmental protein expression profiles of the 4 molecules of interest was done by western blot analysis. Visual cortex samples of cats from 4 different ages (P10, P30, Juv, and Ad) were studied (Fig. 1A). All 4 proteins demonstrated a specific developmental course. Whereas the intensity of the 64-kDa bands of CRMP2 and CRMP4 decreased during maturation, Dyn I protein levels in area 17 gradually increased with age. The Syt I signal augmented during early postnatal ages, peaked in juveniles, and declined thereafter (Fig. 1A).

Upon quantification of all data (Fig. 1B), adult CRMP2 expression levels were found to be approximately 60% of those at P10, and juvenile and adult samples displayed equal CRMP2 levels. The decrease of CRMP4 toward adulthood was more pronounced and showed an explicit drop between P30 and the control (Fig. 1B).
juvenile stage (59%) (Fig. 1B). Dyn I levels increased gradually by 40% from P10 toward the juvenile state and remained constant thereafter. On the other hand, Syt I demonstrated very low levels at P10 and increased almost 70% toward P30. Strongest Syt I protein expression was observed in juveniles, whereas in adult subjects, the Syt I expression again decreased about 55% (Fig. 1B).

To investigate the influence of visual experience on the developmental expression of the 4 molecules, we compared their expression levels in 3 animal models (Fig. 2). Control subjects could normally experience the environment through both eyes from the moment of eye opening until P30 (Fig. 2A). In MD kittens, the left eye was deprived from pattern vision from the time of eye opening (P10) until P30 by means of a linen eye patch that only allowed the detection of differences in light intensity but no accurate form perception (Fig. 2B). BD kittens underwent rearing with a similar mask but that covered both eyes from P10 until P30 (Fig. 2C).

To assess the effect of mask rearing on the activity levels in area 17, we performed in situ hybridization experiments for zif268, a marker for neuronal activity. In control subjects, all layers of area 17 except layer I and layer V labeled positive for zif268 mRNA over the full extent of area 17 (Fig. 2D). In contrast, monocular deprivation resulted in the detection of alternating bands of higher and lower zif268 mRNA expression over all layers of area 17, visualizing the diminished neuronal activity in the ocular dominance columns related to the closed eye (Fig. 2E). In BD kittens (Fig. 2F), the zif268 transcript gave a lower hybridization signal especially in the upper part of layer IV and in layer VI in comparison to normal P30 kittens. As in control kittens, layer IVc of BD kittens’ visual cortex remained highly labeled for zif268.
The possible involvement of CRMP2, CRMP4, Dyn I, and Syt I in plasticity-related reorganization of area 17 as a result of monocular deprivation was then examined with western blotting (Fig. 3). We compared protein levels of area 17 of normal controls (P30; \( n = 3 \)) with the visual cortex ipsilateral and contralateral to the deprived eye of age-matched MD kittens (\( n = 3 \)). Western blot analysis established that CRMP2 expression revealed a statistically significant reduction of the posttranslationally modified 62-kDa form upon monocular deprivation (Fig. 3A, B). As well in the ipsilateral as contralateral visual cortex of MD kittens, this band almost disappeared. The other CRMP2 bands of 66 kDa and 64 kDa remained unaffected (Fig. 3A). Changes in expression after monocular deprivation were detected neither for CRMP4 nor for Dyn I (Fig. 3A, B). In contrast, Syt I expression was significantly downregulated after monocular deprivation in the visual cortex ipsilateral to the deprived eye (Fig. 3A, B).

To evaluate if the expression changes after monocular deprivation could also be observed at the transcriptional level, we performed real-time PCR for crmp2 and syt I in MD kittens with their levels in normal kitten for comparison (Fig. 4). For both crmp2 and syt I mRNA, no obvious expression differences were observed in the normal visual cortex versus the ipsi- or contralateral visual hemisphere of MD kittens. All together our data point toward posttranslational and posttranscriptional regulation of CRMP2 and Syt I expression in area 17 (Figs 3 and 4).

Because our monocular deprivation paradigm included 2 factors that could induce the observed molecular changes, namely visual deprivation and reduced binocular competition, we further investigated the impact of the removal of pattern vision alone. To this end, western blot analysis was performed on area 17 of kittens with both eyes deprived from normal visual experience. The expression of neither CRMP2 nor Syt I was affected in BD kittens compared with control animals (Fig. 5).

The intensity of the immunoreactive bands of both molecules

Table 1

| Gene  | Forward primer (5'−3') | Reverse primer (5'−3') | TaqMan probe (5'−3') |
|-------|------------------------|------------------------|----------------------|
| crmp2 | aagaagggaactgtggtgtatggt | tccagtagtgagccacatca | agcccagggaggagctgg |
| gapdh | tggaaagcccatcaccatct    | ccaggagaggacgctcccac | ccaggagaggacgctcccac |
| syt I | cgtttctccaagcatgacatc   | tcctctccgacgcttca    | tcctctccgacgcttca |

Figure 3. Effect of monocular deprivation on the protein expression of CRMP2, CRMP4, Dyn I, and Syt I. Western blots (A) and graph bars (B) indicate that the expression of CRMP2 and Syt I, but not of CRMP4 and Dyn I, was affected by monocular deprivation. For CRMP2, only the posttranslationally modified 62-kDa form was influenced and almost disappeared in both the left, ipsilateral (I) and right, contralateral (C) visual cortex of MD kittens (respectively: MD I, MD C) in which the left eye was MD. We show a saturated blot for CRMP2 with a long exposure time that could not be used for quantification of the 64-kDa band, but indicated better the disappearance of the 62-kDa band. For Syt I, only major changes in the ipsilateral visual cortex of MD kittens were observed. Changes were indicated to be significant by \( *P < 0.05 \).
was similar in both experimental conditions (Fig. 5), implicating binocular competition as the trigger for the observed CRMP2 and Syt I expression differences upon monocular deprivation.

Discussion

Molecular Evaluation of the Effect of Mask Rearing on Neuronal Activity in Area 17

IEG like zif268 or c-fos have been frequently used to mark sensory stimulated neurons in the mammalian visual cortex (Worley et al. 1991; Rosen et al. 1992; Chaudhuri and Cynader 1993; Zhang et al. 1994; Arckens et al. 2000). Here we successfully applied the activity marker zif268 to assess changes in activity levels as a consequence of the removal of pattern vision by monocular or binocular mask rearing. Under normal visual stimulation conditions, zif268 labeled all layers of area 17 except layer I and V as previously demonstrated by immunocytochemical zif268 staining in young cats (Kaplan et al. 1996). The zif268 labeling of area 17 of MD kittens typically resulted in a columnar pattern of alternating signal intensity over all cortical layers, comparable to results obtained for zif268 transcript and protein in primate visual cortex (Chaudhuri and Cynader 1993; Kaczmarek et al. 1999) and for Fos protein in cat (Mitchell et al. 1995; Van der Gucht et al. 2000). Basically, the ocular dominance columns are detected because the deprived eye loses most of its ability to drive visual cortex neurons, shifting the distribution of visual responses to favor the nondeprived eye (Wiesel and Hubel 1963).

Our experiments further demonstrated that the reduction of binocular visual input by mask rearing resulted in a decreased zif268 signal in area 17, especially in layer IVa,b and in layer VI. Cats binocularly deprived from patterned visual experience contain more unresponsive and monocularly driven cells in area 17 than their normal counterparts (Mower et al. 1981; Sherman and Spear 1982; Michalski et al. 1983). Layer IV and VI are the main input layers of the primary visual cortex, and accordingly, we detected reduced zif268 signals particularly along those layers. In contrast, dark rearing has been shown to decrease zif268 mRNA and protein levels over all cortical layers, except layer IVc (Rosen et al. 1992; Kaplan et al. 1996). The lack of a clear effect of binocular mask rearing on layer IVc is thus consistent with observations in dark-reared kittens (Kaplan et al. 1996). Also in adult cats with retinal lesion, zif268...
mRNA levels resist the manipulation only in layer IVc (Arckens et al. 2000).

We can conclude that zif268 expression levels in area 17 thus clearly reflect the partial versus complete nature of the removal of visual input in our monocular and binocular deprivation paradigms. Apparently, mask rearing is thus a good experimental model for amblyopia.

A Role for Molecules in Area 17 before, during, and after the Critical Period

The molecular machinery in the visual cortex is crucial for its developmental plasticity. Indeed, the blockade of protein synthesis by infusion of a specific inhibitor cycloheximide prevents ocular dominance plasticity in the visual cortex as shown in mice (Taha and Stryker 2002). The N-methyl-D-aspartate receptor (NMDAR) is a typical example of a cortical “plasticity gene” as its developmental expression follows the course of the critical period (Daw 1994; Chen et al. 2000) and the NMDAR1 subunit expression is decreased after monocular deprivation (Murphy et al. 2004). By studying the postnatal expression of molecules, we thus get a better view on their role before, during, and after the critical period when visual experience can dramatically influence the structure of the visual cortex. Together with differential gene expression levels, also the amount of visual activity in both eyes that reaches the cortex is important for the experience-dependent development of the visual cortex (Hubel and Wiesel 1963; Shatz 1990; Katz and Shatz 1996).

We demonstrated that CRMP2 and CRMP4 levels are high early in life (P10 and P30) when anatomical and structural rearrangements are necessary for the normal buildup of the visual cortex, in line with a role for these molecules in neuronal differentiation and dendritic and axonal guidance (Byk et al. 1996; Kamata et al. 1998; Polleux et al. 2000). Their expression, however, cannot be strictly regulated by visual experience because it was already high at eye opening. Also the formation of ocular dominance columns, in contrast to what was believed for a long time, starts experience independently because clustering of lateral geniculate nucleus (LGN) arbors occurs at least a week before the critical period as recently demonstrated by optical imaging techniques (Cairr et al. 2001; Feller and Scanziani 2005). In this so-called “precritical period” (Feller and Scanziani 2005), spontaneous activity already shapes the visual cortex connectivity and visual experience has then little influence on the cortical organization (Katz and Shatz 1996; Bence and Levelt 2005). CRMPs may thus, together with other genes, form a molecular cascade that drives the growth of axons and dendrites in the developing cortex during this experience-independent time window.

Monocular deprivation influenced the posttranslational modification of CRMP2 as no mRNA changes were detected with real-time PCR and only the 62-kDa CRMP2 protein band was significantly affected by this manipulation. These observations probably reflect modifications in the activity status of CRMP2, for instance by (de)phosphorylation or (de)glycosylation. CRMP2 glycosylation has indeed been reported to block CRMP2 phosphorylation, thereby regulating its activity (Cole and Hart 2001). Also other studies demonstrate the involvement of posttranslationally modified CRMP2 forms in Alzheimer-diseased brain (Yoshida et al. 1998; Gu et al. 2000; Castegna et al. 2002) or Down syndrome (Lubec et al. 1999; Weitzdoerfer et al. 2001).

Important structural changes that accompany ocular dominance plasticity are rearrangements of LGN axonal arbors and intracortical afferents, the regulation of retraction of branches, followed by ingrowth of other axonal branches. Those processes are largely regulated not only by chemoaffinity but also by activity (McAllister et al. 1996; Bence and Levelt 2005). We suggest that CRMP2 could induce those reorganization processes in MD kittens. After all, the expression of CRMP2 is also modulated during regeneration of the olfactory nerve after axotomy and bulbectomy (Pasterkamp et al. 1998). Furthermore, as a substrate of CaM kinase II, CRMP2 could be involved in synaptic plasticity (Charrier et al. 2003).

The strong downregulation of CRMP4 with visual cortex maturation correlates to the fact that the closure of the critical period is accompanied by the formation of perineuronal nets through extracellular matrix proteins like chondroitin sulfate proteoglycans that surround predominantly parvalbumin (PV)-containing interneurons (Guimaraes et al. 1990; Hockfeld et al. 1990; Lander et al. 1997; Dityatev and Schachner 2003; Hensch 2005). CRMP4 is known to react with chondroitin sulfates (Franken et al. 2003) and is colocalized with PV in adult cat visual cortex (Cnops et al. 2006). The perineuronal nets form a mesh, which holds secreted proteins like SemA3 (Dityatev and Schachner 2003; Bence and Levelt 2005), possibly resulting in a selective downregulation of CRMP4, one of the intracellular mediators of the SemA3-signaling cascade. Moreover, exactly these PV-containing interneurons seem to be the most important players in regulating the critical period closure (Berardi et al. 2004; Bence and Levelt 2005; Hensch 2005). Thus, reduced CRMP4 expression in normal juvenile and adult correlates well to the stabilization of the network connectivity in the maturing visual cortex by extracellular matrix proteins. Moreover, the fact that CRMP4, in contrast to CRMP2, was not affected by monocular deprivation could probably be related to the cell type-specific expression of CRMP2 in large pyramidal neurons and of CRMP4 in those small PV-positive interneurons (Cnops et al. 2006).

The expression profile of Dyn I shows a clear relationship with its function in the maintenance of the adult cell shape (Nakata et al. 1991; Faire et al. 1992; Noda et al. 1993) in full agreement with previous results obtained in rodent studies. Dyn I mRNA and protein is not expressed in prenatal rat cortex, cerebellum, or hippocampus and is preferentially upregulated from P7 with a principal increase between P7–P15 and P23 that is maintained into adulthood (Nakata et al. 1991; Faire et al. 1992). Its developmental course thus parallels neuronal maturation (Liu and Robinson 1995) after axonal growth and synapse formation are already established (Faire et al. 1992; Powell and Robinson 1995). Dyn I seems not involved in developmental or plasticity-related growth processes in cat visual cortex, as demonstrated here in the MD kitten model. Because Dyn I is suggested to be a microtubule-associated motor protein (Shpeter and Vallee 1989), it is more reasonable to believe that Dyn I stabilizes the microtubule cytoskeleton and modulates the dynamics of actin filaments during endocytosis of mature neurons (Faire et al. 1992; Schafer 2002). Indeed, increasing levels of Dyn I during visual cortex development correspond to the maturation of the neurotransmission system. Once stable synaptic contacts are established, Dyn I-regulated clathrin-mediated endocytosis and vesicle recycling (van der Bieck et al. 1993; Sontag et al. 1994; Urrutia et al. 1997) would then be required for continuous cell-to-cell communication.
Syt I is the only molecule of which the developmental expression profile readily followed the time course of the critical period. In the first phase of the postnatal development, Syt I expression was clearly experience-dependently regulated. Around eye opening (P10), very low levels were detected, but at the height of critical period for monocular deprivation (P30), Syt I levels augmented enormously. Syt I protein expression did not immediately decrease afterward. In juvenile cats, Syt I demonstrated an even more abundant expression, whereas in adults, the expression had declined below P30 levels. Also in rat neocortex, Syt I expression increased during development with a peak around P15–P20 where after the expression again decreased toward the adult state (Bertone et al. 1997). Because Syt I is an abundant synaptic vesicle protein and essential in mediating calcium-triggered neurotransmitter release (Littleton and Bellen 1995; Ullrich and Südhof 1995; Südhof and Rizo 1996), it is not unlikely that Syt I detects a calcium sensor (Littleton and Bellen 1995; Ullrich and Südhof 1996). These effects of monocular deprivation (Cynader et al. 1980). In addition, Winfield (1983) describes that the number of synapses increases during development of the cat visual cortex with a maximal density of synapses between P70 and P110 where after the synaptic density decreases toward adulthood. Also between P8 and P37, an increase in the amount of synapses is observed (Daw et al. 1995 which in turn correlates to the initial increase of Syt I in the visual cortex. Thus, increased Syt I levels correspond well with the boost of synaptic contacts and the correlated increase of neurotransmitter release by Syt I–mediated calcium-triggered neurotransmitter release (Schwab et al. 2001). Because the postnatal visual cortex still undergoes structural changes, upregulation of Syt I protein expression by visual experience possibly engages refinements in synaptic contacts. Also in the chick retinotectal system, Syt I expression correlates well with the period of axon growth and synaptogenesis (Bergmann et al. 1999, 2000). Furthermore, Syt I triggers synchronous and suppresses asynchronous neurotransmitter release (Yoshihara and Littleton 2002; Yoshihara et al. 2003). This could be important for the segregation of LGN afferents in eye-specific columns. The high Syt I expression levels at juvenile ages have never been reported before. Even if experience-dependent ocular dominance plasticity at that age is already reduced, it is known that around the age of 5 months, extragranular cortical layers are still susceptible to changes in visual input, whereas layer IV is not plastic any more (Daw et al. 1992; Daw 1994; Katz and Shatz 1996; Beaver et al. 2001). Furthermore, at the age of 4–6 months, the kitten visual cortex is still susceptible to effects of monocular deprivation (Cynader et al. 1980). In addition, Winfield (1983) describes that the number of synapses increases during development of the cat visual cortex with a maximal density of synapses between P70 and P110 where after the synaptic density decreases toward adulthood. Also between P8 and P37, an increase in the amount of synapses is observed (Daw 1995) which in turn correlates to the initial increase of Syt I in the visual cortex. Thus, increased Syt I levels correspond well with the boost of synaptic contacts and the correlated increase of neurotransmitter release by Syt I-regulated exocytosis.

Upon monocular deprivation, diminished connectivity is detected by reduced size and number of presynaptic terminals, mitochondria, and spines (Tiemann 1991). Syt I levels, which showed a clear experience-dependent expression pattern during the first stages of normal cat visual cortex development, were decreased after monocular deprivation, probably in correlation with the reduction of synaptic contacts. As a calcium sensor (Littleton and Bellen 1995; Ullrich and Südhof 1995; Südhof and Rizo 1996), it is not unlikely that Syt I detects activity changes in the presynaptic compartment.

In contrast to our results, Silver and Stryker (1999) demonstrated that the expression of Syt I protein, among other synaptic vesicle proteins, was not affected in layer IV of kitten primary visual cortex by 2 days or 7 days of monocular deprivation. They detected no changes in Syt I distribution in deprived or nondeprived ocular dominance columns, indicating that monocular deprivation had no effect on the synapse density of geniculocortical axons, in spite of the reduced number of synapses in deprived-eye arbors (Silver and Stryker 1999, 2000). In the present study, however, a longer deprivation period and all cortical layers of area 17 were analyzed, which could explain why we observed a significant decrease of Syt I protein in MD kittens. The discrepancy between Syt I protein and RNA expression as we observed could be explained by a fast turnover activity of mRNA to protein. And, mismatches in mRNA and protein expression levels do occur (Anderson and Seilhamer 1997; Gygi et al. 1999), as reported for brain derived neurotrophic factor (BDNF) in dark-reared and age-matched normal animals (Pollock et al. 2001). Furthermore, the reduction of Syt I expression was more pronounced in the hemisphere ipsilateral to the deprived eye corresponding to the biased input of the contralateral open eye (Hubel and Wiesel 1962; Shatz and Stryker 1978; Daw et al. 1992; Tycshen and Burkhalter 1997; Clair et al. 1998). The same hemisphere-related effect was seen for the β1-adrenergic receptor of which the expression was enhanced in the kitten visual cortex ipsilateral to the deprived eye (Nakadate et al. 2001).

Conclusion
Disruption of normal binocular vision early in life affects the maturation of the cortical circuit and often results in the development of poor visual acuity known as amblyopia (Birch et al. 1998). We here demonstrated that changes in the expression of CRMP2 and Syt I are correlated to competitive mechanisms as induced by monocular deprivation but not by binocular deprivation which is a first step to a better understanding of the molecular mechanisms that underlie the development of amblyopia.

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References
Anderson L, Seilhamer J. 1997. A comparison of selected mRNA and protein abundances in human liver. Electrophoresis. 18:533–537.
Arckens L, Van der Gucht E, Eysel UT, Orban GA, Vandesande E. 2000. Investigation of cortical reorganization in area 17 and sinue extrastriate visual areas through the detection of changes in immediate early gene expression as induced by retinal lesions. J Comp Neurol. 425:531–544.
Beaver CJ, Ji Q, Daw ND. 2001. Layer differences in the effects of monocular vision in light- and dark-reared kittens. Vis Neurosci. 18:811–820.
Bence M, Leveilly CN. 2005. Structural plasticity in the developing visual system. Prog Brain Res. 147:125–139.
Dityatev A, Schachner M. 2003. Extracellular matrix molecules and plasticity in the retinotectal system. J Histochem Cytochem. 47:1297–1306.

Bergmann M, Grabs D, Rager G. 1999. Developmental expression of dynamin in the chick retinotectal system. J Histochem Cytochem. 47:1297–1306.

Bergmann M, Grabs D, Rager G. 2000. Expression of presynaptic proteins is closely correlated with the chronotopic pattern of axons in the retinotectal system of the chick. J Comp Neurol. 418:361–372.

Birch EE, Stager D, Effler J, Weakley D. 1998. Early treatment of congenital unilateral cataract minimizes unequal competition. Invest Ophthalmol Vis Sci. 39:1560–1566.

Burkert K, Stiers P, Arcenni L, Vandenbussche E, Zernicki B. 2005. Global form perception in cats early deprived of pattern vision. Neuropeport. 16:751–754.

Burnet K, Vandenbussche E, Zernicki B. 2002. Global motion detection is impaired in cats deprived early of pattern vision. Behav Brain Res. 134:59–65.

Byk T, Dobransky T, Ciuffi-Ciussi D, Sobel A. 1996. Identification and molecular characterization of unc-33-like phosphoprotein (Ulip), a putative mammalian homolog of the axonal guidance-associated unc-33 gene product. J Neurosci. 16:688–701.

Byk T, Ozen S, Sobel A. 1998. The Ulip family phosphoproteins, common and specific properties. Eur J Biochem. 254:14–24.

Castejón A, Aksenov M, Thongboonkerd V, Klein JB, Pierce WM, Booze R, Marksberry WR, Butterfly DA. 2002. Proteomic identification of oxidatively modified proteins in Alzheimer’s disease brain. Part II: dihydropyrimidinase-related protein-2, α-enolase and heat shock cognate 71. J Neurochem. 82:1524–1532.

Charrier E, Reibel S, Rogemond V, Aguera M, Thomasset N, Honnorat J. 2003. Collapsin response mediator proteins (CRMPs) involvement in nervous system development and adult neuroregenerative disorders. Mol Neurobiol. 28:51–64.

Chaudhuri A, Cynader MS. 1993. Activity-dependent expression of the transcription factor Zif268 reveals ocular dominance columns in monkey visual cortex. Brain Res. 605:349–353.

Chen L, Cooper NG, Mower GD. 2000. Developmental changes in the expression of NMDA receptor subunits (NR1, NR2A, NR2B) in the cat visual cortex and the effects of dark rearing. Brain Res Mol Brain Res. 78:196–200.

Cnops L, Hu TT, Burkert K. Van der Gucht M, Thomasset N, Honnorat J. 2003. Collapsin response mediator proteins (CRMPs) involvement in nervous system development and adult neuroregenerative disorders. Mol Neurobiol. 28:51–64.

Cole RN, Hart GW. 2001. Cytosolic O-glycosylation in abundant in nerve terminals. J Neurochem. 79:1080–1089.

Crair MC, Gillespie DC, Stryker MP. 1998. The role of visual experience in the functional architecture in the cat’s visual cortex. J Physiol. 510:143–154.

Crair MC, Antonini A, Stryker MP. 2001. Emergence of ocular dominance columns in the cat visual cortex. J Neurosci. 21:134:519–1438.

Kaczmarek L, Zangenehpour S, Chaudhuri A. 1999. Sensory regulation of immediate-early genes c-fos and zif268 in monkey visual cortex at birth and throughout the critical period. Cereb Cortex. 9:179–187.

Kennedy MJ, Ehlers MD. 2006. Organelles and trafficking machinery for postsynaptic plasticity. Annu Rev Neurosci. 29:325–351.

Kossut M, Michalski A, Zernicki B. 1978. The ocular following reflex in cats deprived of pattern vision from birth. Brain Res. 141:77–87.

Lander C, Kind P, Maleski M, Hockfield S. 1997. A family of activity-dependent neuronal cell-surface chondroitin sulfate proteoglycans in cat visual cortex. J Neurosci. 17:1928–1939.

LeVay S, Stryker MP, Shatz CJ. 1978. Ocular dominance columns and their development in layer IV of the cat’s visual cortex: a quantitative study. J Comp Neurol. 179:223–244.

Littleton JT, Bellen HJ. 1995. Synaptotagmin controls and modulates synaptic-vesicle fusion in a Ca(2+)-dependent manner. Trends Neurosci. 18:177–183.

Liu JP, Robinson PJ. 1995. Dynamin and endocytosis. Endocr Rev. 16:590–607.

Lubec G, Nonaka M, Krapfenbauer K, Glaser M, Cairns N, Fountoulakis M. 1999. Expression of the dihydropyrimidinase related protein 2 (DRP-2) in Down syndrome and Alzheimer’s disease brain is downregulated at the mRNA and dysregulated at the protein level. J Neural Transm. 57:161–177.

Faire K, Trent F, Tepper JM, Bonder EM. 1992. Analysis of dynamin isoforms in mammalian brain: dynamin-1 expression is spatially and temporally regulated during postnatal development. Proc Natl Acad Sci USA. 89:8376–8380.

Feller MB, Scanziani M. 2005. A precritical period for plasticity in visual cortex. Curr Opin Neurobiol. 15:94–100.

Franken S, Junghans U, Rosslenbroich V, Baader SL, Hoffmann R, Gieselmann V, Viebahn C, Konnerth A, Karschin A, Basler K, Kimura T. 2000. Functions of semaphorins in axon guidance and neuronal regeneration. Jpn J Pharmacol. 82:273–279.

Guimaraes A, Zaremba S, Hockfield S. 1997. Molecular and morphological changes in the cat lateral geniculate nucleus and visual cortex induced by visual deprivation are revealed by monoclonal antibodies Cat-304 and Cat-301. J Neurosci. 17:3014–3028.

Goyet SP, Aronon F, Frank SB, Acrivos RD. 1999. Correlation between protein and mRNA abundance in yeast. Mol Cell Biol. 19:1720–1730.

Hensch TK. 2005. Critical period plasticity in local cortical circuits. Nat Rev Neurosci. 6:877–888.

Hockfield S, Kalb RG, Zaremba S, Fryer H. 1990. Expression of neural proteoglycans correlates with acquisition of mature neuronal properties in the mammalian brain. Cold Spring Harbor Symp Quant Biol. 55:505–513.

Hubel DH, Wiesel TN. 1962. Receptive fields, binocular interaction and functional architecture in the cat’s visual cortex. J Physiol. 160:106–154.

Hubel DH, Wiesel TN. 1963. Receptive fields of cells in striate cortex of very young, visually inexperienced kittens. J Neurophysiol. 26:994–1002.

Hubel DH, Wiesel TN. 1970. The physiological period of the susceptibility effects of unilateral eye closure in kittens. J Physiol. 206:519–1438.

Kennedy MJ, Ehlers MD. 2006. Organelles and trafficking machinery for postsynaptic plasticity. Annu Rev Neurosci. 29:325–362.

Kemp T, Subleski M, Hara Y, Yahagi K, Naka K, Copeland NG, Jenkins NA, Yoshimura T, Modl W, Copeland NT. 1998. Isolation and characterization of a bovine neural specific protein (CRMP2) cDNA homologous to unc-33, a C elegans gene implicated in axonal outgrowth and guidance. Brain Res Mol Brain Res. 54:219–239.

Kaplan IV, Guo Y, Mower GD. 1996. Immediate early gene expression in cat visual cortex during and after the critical period: differences between EGR-1 and Fos proteins. Brain Res Mol Brain Res. 36:12–22.

Kat LC, Shatz CJ. 1996. Synaptic activity and the construction of cortical circuits. Science. 274:1133–1138.

Katz LC, Shatz CJ. 1996. Synaptic activity and the construction of cortical circuits. Science. 274:1133–1138.

Kennedy MJ, Ehlers MD. 2006. Organelles and trafficking machinery for postsynaptic plasticity. Annu Rev Neurosci. 29:325–362.

Kessels M, Michalski A, Zernicki B. 1978. The ocular following reflex in cats deprived of pattern vision from birth. Brain Res. 141:77–87.

Lander C, Kind P, Maleski M, Hockfield S. 1997. A family of activity-dependent neuronal cell-surface chondroitin sulfate proteoglycans in cat visual cortex. J Neurosci. 17:1928–1939.

LeVay S, Stryker MP, Shatz CJ. 1978. Ocular dominance columns and their development in layer IV of the cat’s visual cortex: a quantitative study. J Comp Neurol. 179:223–244.

Littleton JT, Bellen HJ. 1995. Synaptotagmin controls and modulates synaptic-vesicle fusion in a Ca(2+)-dependent manner. Trends Neurosci. 18:177–183.

Liu JP, Robinson PJ. 1995. Dynamin and endocytosis. Endocr Rev. 16:590–607.

Lubec G, Nonaka M, Krapfenbauer K, Glaser M, Cairns N, Fountoulakis M. 1999. Expression of the dihydropyrimidinase related protein 2 (DRP-2) in Down syndrome and Alzheimer’s disease brain is downregulated at the mRNA and dysregulated at the protein level. J Neural Transm. 57:161–177.
McAllister AK, Katz LC, Donald CL. 1996. Neurotrophin regulation of cortical dendritic growth requires activity. Neuron. 17:1057–1064.

Michalski A, Kossut M, Chmielowska J. 1983. Responses of area 17 neurons in cats binocularly deprived by rearing in hoods. Acta Neurobiol Exp. 43:263–272.

Minturn JE, Fryer HJL, Geschwind DH, Hoﬀeld S. 1995. TOAD-64, a gene expressed early in neuronal diﬀerentiation in the rat, is related to unc-33, a C. elegans gene involved in axon outgrowth. J Neurosci. 15:6757–6766.

Mitchell DE, Beaver CJ, Ritchie PJ. 1995. A method to study changes in eye-related columns in the visual cortex of kittens during and following early periods of monocular deprivation. Can J Physiol Pharmacol. 73:1352–1363.

Mower GD, Berry D, Burchﬁel JL, Duffy FH. 1981. Comparison of the eﬀects of dark rearing and binocular suture on development and plasticity of cat visual cortex. Brain Res. 220:255–267.

Murphy KM, Duffy KR, Jones DG. 2004. Experience-dependent changes in NMDAR1 expression in the visual cortex of an animal model for amblyopia. Vis Neurosci. 21:653–670.

Nakadate K, Imamura K, Watanabe Y. 2001. Eﬀects of monocular deprivation on the expression pattern of alpha-I and beta-I adrenergic receptors in kitten visual cortex. Neurosci Res. 40:155–162.

Nakata T, Iwamoto A, Noda Y, Takemura R, Yoshikura H, Hirokawa N. 1991. Predominant and developmentally regulated expression of dynamin in neurons. Neuron. 7:461–469.

Napolitano M, Marﬁa GA, Vacca A, Centonze D, Bellavia D, Di Marcotullio L, Fratti L, Bernardi G, Gulino A, Calabresi P. 1999. Modulation of gene expression following long-term synaptic depression in the striatum. Mol Brain Res. 72:89–96.

Noakes PG, Chin D, Kim SS, Liang S, Phillips WD. 1999. Expression and localization of dynamin and syntaxin during neural development and neuromuscular synapse formation. J Comp Neurol. 410:513–540.

Noda Y, Nakata T, Hirokawa N. 1993. Localization of dynamin: widespread distribution in mature neurons and association with membrane organelles. Neuroscience. 55:113–127.

Pasterkamp RJ, De Winter F, Holtmaat AJ, Verhaagen J. 1998. Evidence for the role of the chemorepellent semaphorin III and its receptor neuropilin-1 in the regeneration of primary olfactory axons. J Neurosci. 18:9962–9976.

Payne BR, Peters A. 2002. The cat primary visual cortex. 6th ed. San Diego: Academic Press.

Polleux F, Morrow T, Ghosh A. 2000. Semaphorin 3A is a chemotactant for cortical apical dendrites. Nature. 404:567–573.

Pollock GS, Vernon E, Forbes ME, Yan Q, Ma YT, Hsieh T, Robichon R, Frost D, Johnson JE. 2001. Eﬀects of early visual experience and diurnal rhythms on BDNF mRNA and protein levels in the visual system, hippocampus, and cerebellum. J Neurosci. 21:3923–3931.

Powell KA, Robinson PJ. 1995. Diphosphoinositol is a neuronal phosphophoprotein concentrated in nerve terminals: evidence for rat cerebellum. Neurosci. 64:821–833.

Quinn CC, Gray GE, Hoﬀeld S. 1999. A family of proteins implicated in axon guidance and outgrowth. J Neurobiol. 41:158–164.

Rosen KM, McCormack MA. Villa-Komaroff L, Mower GD. 1992. Brief bilateral eye closure on cortical unit responses in kittens. J Neurophysiol. 26:1082–1084.

Taha S, Stryker MP. 2002. Rapid ocular dominance plasticity requires cortical but not geniculate protein synthesis. Neuron. 34:425–436.

Tieman SB. 1991. Morphological changes in the geniculocortical pathway associated with monocular deprivation. Ann NY Acad Sci. 627:212–230.

Tychsen L, Burkhalter A. 1997. Nasotemporal asymmetries in V1: ocular dominance columns of infant, adult, and strabismus macaque monkeys. J Comp Neurol. 388:32–46.

Ullrich B, Sudhof TC. 1995. Diﬀerential distributions of novel synaptotagmins: comparison to synapsins. Neuropharmacology. 34:1371–1377.

Urrutia R, Henley JR, Cook T, McNiven MA. 1997. The dynamins: redundant or distinct functions for an expanding family of related GTPases. Proc Natl Acad Sci USA. 94:377–384.

Van den Bergh G, Clerens S, Cnops I, Vandensande F, Arckens L. 2003. Fluorescent two-dimensional diﬀerence gel electrophoresis and mass spectrometry identify age-related protein expression diﬀerences for the primary visual cortex of kitten and adult cat. J Neurochem. 85:193–205.

Van den Bergh G, Clerens S, Vandensande F, Arckens L. 2003. Reverse-assay high-performance liquid chromatography pre-fractionation prior to 2D diﬀerence gel electrophoresis and mass spectrometry identifies new diﬀerentially expressed proteins between strate cortex of kitten and adult cat. Electrophoresis. 24:1471–1481.

Van der Blik AM, Redelmeier TE, Damke H, Tisdale EJ, Meyerowitz EM, Schmid SL. 1993. Mutations in human dynamin block an intermediate stage in coated vesicle formation. J Cell Biol. 122:1387–1377.

Van der Gucht E, Vandenbussche E, Orban GA, Vandesande F, Arkens L, Van den Bergh G, Clerens S, Cnops I, Vandensande F, Arckens L. 2000. A new cat Fos antibody to localize the immediate early gene expression. J Neurosci. 20:6031–6038.

Schafer DA. 2002. Coupling actin dynamics and membrane dynamics during axon guidance. Curr Opin Cell Biol. 14:76–81.

Schwab Y, Mouton J, Chasserot-Golaz S, Marty I, Maulet Y, Jover E. 2001. Calcium-dependent translocation of synapticaptomin to the plasma membrane in the dendrites of developing neurones. Mol Brain Res. 96:1–13.

Shatz CJ. 1990. Impulse activity and the patterning of connections during CNS development. Neuron. 5:745–756.

Shatz CJ, Luskin MB. 1986. The relationship between geniculocortical afferents and their cortical target cells during development of the cat’s primary visual cortex. J Neurosci. 6:3655–3668.
Worley PF, Christy BA, Nakabeppu Y, Bhat RV, Cole AJ, Baraban JM. 1991. Constitutive expression of zif268 in neocortex is regulated by synaptic activity. Proc Natl Acad Sci USA. 88: 5106-5110.

Yao PJ, Zhu M, Pyun EI, Brooks AI, Therianos S, Meyer VE, Coleman PD. 2003. Defects in expression of genes related to synaptic vesicle trafficking in frontal cortex of Alzheimer’s disease. Neurobiol Dis. 12:97-109.

Yoshida H, Watanabe A, Ihara Y. 1998. Collapsin response mediator protein-2 is associated with neurofibrillary tangles in Alzheimer’s disease. J Biol Chem. 273:9761-9768.

Yoshihara M, Adolfsen B, Littleton JT. 2003. Is synaptotagmin the calcium sensor. Curr Opin Neurobiol. 13:315-323.

Yoshihara M, Littleton JT. 2002. Synaptotagmin I functions as a calcium sensor to synchronize neurotransmitter release. Neuron. 36:897-908.

Zernicki B. 1991. Visual discrimination learning in binocularly deprived cats. 20 years of studies in the Nencki Institute. Brain Res Rev. 16:1-13.

Zhang F, Halleux P, Arckens L, Vanduffel W, Van Brée L, Mailleux P, Vandesande F, Orban GA, Vanderhaeghen JJ. 1994. Distribution of immediate early gene zif-268, c-fos, c-jun and jun-D mRNAs in the adult cat with special references to brain region related to vision. Neurosci Lett. 176:137-141.

Zhang F, Vanduffel W, Schiffmann S, Malleux P, Arckens L, Vandesande F, Orban GA, Vanderhaeghen JJ. 1995. Decrease of zif-268 and c-fos and increase of c-jun mRNA in the cat areas 17, 18 and 19 following complete visual deafferentation. Eur J Neurosci. 7:1292-1296.