Role of Calcineurin in Activity-Dependent Pattern Formation in the Dorsal Lateral Geniculate Nucleus of the Ferret

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ABSTRACT: In the retinogeniculate pathway of the ferret, in addition to the separation of the inputs from the two eyes to form eye-specific layers, there is also an anatomical segregation of the terminal arbors of on-center retinal ganglion cells from the terminal arbors of off-center retinal ganglion cell axons to form on/off sublaminae. Sublamination normally occurs during postnatal weeks 3–4 and requires the activity of retinal afferents, *N*-methyl-D-aspartate receptors, nitric oxide synthase, and a target of nitric oxide, cyclic guanosine monophosphate. Calcineurin is a calcium/calmodulin dependent serine, threonine protein phosphatase suggested to mediate NMDA-receptor dependent synaptic plasticity in the hippocampus. We have examined whether calcineurin plays a role during on/off sublamination in the dorsal lateral geniculate nucleus (dLGN) of the ferret. Immunohistochemistry showed that calcineurin expression is transiently up-regulated in dLGN cells and neuropil during the period of on/off sublamination. A functional role for calcineurin during sublamination was investigated by blocking the enzyme locally via intracranial infusion of FK506. Treatment with FK506 during postnatal weeks 3–4 disrupted the appearance of sublaminae. These results suggest that calcineurin may play a role during this process of activity-dependent pattern formation in the visual pathway.
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Keywords: **FK506; visual thalamus; synaptic plasticity; development; protein phosphatase 2B**

INTRODUCTION

The precise pattern of neuronal connections that underlies visual processing in adult mammals arises from the refinement of less specific connections that are present during development. The mechanisms by which the connections are refined rely, at least in part, on neuronal activity. In the ferret, retinogeniculate connections undergo two distinct phases of refinement. During the first postnatal week, retinal axons within the dorsal lateral geniculate nucleus (dLGN) segregate into eye-specific layers (Linden et al., 1981). During postnatal weeks 3–4, afferents to the A and A1 layers (which receive input from either the contralateral or ipsilateral eye, respectively) further segregate into sublaminae. The inner sublamina receives inputs from on-center retinal ganglion cells, whereas the outer sublamina receives inputs from off-center retinal ganglion cells (Hahm et al., 1991; Stryker and Zahs, 1983). Segregation of eye-specific layers in the dLGN can be disrupted by intracranial infusion of the sodium channel blocker tetrodotoxin (TTX) (Shatz and Stryker, 1988), or intravitreal ad-

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ministration of cholinergic blockers (Penn et al., 1998). On/off sublamination is also prevented by blockade of retinal afferent activity with TTX (Cramer and Sur, 1997). Furthermore, blockade within the dLGN of *N*-methyl-D-aspartate (NMDA)-receptors (Hahm et al., 1991, 1999), nitric oxide synthase (NOS) (Cramer et al., 1996; Cramer and Sur, 1999), soluble guanylyl cyclase, or protein kinase G (PKG) (Leamey et al., 2001) disrupts sublamina formation. A hypothesis consistent with these data is that NMDA receptors and signals downstream of them have a key role in retinogeniculate patterning.

It is still unclear, however, how the activation of NMDA-receptors is transformed into specific patterns of connectivity. Several studies suggest that the mechanisms responsible for the activity-dependent consolidation and refinement of connections that occurs during development have much in common with the extensively studied NMDA-receptor-dependent forms of synaptic plasticity in the hippocampus (Bear, 1996; Cramer and Sur, 1996; Katz and Shatz, 1996). In NMDA-receptor-dependent synaptic plasticity, the postsynaptic influx of Ca^{2+} binds calmodulin, which recruits several second messenger phosphatases and kinases, including calcineurin (Mulkey et al., 1994; Wang and Kelly, 1996, 1997), which is also known as protein phosphatase 2B (PP2B).

Calcineurin is a calcium and calmodulin-dependent serine/threonine protein phosphatase. There is accumulating evidence that calcineurin is a major mediator of synaptic transmission and plasticity (reviewed in Winder and Sweatt, 2001). Recent studies, using transgenic mice and antisense oligonucleotides to down-regulate calcineurin expression in the adult brain, have demonstrated that endogenous calcineurin activity constrains the induction of NMDA-receptor- dependent long-term potentiation (LTP) (Ikegami and Inokuchi, 2000; Malleret et al., 2001; Zeng et al., 2001). Conversely, down-regulation of endogenous calcineurin activity diminishes long-term depression (LTD) (Funauchi et al., 1994; Mulkey et al., 1993, 1994; Wang and Kelly, 1996, 1997; Zeng et al., 2001). Changes in performance levels on tasks that require specific forms of learning and memory were also described in these studies. It has been suggested that calcineurin, in combination with two of its associated phosphatases (PP1 and PP2A), plays a pivotal role in synaptic plasticity by setting the balance between the phosphorylation and dephosphorylation, and so provides dynamic control of physiological synaptic strength (Wang and Kelly, 1996; Winder and Sweatt, 2001).

Calcineurin has recently been reported to mediate a developmental decrease in the time course of NMDA receptor currents in the visual system of the developing rat (Shi et al., 2000). Other work has shown that calcineurin regulates the phosphorylation state of the microtubule-associated protein MAP2. This determines the interaction between MAP2, microtubules, and actin filaments, and is thus a mechanism by which neural activity may be transduced into modifications of dendritic structure (Morales and Fifkova, 1989; Nishio et al., 1995; Quinlan and Halpain, 1996). FK506, an inhibitor of calcineurin function, augments neurotransmitter release (Liu et al., 1994; Nichols et al., 1994; Steiner et al., 1996). FK506 has also been shown to enhance NOS phosphorylation, decreasing NOS activity (Bredt et al., 1992; Dawson et al., 1993; Sharkey and Butcher, 1994). Because NOS activity has been shown to be required for sublamination (Cramer et al., 1996; Cramer and Sur, 1999) the effect of FK506 on NOS activity may have particular significance for the current study. In the previous studies it was postulated that postsynaptic NOS produces NO, which can then act as a retrograde messenger to signal changes in postsynaptic activity to presynaptic structures. These signals may then regulate changes in the pattern of axonal arborizations, resulting in the formation of on/off sublaminae. It should also be pointed out, however, that other, NOS independent signaling pathways have also been suggested for calcineurin (see Discussion).

The potential functions of calcineurin in nervous system are consistent with this protein playing a role in activity-dependent synaptic plasticity and development. Here we examine whether calcineurin plays a role in the activity-dependent rearrangement of connections in the retinogeniculate pathway of the ferret. We show that there is a transient increase in calcineurin expression in both cells and neuropil during the period of on/off sublamination. Moreover, intracranial infusion of the calcineurin inhibitor FK506 disrupts the appearance of sublaminae, suggesting that calcineurin is involved in this process.

MATERIALS AND METHODS

Immunohistochemistry

Seventeen ferret kits ranging in age from postnatal day (P)0 to 7 postnatal weeks were included in this study. At least two animals were included in each age group (P0–2, P7, P14, P21–22, P28–29, P35, and P42). Kits were euthanized with sodium pentobarbital (>100 mg/kg) and perfused intracardially with 0.9% saline followed by 4% paraformaldehyde in 0.1 *M* sodium phosphate buffer (PB; pH 7.4). Tissue was postfixed overnight in 1% paraformaldehyde at 4°C, cryoprotected in 20% sucrose in PB, and sectioned in the horizontal plane at 50 μm. Sections from ferrets of different ages were reacted in parallel to ensure results were comparable across ages. Sec-

tions were washed in phosphate buffered saline (PBS) and placed in 10% normal goat serum (NGS) in PBS for 1 h before being incubated overnight in the primary antibody (affinity purified rabbit anticalcineurin pan-A from Chemicon, Temecula, CA) diluted 1:500 in PBS containing 0.5% Triton X-100, 2.5% bovine serum albumin (BSA), and 2% NGS. After rinsing, tissue was placed in a biotinylated goat antirabbit secondary antibody diluted 1:200 for 1 h, rinsed, and the reaction product developed using the Vectastain ABC elite and the Vector VIP kits as per the manufacturer's instructions. Tissue-sections were analyzed using bright-field microscopy. Cell density measurements were obtained by counting all the stained cells in one field of view from the medial, binocular, part of the dLGN viewed with a 40X objective lens. Counts focused on the A layers but also included the C layer at all ages and were made from two or more sections from at least two different animals. All cells that had nuclei visible within the plane of section and showed staining that was clearly above background were included. Soma diameter was taken as the average of the longest axis and the axis perpendicular to the long axis.

Demonstration of Antibody Specificity

The specificity of the primary polyclonal antibody was demonstrated by a peptide blocking procedure. The primary antibody incubation solution was preincubated with either 1 mg/mL synthetic, the original immunogenic peptide (AAVPFPPSHRLT), or 6 mg/mL BSA at room temperature for 3 h. The subsequent immunostaining was carried out as described above.

On/Off Sublamination

FK506 (Fujisawa, Deerfield, IL) or control vehicle solution [50% normal saline/50% dimethyl sulphoxide (DMSO)] was focally administered to the dLGN via osmotic minipumps (Alzet, Palo Alto, CA) as previously described (Cramer et al., 1996) with a slight modification. Briefly, the ferret kits (P14–15) were premedicated with atropine (0.04 mg/kg, s.c.) and then anaesthetized with 2–4% isofluorane in oxygen. An incision was made in the scalp, and a primed osmotic minipump (Alzet model 2002) filled with 0.5 m*M* FK506 or vehicle solution was inserted underneath the skin on the back of the neck. The minipump was connected to a 22 gauge cannula. A small, vertical hole was made in the cranium with a 26 gauge needle, and the cannula was inserted through the hole from the dorsal surface through the cortex and above the thalamus (2 mm posterior and 2 mm to the right of bregma). This is slightly anteromedial, but in close proximity, to the right dLGN. The issue of mechanical or surgical damage was addressed directly in control animals that were implanted with minipumps containing vehicle control solution. The cannula was first glued in place and then covered with dental acrylic and the open wound was sutured. During the postsurgical period, animals were treated prophylactically with amoxycillin. On P24–25, animals were anaesthetized as above, and an intravitreal injection of 8–9 μL of 4% horseradish peroxidase conjugated to wheat germ agglutinin (WGA-HRP) in 0.9% saline was made into the left eye. After a survival period of 26–38 h, at P25–26, animals were euthanized with sodium pentobarbital (>100 mg/kg, i.p.) and perfused intracardially with 0.9% saline followed by 1% paraformaldehyde/1.25% glutaraldehyde in 0.1 *M* PB for at least 15 min, followed by 10–15% sucrose in 0.1 *M* PB. P25–26 was the day chosen to sacrifice the animals, as our observations have found that at this time retinogeniculate axons have segregated into on/off sublaminae but the interlaminar space cannot yet be visualized in Nissl stains. Brains were removed, equilibrated in 30% sucrose in 0.1 *M* PB, and sectioned at 50 μm in the horizontal plane using a freezing microtome. Serial sections were processed using the tetramethylbenzidine (TMB) reaction to reveal HRP (Mesulam, 1978).

To assess sublamination, approximately seven sections through the middle of the dLGN along the dorsoventral axis were given scores on a scale of 0 to 3, based on the proportion of the labeled A layer, which was clearly divided by a palely stained intersublaminar zone that approximately bisected the A layer longitudinally (Cramer et al., 1996). This assessment was performed objectively by an observer who was blind as to which treatment a particular animal had received. An average score for each animal was obtained by dividing the total scores by the number of sections scored.

RESULTS

Expression of Calcineurin Subunit A in the Developing dLGN

The specificity of the polyclonal antibodies was examined by the peptide blocking procedure. Preincubation with 1 mg/mL immunogenic peptide completely blocked immunoreactivity in all brain areas examined, including the otherwise densely stained cortex [Fig. 1(B)], while 6 mg/mL BSA had little effect on immunoreactivity [Fig. 1(A)]. The rostral cortex is shown here to demonstrate the specificity of the staining, as it typically showed more intense staining than seen in other brain regions.

At P0–2, no immunoreactivity for calcineurin was detected in the dLGN [Fig. 2(A)]. By P7, some diffuse staining was seen throughout the neuropil of the nucleus [Fig. 2(B)]. There was also some staining of cell bodies, predominantly in the lateroposterior pole of the dLGN, in the C laminae adjacent to the optic tract. There was a marked increase in the degree of staining seen at P14. Many calcineurin positive cells were seen stained throughout the nucleus. There was also an increase in the amount of staining seen in the neuropil [Fig. 2(C)]. The neuropil staining demarcated the border between the A and A1 layers. Immunoreactivity for calcineurin was retained in both cells and neuropil throughout the fol-

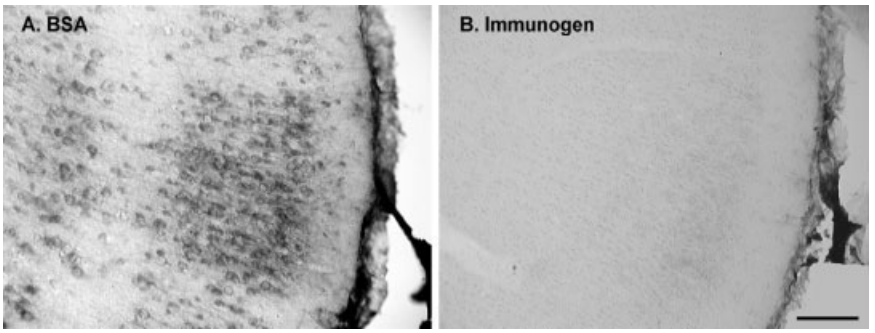

Figure 1 Specificity of antibodies for the catalytic subunit A of calcineurin. In tissue stained with antibodies that were preincubated with 6 mg/mL BSA intense labeling is present (A). In contrast, staining is absent in tissue stained with antibodies that were preincubated with 1 mg/mL immunogenic peptide staining (B). Pictures were taken at comparable cortical regions. Scale bar: 100 μm.

lowing week [Fig. 2(D)]. By P21 the division between the inner and outer sublaminae was beginning to appear in the neuropil staining. In many of the cells that were stained during this period, the axon and primary dendrites were labeled; this can be seen more clearly in the images shown at higher power in the A layer of the dLGN [Fig. 3(A)]. Labeled cells had various morphologies including bipolar and multipolar somata. Both the cellular and neuropil staining was still present at P29 although it had begun to decline in intensity. Although less intense by this stage, the neuropil staining now clearly demarcated both the eye-specific layers and on/off sublaminae [Fig. 2(E)]. The decrease observed in the cellular staining reflected not only a reduction in the number of cells that actually expressed calcineurin (see Fig. 4), but also the fact that the dendritic staining of calcineurin positive cells was reduced; by this stage the protein was expressed primarily in the somata and a little in the most proximal regions of primary dendrites, but it was not expressed along the length of the primary dendrites as seen at younger ages [Fig. 3(B)]. The decrease in neuropil staining continued through P35 (Fig. 4 and results not shown), and by P42, much of the cellular and neuropil staining had disappeared [Fig. 2(F)]. Thus, the time course of calcineurin expression in the dLGN of the ferret resembles the period of on/off sublamination.

From P14 through P28, clearly outlined cell bodies in the dLGN were labeled. Cells and neuropil were also stained in other thalamic nuclei during development, including the perigeniculate nucleus (Fig. 2). A histogram summarizing the changes in the number of stained cell bodies in the dLGN is presented in Figure 4. There was a transient increase in the density of labeled cells between the first and the third postnatal weeks; this was followed by a decline in staining such that by postnatal week 6 very few cell bodies were labeled. (Note that the density is expressed as cells per square millimeter. Because the dLGN grows substantially over the period examined, a lesser proportion of the dLGN is included at progressively later ages.) The distribution of somal diameters in the dLGN was sampled at 3, 4, and 5 weeks postnatal. At P14, soma diameters ranged from 6 to 14 μm; at P21, 8.5 to 14.5 μm; at P28, 9.5 to 20 μm. These figures closely reflect the range of cell body sizes previously reported in the dLGN during development (Rocha and Sur, 1995; Sutton and Brunso-Bechtold, 1991).

Effect of FK506 on On/Off Sublamination

In the second half of the study, a possible functional role for calcineurin during sublamination was examined. Ferrets received an intracranial infusion of either FK506 or vehicle control solution from P14–26. Following infusion of FK506 there were no observable differences in the appearance of the animals or in brain morphology compared to controls. Eye injections demonstrated that within the thalamus, retinal axons were clearly confined to the dLGN. There was no observable difference in the appearance or degree of segregation present in the eye-specific layers (Fig. 5), suggesting FK506 infusion between weeks 3–4 has no impact on the maintenance of eye-specific layers. There was, however, a pronounced difference in the degree of sublamination present in the dLGN. In control animals, a palely staining intersublaminar zone was clearly visible in horizontal sections through the dLGN [arrow in Fig. 5(B)] separating the inner (A_i) and outer (A_o) sublaminae. Because the space that will separate the sublaminae in the adult has not formed at this stage of development, the intersublaminar zone seen following bulk-labeling from the retina in control animals reflects the lack of retinogeniculate terminal arbors in this region. The intersublaminar zone was not visible in animals that had been treated

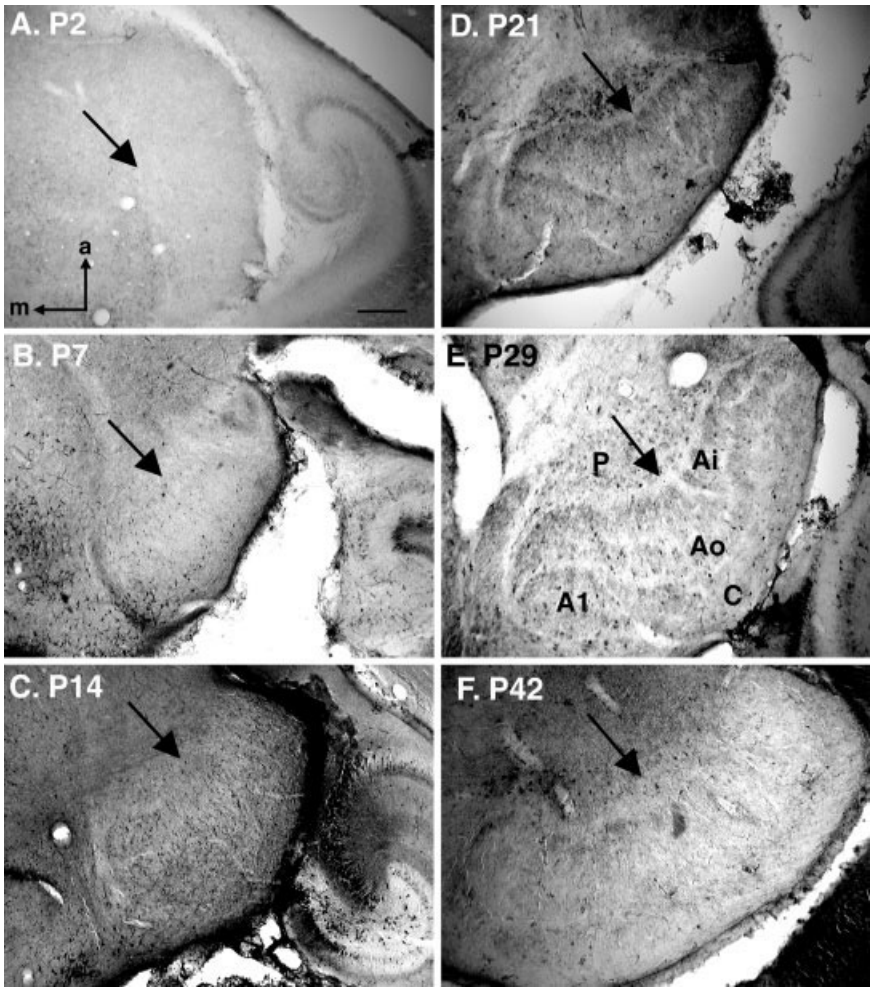

Figure 2 Expression of calcineurin A in the dLGN of developing ferrets. Horizontal sections of ferret brains of ages marked stained with antibodies specific to the catalytic A subunit of calcineurin. The arrow points to the dLGN. No immunoreactivity is seen at P2 (A). Staining is first evident at P7 (B) and becomes marked in cells and neuropil by P14 (C). Calcineurin immunoreactivity is retained through P21 (D), and is still present in cells and neuropil at P29 (E) although it has decreased by this time. Much of the cellular and neuropil staining has disappeared by P42 (F). Abbreviations: P, perigeniculate nucleus. The eye-specific layers A and A1 are evident in (C–E) and marked in (E). The on/off sublaminae of the A lamina (A_i and A_o) are also evident in (D–E) and marked in (E). Scale bar : 200 μm.

with FK506, strongly suggesting that the drug treatment disrupted afferent segregation [Fig. 5(A)]. Sublamination scores between 0 and 3 were assigned objectively to control and FK506 animals, with a score of 0 indicating no sublamination and a score of 3 indicating complete sublamination. The mean sublamination score for animals treated with FK506 (mean = 0.2; $n = 5$; S.D. = 0.15) was significantly lower (Mann-Whitney U-test, $p < 0.01$) than that seen in animals infused with vehicle control solution (mean = 1.9; $n = 6$; S.D. = 0.4). The scores for control animals were essentially the same as previously reported for normal animals (mean = 2.1; $n = 6$; Cramer et al., 1996). In FK506-treated animals, the disruption of sublamination was restricted to the infused side. Sublamination in the dLGN contralateral to the minipump appeared normal in both FK506-treated [Fig. 5(C)] and control [Fig. 5(D)] animals, confirming the local nature of drug administration.

DISCUSSION

This study has shown that there is a transient up-regulation of calcineurin immunoreactivity in both the

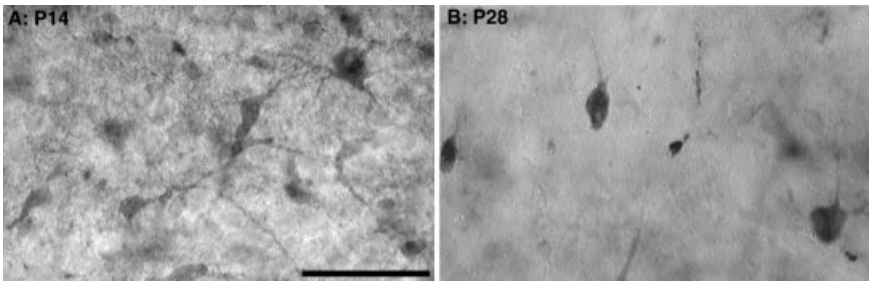

Figure 3 High power view of calcineurin staining in the A layer of the dLGN at P14 (A) and P28 (B). Staining is present in the cell bodies and processes at P14. Staining is still present in somata at P28 though dendritic staining has decreased. Scale bar: 50 μm

cells and neuropil of the dLGN of the ferret, which resembles the period of on/off sublamination. Further, infusion of the calcineurin inhibitor FK506 disrupted the appearance of sublaminae, suggesting that calcineurin may play a functional role during the formation of on/off sublaminae. To our knowledge this is the first time that a role for calcineurin has been demonstrated in the activity-dependent rearrangement of connections *in vivo*.

The range of soma diameters of calcineurin-stained cells suggests that this protein is expressed by a number of different cell types in the dLGN (Rocha and Sur, 1992; Sutton and Brunso-Bechtold, 1991). The greatest density of labeled cell bodies occurs between P14 and P21, with labeling evident from P7–28. Neuropil staining was also up-regulated during on/off sublamination (P14–28). The comparison between different ages is valid as brain sections were reacted in parallel. Strong neuropil staining demarcates the A, A1, and C layers and on/off sublaminae from P21–28, and subsequently declines. Interestingly, this pattern of neuropil staining resembles that observed following retinogeniculate labeling, suggesting the possibility that calcineurin may be expressed in retinal terminals. Further study will be required to determine the source(s) of innervation accountable for the neuropil staining seen here in the dLGN of the developing ferret. Some cellular and neuropil staining is retained in the dLGN of older ferrets (P42, Fig. 2, and results not shown).

Eye-specific laminae in ferrets are reported to arise from the refinement of more diffuse retinogeniculate projections during the first and second postnatal weeks (Linden et al., 1981). These projections are further refined during postnatal weeks 3–4 (Hahm et al., 1991, 1999), such that axonal arbors become restricted to sublaminae that contain either on- or off-center afferents and their target cells (Roe et al., 1989; Stryker and Zahs, 1983). The formation of on/off sublaminae requires the activation of retinal afferents (Cramer and Sur, 1997), NMDA-receptors (Hahm et al., 1991), and the neuronal form of NOS (Cramer and Sur, 1996); blockade of NMDA-receptors (Hahm et al., 1991) or NOS (Cramer and Sur, 1996) during this time results in retinogeniculate arbors that are inappropriately positioned in the dLGN.

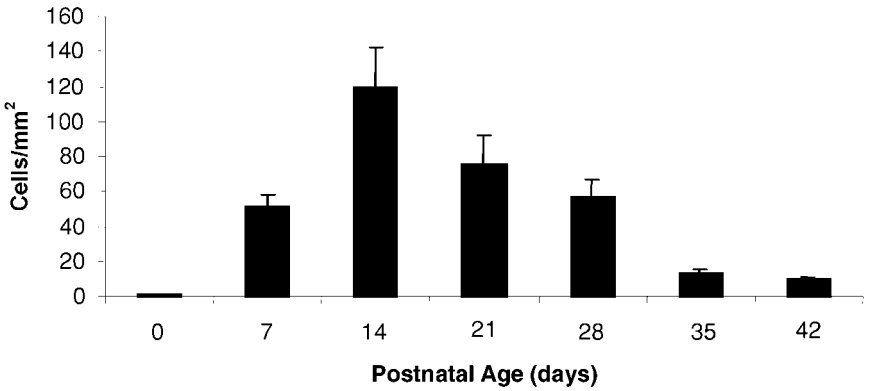

Figure 4 Density of cell bodies labeled in the ferret dLGN at ages included in the study using immunohistochemical staining with antibodies specific to calcineurin A. Error bars denote standard errors when each animal is treated as one data point; $n = 2$ at each age.

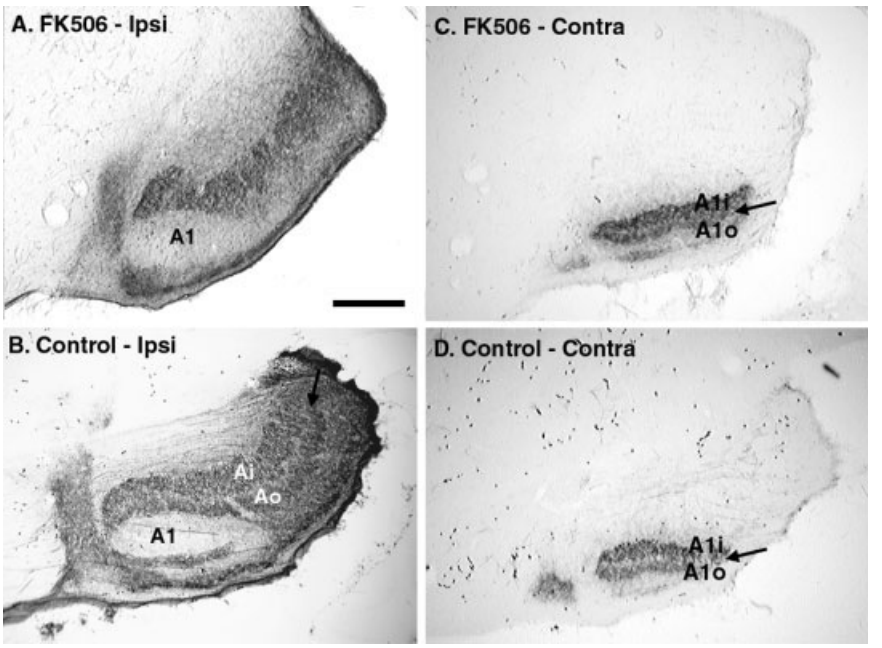

Figure 5 Horizontal sections through the dLGN of P26 ferrets that received minipump implants following anterograde transport of WGA-HRP from the eye contralateral to the minipump implantation site, viewed with bright-field microscopy. (A) Section through the dLGN of an FK506-treated animal ipsilateral to the minipump implantation site. No palely staining intersublaminar zone can be seen bisecting the A layer. (B) Section from the dLGN of a control animal treated with vehicle solution ipsilateral to the pump implantation site. A palely staining intersublaminar region (arrow) can be seen separating the inner (A_i) and outer (A_o) sublaminae. (C) Section through the dLGN of an FK506-treated animal contralateral to the minipump implantation site (and ipsilateral to the injected eye). A palely staining intersublaminar zone can be seen bisecting the A1 layer into inner ($A1_i$) and outer ($A1_o$) sublaminae (arrow). (D) As for (C) but contralateral to the pump implantation site of a control animal. A palely staining interlaminar zone (arrow) is also visible. Scale bar in (A): 150 μm, applies to (B–D).

Thus, calcineurin-A expression in the dLGN cells peaks when afferent activity, activation of NMDA-receptors, and NOS are all required for the normal refinement of presynaptic connections. In addition, the temporal pattern of calcineurin-A expression in the cells of the dLGN resembles that of NOS (Cramer et al., 1995). The expression of calcineurin within postsynaptic structures is consistent with the possibility that the effects of calcineurin could be mediated by NOS.

It has previously been shown that the application of FK506 or cyclosporin A (CsA, a calcineurin inhibitor that is structurally different from FK506) to primary cortical cultures results in enhanced NOS phosphorylation. Phosphorylation of NOS reduces its activity and results in a decrease in cyclic guanosine monophosphate (cGMP) production (Dawson et al., 1993). The results of the current study are therefore consistent with previous work from our laboratory that demonstrated that NOS activity is required for on/off sublamination (Cramer et al., 1996), together with recent work that showed NOS activity downstream of NMDA receptors regulates cGMP production in the dLGN of the ferret (Leamey et al., 2001). The latter study also showed that cGMP expression is normally up-regulated during the period of on/off sublamination, and that the blockade of the synthetic enzyme for cGMP, soluble guanylyl cyclase, or one of its potential downstream targets, PKG, prevents on/off sublamination. Together, these results suggest that the activity-dependent regulation of NOS downstream of NMDA receptors is required for sublamination; interfering with the activity of NMDA receptors, NOS (either directly or by blockade of one its regulatory enzymes, calcineurin), or downstream effectors of NOS signaling such as soluble guanylyl cyclase or PKG all prevent on/off sublamination. Importantly, following activation of NMDA receptors, NO can diffuse to the presynaptic terminal where it can transmit information about postsynaptic activity, which may result in the regulation of presynaptic arbor size and position.

Patterns of neuronal activity produce bidirectional changes in synaptic efficacy in the hippocampus and cerebral cortex (Bear and Malenka, 1994; Bliss and Collingridge, 1993). Recent studies using antisense oligonucleotides or genetic manipulations to specifically reduce endogenous calcineurin function in adult mice showed enhanced LTP and reduced LTD in the hippocampus (Ikegami and Inokuchi, 2000; Malleret et al., 2001; Zeng et al., 2001). Previous studies using pharmacological agents such as FK506 and CsA reported similar findings (Funauchi et al., 1994; Hodgkiss and Kelly, 1995; Lu et al., 1996; Mulkey et al., 1994; Torii et al., 1995). NOS activity and cGMP signaling are also required for the induction of LTP and/or LTD in a number of brain regions under specific conditions (Arancio et al., 1996, 2001; Calabresi et al., 1999; Son et al., 1996; Wu et al., 1997, 1998). These various studies demonstrate that NOS and calcineurin modulate synaptic plasticity in the adult brain and can similarly do so in development.

It should be pointed out that both mechanisms of action that are independent of NOS have been proposed for calcineurin (reviewed in Winder and Sweatt, 2001) and that these may be important in mediating its effects. Postsynaptically, calcineurin is known to dephosphorylate the PP1 inhibitor I-1, suppressing PP1 activity and permitting induction of LTP. Calcineurin may also associate with dynamin I to dephosphorylate a number of endocytic proteins (Lai et al., 1999; Liu et al., 1994; Marks and McMahon, 1998). This could then lead to changes in the internalization of AMPA receptors and result in LTD. Application of FK506 prolongs the opening of NMDA-receptor channels in adult hippocampal neurons (Lieberman and Mody, 1994; Tong et al., 1995) and prevents a developmental decrease in the decay time of NMDA receptor currents in the superior colliculus of the rat (Shi et al., 2000). Calcineurin can alter the phosphorylation status of MAP-2, and thus change the state of microtubule and/or actin polymerization; this provides a means whereby calcineurin can regulate dendritic structure (Halpain and Greengard, 1990; Morales and Fifkova, 1989; Quinlan and Halpain, 1996). Calcineurin can also regulate postsynaptic calcium (Brillantes et al., 1994; Cameron et al., 1995; Genazzani et al., 1999; Jayaraman et al., 1992). Calcineurin is also known to have presynaptic effects, through which it can modulate synaptic transmission (Wu and Saggau, 1995; Zucker, 1993). Calcineurin may also influence axonal structure and remodeling via its association with GAP-43 (Steiner et al., 1992) and its influence on presynaptic calcium (Burley and Sihra, 2000; Mulkey et al., 1994). The calcineurin staining seen here in the neuropil is consistent with the possibility that calcineurin may play a direct presynaptic role in on/off sublamination. Because it is also expressed in a subset of dLGN cells it is in a position to exert both pre- and postsynaptic effects, and may thus be able to coregulate pre- and postsynaptic changes in response to patterns of activity.

CONCLUSION

This study has presented evidence that calcineurin immunoreactivity is present in the dLGN of the ferret during the period of on/off sublamination, and that infusion of FK506 disrupts the appearance of sublaminae. Together these findings suggest a possible functional role for calcineurin during the activity-dependent refinement of connections in the ferret dLGN. One possibility is that calcineurin may act through the NOS-cGMP pathway, which we have previously shown to be important for on/off sublamination in the ferret. Alternatively, or in addition, calcineurin may act through NOS independent pathways. The effects of calcineurin activation in the developing retinogeniculate pathway may be to alter the efficacy of normal synaptic transmission, facilitate or depress transmission in the long term, and influence the reorganization of synapses and terminal arbors of retinal axons during pattern formation.

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