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Thalamocortical cells in the cat pulvinar nucleus transiently express nitric oxide synthase during development

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Abstract

We examined the postnatal expression of the neuronal form of nitric oxide synthase (nNOS) within the pulvinar and lateral posterior (LP) nuclei of the cat thalamus using immunocytochemical techniques. During the first postnatal month, nNOS was expressed in many cells within the pulvinar nucleus and medial subdivision of the LP nucleus; fewer neurons in the lateral LP nucleus were stained by the nNOS antibody. We examined the pulvinar nucleus to determine what cell types express nNOS. A comparison of the soma sizes of nNOS-stained cells to the overall population of Nissl-stained cells and interneurons (stained with an antibody against glutamic acid decarboxylase) suggests that within the pulvinar nucleus, thalamocortical cells express nNOS during development. In addition, the nNOS antibody stained axon bundles that traverse the pulvinar nucleus to enter the optic radiations, suggesting that thalamocortical cell axons also contain nNOS during development. However, this staining pattern was dramatically reduced by postnatal day 42 and later ages; the size of the remaining nNOS-stained cells was closer to that of interneurons, a subset of which contain nNOS in the adult pulvinar nucleus. This contrasts with our previous findings that nNOS is specifically expressed within interneurons in the developing dorsal lateral geniculate nucleus (LGN) and serves as further confirmation that the pulvinar nucleus and LGN represent distinct categories of thalamic nuclei.

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Previous studies have noted that the neuronal form of nitric oxide synthase (nNOS) is expressed within interneurons during the postnatal development of the dorsal lateral geniculate nucleus (LGN). This expression declines dramatically during the month following eye opening, and parallels an increase in the expression of acetylcholine and nNOS within axons and terminals that arise from the parabrachial region [4,12,18]. In contrast, in the pulvinar, lateral posterior (LP) nuclei, and ventrobasal complex, a subset of interneurons express nNOS in the adult [3,6,19]. We were therefore interested in determining how the expression of nNOS levels in the developing pulvinar/LP complex compares to that reported for the LGN.

A comparison of the development of the pulvinar/LP complex to that of the LGN is of interest because it has been

proposed these nuclei can be divided into two categories based on the origin of their primary or driving input [13]. The LGN is considered a ‘first order’ nucleus because it is driven by relatively direct sensory inputs. That is, the response properties of cells in the LGN are very similar to the retinal ganglion cells that innervate them, while the cortical inputs to the LGN, which arise exclusively from layer VI [10], elicit more subtle, modulating effects. In contrast, the pulvinar and LP nuclei receive input from both cortical layers V and VI [1], and the morphology and synaptic connections of terminals that originate from cortical layer V closely resemble those of retinogeniculate terminals [23]. Thus, it has been postulated that the pulvinar and LP nuclei may be driven by cortical inputs, and they have thus been described as ‘higher order’ nuclei [21,22]. Because layer V neurons establish the first corticothalamic connections in the developing brain [7], and because nitric oxide plays a putative role in synaptic stabilization [9], early expression of nNOS in the pulvinar/LP complex may

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contribute to the establishment of corticothalamic connections.

We examined the development of nNOS expression using 18 kittens, postnatal day 0 (P0; $n = 3$), P7 ($n = 1$), P13 ($n = 3$), P21 ($n = 2$), P28 ($n = 2$), P35 ($n = 3$), P42 ($n = 2$), and P56 ($n = 2$), and 11 adult cats, greater than 1 year old. These animals were also used for our previous studies [3–6]. Cats and kittens were deeply anesthetized and perfused with saline followed by either 2–4% paraformaldehyde or 0–0.5% glutaraldehyde in 0.1 M phosphate buffer (PB) (pH 7.4). Brains were cut into 50 μm sections using a vibratome, or rapidly frozen and cut using a cryostat. Sections from the same brains were alternately stained with either a nNOS antibody (mouse monoclonal, Sigma) at a dilution of 1:1000, or a glutamic acid decarboxylase (GAD) antibody (rabbit polyclonal, Chemicon, Temecula, CA) at a dilution of 1:2000, or mounted on slides and stained for Nissl substance. Using previously reported immunocytochemical techniques [3,4,6], the antibodies were tagged with a biotinylated goat-anti-mouse antibody (for BNOS) or a biotinylated goat-anti-rabbit antibody (for GAD), a complex of avidin and biotinylated horseradish peroxidase, and revealed with a nickel-enhanced diaminobenzidine reaction.

Fig. 1 shows the staining pattern within the pulvinar/LP complex at P13. A similar staining pattern was observed in tissue obtained at P0, P7, P21 and P28. The overall pattern of staining was similar to that obtained with an acetylcholinesterase reaction [11], i.e. many neurons within the pulvinar nucleus and medial subdivision of the LP nucleus were densely stained with the nNOS antibody. Cells were also stained within the lateral subdivision of the LP nucleus, but the overall staining of this region was considerably reduced in comparison to that in the pulvinar and medial LP nuclei. Additional nNOS staining was also observed within axon bundles in tissue from ages P0 to P35. These nNOS-positive axons were observed within the pulvinar nucleus, as well as coursing through the anterior ventral nucleus and thalamic reticular nucleus into the optic radiations; this staining pattern was not present in tissue collected at later ages.

Fig. 2 illustrates the morphology of nNOS-stained neurons and axons in the pulvinar nucleus at birth, P13, P21 and in the adult. In tissue collected during the first postnatal month, the stained cells were relatively large, and the immunohistochemical staining generally revealed three to five proximal dendrites. In many cases, the staining was sufficiently intense to reveal multiple secondary and tertiary branches. The size of the nNOS-stained neurons increased with age until approximately P28. Thereafter, the size and number of nNOS-stained cells within the pulvinar nucleus were considerably reduced (Fig. 2D).

Fig. 3 compares the mean soma sizes of cells stained with nNOS to those stained for Nissl substance (all neurons) and for GAD (interneurons). In P0–P28 tissue, there was no significant difference between the size of nNOS-stained

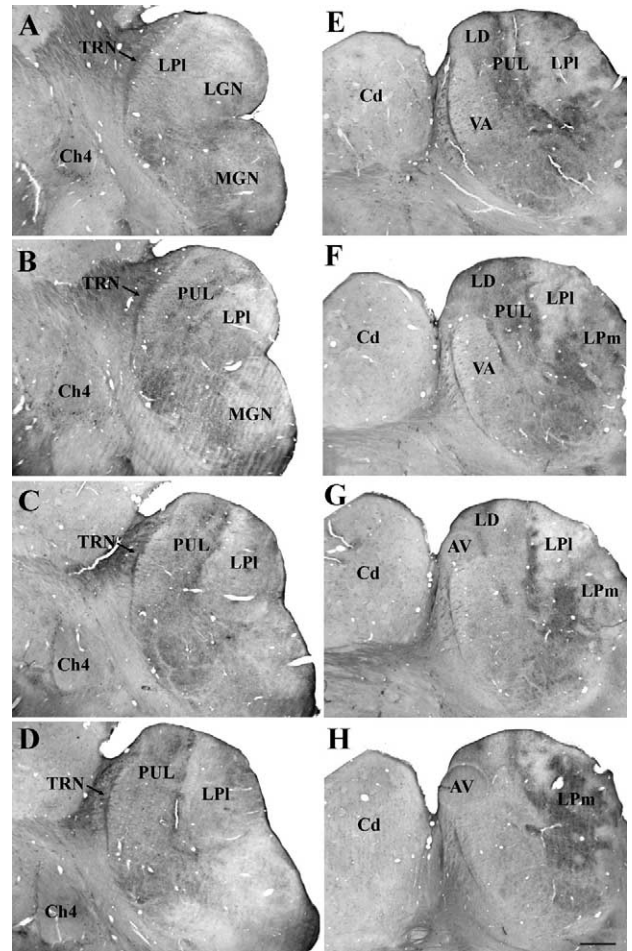


Fig. 1. During development the pulvinar nucleus (PUL) is densely stained with an antibody against nNOS. Shown is a series of parasagittal sections through the pulvinar/LP complex at postnatal day 13 arranged from lateral (A) to medial (H). Dense staining is also seen within the medial subdivision of the lateral posterior nucleus (LPm), but staining within the lateral subdivision of the lateral posterior nucleus (LPI) is relatively light. Also stained are presumed cholinergic cells of the basal forebrain (Ch4), and axon bundles that traverse the thalamic reticular nucleus (TRN). AV, anterior ventral nucleus; Cd, caudate nucleus; LD, lateral dorsal nucleus; LGN, lateral geniculate nucleus; MG, medial geniculate nucleus; VA, ventral anterior nucleus. Scale bar, 1 mm (applies to A–H).

cells and the size of Nissl-stained cells (Student's t -test; $P > 0.05$). However, for all later ages examined (P35, P42, P56, and adult), nNOS cells were significantly smaller than the overall population of Nissl-stained cells (Student's t -test; $P < 0.001$). Nevertheless, at all ages, nNOS-stained cells were significantly larger than the overall population of GAD-stained cells. This corresponds with our previously reported data; in the adult pulvinar nucleus nNOS-stained cells make up a subset of interneurons that are slightly larger than the overall population of GAD-stained cells [3]. In addition, as previously reported for the LGN [4], the developing interneurons were consistently the smallest cells at each postnatal age, and reached their adult size by approximately the third postnatal week. We also noted that the average size of Nissl-stained pulvinar neurons (267 ± 9

μm^2 in the adult) was significantly ($P < 0.001$) smaller than Nissl-stained LGN cells measured in our previous studies ($382 \pm 9 \mu\text{m}^2$ in the adult) [2,4].

Our results suggest that during development, nNOS is expressed within pulvinar thalamocortical cells as well as their axons. The timing of nNOS changes in the pulvinar

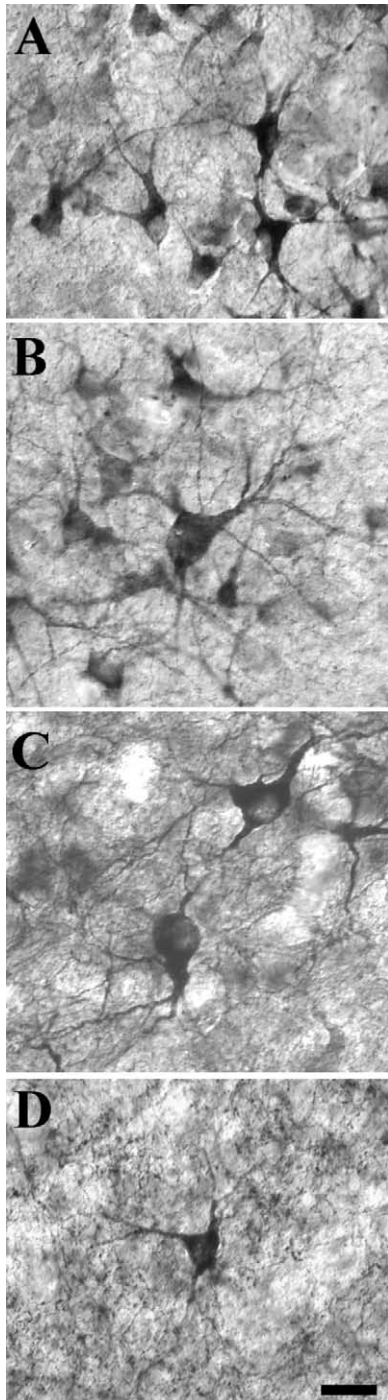


Fig. 2. During development, large cells in the pulvinar nucleus are densely stained with an antibody against nNOS. Shown are photomicrographs of nNOS-stained cells in the pulvinar nucleus of P0 (A), P13 (B) and P21 (C) kittens. In the adult, nNOS staining is confined to presumed interneurons (D). Scale bar, 20 μm (applies to A–D).

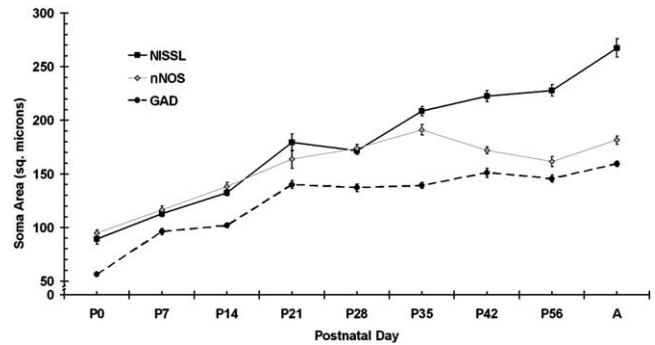


Fig. 3. From P0 to P28, the mean soma area of pulvinar cells stained with the nNOS antibody (diamonds; $n \geq 100$ at each age) is similar to the mean soma area of cells stained for Nissl substance (squares; $n \geq 300$ at each age), and greater than the mean soma area of GAD-stained cells (circles; $n \geq 100$ at each age). At P35 and later ages, the mean soma area of nNOS cells in the pulvinar nucleus decreases in size and is closer to that of GAD-stained cells.

nucleus coincides with several different developmental events. At birth, the cat pulvinar/LP complex sends dense projections to layer I of the lateral suprasylvian visual areas, but after 1 month, projections to this area are largely confined to layer IV [14,16]. Parallel refinements in the cortical innervation of the pulvinar/LP complex also occur postnatally. During the first 1–2 postnatal months, the number of layer V cells projecting to the pulvinar/LP complex decreases, and their size increases [7,15]. This refinement of cortical projections to the pulvinar/LP complex may include a corresponding increase in the proportion of inputs from layer VI, which in the adult pulvinar nucleus far outnumber the projections from layer V [24]. It is possible that, similar to previous reports of the involvement of nitric oxide in the refinement of the retinogeniculate and retinotectal pathways [8,25], the release of nitric oxide from pulvinar thalamocortical cells may contribute to the refinement of thalamocortical and/or corticothalamic projections.

Alternatively, the expression of nNOS in the pulvinar nucleus may reflect more general developmental differences in the neurons that populate the pulvinar nucleus and LGN. We have recently found that neurons in the rat LP nucleus display firing properties that are distinct from those of LGN cells [17]. Cells in the pulvinar/LP complex also differ from LGN cells in that they show little staining for the high molecular weight neurofilament protein revealed with the SMI-32 antibody, whereas a subset of LGN cells stain intensely for this protein [2,5]. Finally, the results of the present study, when compared to our previous studies, suggest that neurons in the pulvinar nucleus are significantly smaller than those in the LGN, both during development and in the adult. Data from the primate further suggest that the pulvinar nucleus is a unique region of the thalamus because at least some of its neurons may originate from the telencephalon [20]. Thus, although the reason the pulvinar nucleus and LGN exhibit differential patterns of nNOS expression remains to be determined, the contrasting

developmental patterns of nNOS expression within these two nuclei serve as further confirmation that the pulvinar nucleus and LGN represent distinct categories of thalamic nuclei.

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