Postnatal Development of Brainstem Cholinergic Inputs to the Dorsal Lateral Geniculate Nucleus of the Domesticated Ferret, *Mustela putorius furo*

by

Sherri Lynn Hitz

B.S., Biology
B.A., French
Cleveland State University, 1993

SUBMITTED TO THE DEPARTMENT OF BRAIN AND COGNITIVE SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN BRAIN AND COGNITIVE SCIENCES

AT THE

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

SEPTEMBER 1998

Signature of Author: _____
Department of Brain and Cognitive Sciences
August 8, 1998

Certified by: _____
Mriganka Sur
Fairchild Professor of Neuroscience
Thesis Supervisor

Accepted by: _____
Gerald Schneider
Professor of Neuroscience
Chairman, Department Graduate Committee

Postnatal Development of Brainstem Cholinergic Inputs to the Dorsal Lateral Geniculate Nucleus of the Domesticated Ferret, *Mustela putorius furo*

by

Sherri Lynn Hitz

Submitted to the Department of Brain and Cognitive Sciences
on August 8, 1998 in Partial Fulfillment of the
Requirements for the Degree of Master of Science in
Brain and Cognitive Sciences

ABSTRACT

The ferret dorsal lateral geniculate nucleus (dLGN) undergoes two periods of retinal afferent segregation during postnatal development. The first establishes the eye-specific laminae, A and A1, and the second establishes ON/OFF sublaminae within laminae A and A1. In contrast to eye-specific segregation, which seems to rely only on presynaptic activity, ON/OFF sublamination requires both pre- and postsynaptic activity. Because of its dependence on postsynaptic (relay cell) activity, sublamination may be influenced by extraretinal inputs which alter relay cell excitability. We have examined the postnatal development of cholinergic brainstem inputs to the dLGN to determine whether these inputs arrive in time to influence sublamination and whether the cholinergic innervation is present in laminar zones (i.e., whether it might target relay cells). Choline acetyltransferase (ChAT) immunoreactivity is not detected in the dLGN until a few days after the second postnatal week (just after sublamination begins), at which time it can be seen in both A and C laminae. ChAT labeling increases in intensity until two days before the end of the fourth postnatal week (when ON/OFF sublamination is complete), when it drops dramatically throughout the dLGN . ChAT labeling returns a few days later, but appears in the interlaminar and inter-sublaminar zones instead of within the A and C laminae. However, the pattern of ChAT labeling reverses once more, so that in the adult, ChAT labeling appears in the A and C laminae and is relatively absent from interlaminar zones. Acetylcholinesterase (AChE) labeling in the dLGN shows a similar ontogenetic pattern and time course. Retrograde labeling of brainstem cholinergic nuclei demonstrates that these inputs are in place in the dLGN after the second postnatal week. Thus, cholinergic inputs to A and A1 laminae of the ferret dLGN do arrive in time to influence ON/OFF sublamination.

Thesis Supervisor: Mriganka Sur
Title: Fairchild Professor of Neuroscience

CONTENTS

ACKNOWLEDGEMENTS

First, I would like to thank Diana Smetters for passing along the following words of wisdom, which turned out to be more true than I would have ever believed.

MIT is not a very nice place.
But once you've been there, nothing will ever scare you again.
– Steve Kosslyn, Harvard Professor, 1989

I would like to thank the ferrets who sacrificed their lives for my research. Henceforth, I shall certainly be more kind to my fellow animals.

I'd also like to thank quite a few people who have contributed to my personal and academic development at MIT and beyond:

Carsten Hohnke, for being there for me during the "critical periods" with just what I needed – an honest-to-goodness intellectual tussle, a shoulder to cry on, an ear for my fairly constant rantings and ravings, and for discovering with me that love at first sight is a real and splendid thing.

My parents, Patricia and Nelson Bernauer, for always discouraging me from being politically correct or otherwise a sheep, even when it might seem a much easier path to follow. My brothers, Curt, Joe, Doug and Mike, for being four unique and terrific companions on the tough path.

Mike Meredith, for continuing to be my friend and advisor long after anyone else would have quit; I cannot ever fully express my gratitude for your patience and generosity. And, thank you for showing me how wonderful science can be when it's done properly.

Bernardo Aumond, for taking me out to play – in hurricanes, no less –, and for being my best friend and confidant even during the worst of times. Thanks, too, for reminding me that the ocean is where I really belong.

Karen Fu, for your friendship and your no-BS attitude towards life in general. I always hope to share both of these things.

Gina Rendon, who will someday be the finest large mammal veterinarian in the world, for making me take the qualifiers early, for taking on the IAP Neuroanatomy course with me, for sometimes reminding me how completely insane I am, and for being such a terrific friend through it all.

Terry D'Souza, who is quite possibly the coolest chick and best scientist I will *ever* know, for your wonderful friendship, your honesty, your knock-'em-dead attitude, and your interminable energy.

Justin Grubich, for rekindling my love of the ocean by teaching me to surf, for encouraging me to "carpe diem", and for simply being one of the best human beings I will ever be fortunate enough to know.

Diana Smetters, again, for honest friendship, excellent advice in all realms, and for giving me a good kick in the pants whenever I needed it. And, believe me, I have needed it.

Tara McHugh, for invaluable assistance with all things experimental, especially when I just couldn't do it alone. I really couldn't have gotten through this without your help.

And last, but certainly not least, my advisor, Mriganka Sur, for forcing me to develop clear scientific objectives, dogged determination, and a self-discipline and motivation I never thought I would have.

Finally, I would like to acknowledge that this research was supported by NIH grant EY11512 and NSF grant IBN-9602143 to M. Sur.

INTRODUCTION

The dorsal lateral geniculate nucleus (dLGN) relays visual information from retinal ganglion cells (RGCs) to visual cortex. It receives visually-driven excitatory inputs from the retina, visual cortex and superior colliculus, inhibitory inputs from intrageniculate and perigeniculate interneurons, and modulatory inputs from several pontomesencephalic brainstem nuclei, the largest of which is cholinergic. [A closely related structure, the ventral lateral geniculate nucleus (vLGN), also receives retinal and neuromodulatory inputs and may be involved in visuomotor responses and circadian rhythms (Harrington, 1997).] The ferret dLGN is composed of six layers, the characteristic organization in carnivores: A, C and C2 receive inputs from contralateral retina; A1 and C1 receive ipsilateral input, while C3 receives only nonretinal input (Linden et al., 1981). Layers A and A1 receive input from X- and Y-type RGC inputs, while layers C, C1 and C2 receive W-type RGC input (Linden et al., 1981).

The ferret retinogeniculate pathway has been used as a model system in developmental plasticity (despite the fact that ferrets do not seem to be highly visual animals and instead seem to rely mainly on olfaction; Apfelbach and Wester, 1977)) because the pathway is relatively immature at birth, its development is at least in part activity-dependent and this activity is relatively easy to manipulate via ocular treatments. The cat dLGN is also extensively used in investigations of developmental plasticity for the same reasons, though cats are born after 65 days of gestation, while ferrets are born after only 41 days. Retinogeniculate development occurs at roughly the same rate in both species; therefore, the cat's retinogeniculate pathway is more mature at birth and many investigators

have resorted to embryonic studies to examine early events in retinogeniculate development. In this paper, cat and ferret data will be discussed interchangeably; however, when quantitative information is presented from the cat, this will be explicitly noted.

Most studies of retinal afferent segregation within the dLGN have focused on the classical excitatory (glutamatergic) retinogeniculate neurotransmitter system (see Shatz, 1990; Cramer and Sur, 1996 for reviews); here we suggest that a neuromodulatory (cholinergic) system arising in the pontomesencephalic tegmental region of the brainstem might influence segregation of on and off retinal afferents. In addition, we provide anatomical evidence that cholinergic inputs arising from the pedunculopontine tegmental and laterodorsal tegmental nuclei (PPTg and LDTg, respectively) arrive in the dLGN early enough to influence on/off segregation in the ferret dLGN.

Ontogeny of the Retinogeniculate Pathway

Embryonic Development

In ferrets, neurogenesis of retinal ganglion cells (RGC) and dorsal lateral geniculate (dLGN) neurons begins at embryonic day (E) 20; retinal ganglion cell neurogenesis continues through postnatal day (P) 2 (Greiner and Weidman, 1981), while dLGN neurogenesis concludes by E30 (Peduzzi, 1989). RGC axon outgrowth from the eye has been detected as early as E24, though some exit the eye earlier since RGC axons are present in the optic chiasm by this age (Johnson and Casagrande, 1993). Axons from both nasal and temporal retina grow into the optic chiasm in roughly two stages; the first is apparently independent of midline cues while the second relies on some as yet undetermined cue or combination of cues at the midline (Baker and Reese, 1993). During the second stage of axon growth into the chiasm,

axons from nasal RGCs cross to innervate contralateral LGN; however, temporal axons are prevented from crossing the midline and, instead, they turn back from the midline to innervate ipsilateral LGN (Stretavan and Reichardt, 1993; Godement et al., 1994).

As a result of the segregation of nasal and temporal retinal inputs at the chiasm, each dLGN receives inputs from both eyes. In the cat, contralateral inputs arrive first (at E32) and grow across most of the dLGN while ipsilateral inputs arrive around E35 and grow into the caudomedial portion of the LGN (Shatz, 1983); thus, the overlap of ipsi- and contralateral inputs is extensive, though never complete, as was initially suggested (Shatz, 1983; but see Shatz, 1996 for a more recent assessment). In the ferret, retinal afferents arrive at the dLGN between E25 and E27 and invade the nucleus by E32, with contralateral inputs invading before ipsilateral (Cucchiaro and Guillery, 1984; Johnson and Casagrande, 1993). The pattern of contra- and ipsilateral innervation of ferret dLGN is very similar to that seen in the cat, though Hutchins and Casagrande (1990) have explicitly noted that contralateral fibers innervate the future A1 area less densely than they do the rest of the dLGN and that ipsilateral fibers are the first to restrict themselves to their appropriate territory.

Postnatal Development

On the day of birth (embryonic day 41; E41 = postnatal day zero; P0), the overlap between ipsilateral and contralateral inputs is extensive in the dLGN and contralateral inputs have extended into the adjacent perigeniculate nucleus (PGN) (Linden et al., 1981; Cucchiaro and Guillery, 1984; Hahm, 1991). By P8, eye-specific inputs have segregated into fairly distinct laminae in the dLGN so that contralateral inputs terminate mainly in the A layer and ipsilateral inputs terminate mainly in the A1 layer; however,

some axons still extend processes across laminar borders until between P15 and P19 (Linden et al., 1981; Cucchiaro and Guillery, 1984; Hahm, 1991b).

ON/OFF segregation in the ferret dLGN begins around P14 and is complete by P28 (several days before eye opening). During this second segregation period, inputs from ON-center and OFF-center RGCs, whose dendrites stratify in the inner inner plexiform layer (iIPL) and outer IPL (oIPL) of the retina, respectively, segregate to form distinct sublaminae within the eye-specific (i.e., A and A1) layers of the dLGN (Nelson et al., 1978). ON-center and OFF-center RGCs are named for their responses to light: ON-center cells are maximally depolarized when the center of their receptive field is illuminated while the surround is dark; OFF-center cells are maximally depolarized when the surround is illuminated and the center is dark. Stryker and Zahs (1983) were the first to demonstrate that sublaminae in A and A1 represent the anatomical segregation of ON and OFF inputs in the ferret dLGN.

Activity-Dependence of Retinal Afferent Segregation in the dLGN

A central question in developmental neurobiology is whether the mechanisms which refine neuronal connectivities depend on neuronal activity. In 1949, Hebb postulated a mechanism for synaptic strengthening. This so-called Hebbian plasticity requires that postsynaptic neurons detect coincidence of presynaptic inputs and respond to that coincidence by specifically strengthening synaptic responses to those inputs. Hebbian plasticity is thought to play a central role in developmental changes in connectivities and N-methyl-D-aspartate (NMDA) receptors, which may act as coincidence detectors, have been implicated in retinogeniculate plasticity (Stent, 1973; Constantine-Paton et al., 1990; Mooney et al., 1993). The proposed mechanism of NMDA

receptor involvement in neuronal plasticity has been widely studied, especially in the rat hippocampus (see Bliss and Collingridge, 1993 for review). Briefly, both alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and NMDA receptors are activated by glutamatergic input. NMDA receptors differ from AMPA receptors in two critical ways: 1. They do not become activated until the postsynaptic neuron is sufficiently depolarized to relieve a voltage-dependent magnesium block of the NMDA receptor's cation channel and 2. During adequate depolarization, they conduct calcium cations. Sufficient influx of calcium is required for activation of nitric oxide synthase (NOS), which produces a putative retrograde messenger, nitric oxide (NO), which may diffuse across the synaptic cleft to the presynaptic neuron and cause an increase in glutamate release, increasing synaptic efficacy (Schuman and Madison, 1991). In addition, calcium activates a number of second messenger pathways and may cause morphological and/or physiological changes in the postsynaptic neuron (e.g., see Chen et al., 1997).

Because eye-specific segregation is complete before photoreceptor maturation and eye opening in both ferret and cat, there is no visually-driven activity in the retina during eye-specific segregation; however, spontaneous waves of action potential activity have been detected in the RGCs of fetal rats (Galli and Maffei, 1988; Maffei and Galli-Resta, 1990) and neonatal ferrets (Meister et al., 1991; Wong et al., 1993; Wong and Oakley, 1996). *In vitro* electrophysiological recordings in cat dLGN have established that RGC axons make functional synapses onto relay cells even before eye-specific segregation begins; thus retinal activity (presynaptic activity) can be transmitted to relay cells (postsynaptic activity) during both eye-specific and ON/OFF segregation periods (Shatz and Kirkwood, 1984; Shatz, 1996). Electron micrographs of retinal inputs to dLGN relay cells (also in

cat) provide anatomical evidence that retinogeniculate synapses are already established during eye-specific segregation (Campbell and Shatz, 1992). Thus, it is conceivable that spontaneous activity of retinal ganglion cells activates NMDA receptors on dLGN relay cells, strengthening and perhaps physically stabilizing "appropriate" synapses to create a functional adult retinogeniculate system.

Eye-Specific Segregation

Shatz and Stryker (1988) disrupted eye-specific segregation in kittens via minipump delivery of tetrodotoxin (TTX; a neurotoxin which prevents action potential activity) near the optic chiasm. They concluded that presynaptic activity was required for eye-specific segregation to proceed normally; however, the TTX was not restricted to the chiasm. Therefore, it is more accurate to conclude that action potential activity is required for eye-specific segregation, but the critical activity might be presynaptic, postsynaptic or a combination of both. [An alternate interpretation is that TTX influences segregation not by blocking activity, but instead by influencing axon growth. Wilkemeyer and Angelides (1996) demonstrated that TTX stimulates axon branching and the formation of varicosities in organotypic cocultures of rat thalamic and cortical tissue; however, since TTX concurrently blocks activity and causes changes in axonal morphology, it is perhaps impossible to separate these effects.] Preliminary data from Cook et al. (1996) have shown that focal blockade of presynaptic activity, achieved by repeated intraocular applications of TTX, does not disrupt eye-specific segregation in neonatal ferrets. Penn et al. (1998) have perhaps provided some resolution to these conflicting results with their recent finding that focal blockade of RGC action potential activity does in fact prevent eye-specific segregation in the ferret. Their elegant experimental design involves the use of cholinergic antagonists to block action potential activity of RGCs indirectly, that is, without changing any other aspect of RGC structure

or function. Since they did not show any single-axon anatomy, however, they did not address the issue of whether activity blockade is sufficient to alter axonal morphology, even in the absence of TTX. In conclusion, it seems that presynaptic action-potential activity is required for the normal formation of eye-specific laminae in ferret dLGN.

It is not clear, however, whether postsynaptic activity (i.e., dLGN relay cell activity) is required for eye-specific segregation. Supporting the notion that eye-specific segregation is independent of postsynaptic activity, Smetters et al. (1994) have demonstrated that blockade of N-methyl-D-aspartate (NMDA) receptor function, by systemic delivery of CPP (a competitive antagonist of glutamate at the NMDA receptor), does not disrupt eye-specific segregation in the ferret dLGN. It is worth noting that blockade of NMDA receptors in developing relay cells blocks a major component of postsynaptic activity, so that NMDA receptor blockade severely attenuates relay cell activity (Sillito et al., 1990; Heggelund and Hartveit, 1990; Esguerra et al., 1992; Kwon et al., 1992; Ramoa and McCormick, 1994b). Additional evidence that eye-specific segregation is postsynaptic activity-independent comes from Cramer et al. (1996), who were unable to disrupt eye-specific segregation in ferret dLGN by systemic delivery of L-nitroarginine (L-NoArg, an arginine analog) to inhibit NOS. Thus, it seems that eye-specific segregation may be presynaptic activity-dependent but postsynaptic activity-independent.

ON/OFF Sublamination

Cramer and Sur (1997) have shown that presynaptic activity blockade, via repeated intraocular injections of TTX from P12-P24, is sufficient to prevent ON/OFF sublamination in ferret dLGN. Thus, it seems that sublamination is dependent on presynaptic action potential activity. Wong and Oakley (1996)

showed that ON and OFF RGC activity in the neonatal ferret is correlated until sublamination begins and then they develop distinct burst frequencies which might be used to drive segregation. Evidence that ON/OFF sublamination is also postsynaptic activity-dependent comes from Hahm et al. (1991a) who blocked sublamination in ferret dLGN via osmotic minipump delivery of D-APV or systemic delivery of MK-801 to inhibit NMDA receptor function. In addition, Cramer et al. (1996) demonstrated that NOS blockade, again by systemic delivery of L-NoArg, is sufficient to disrupt ON/OFF sublamination.

Extraretinal inputs to the dLGN

Intrathalamic Inputs

γ-aminobutyric acid (GABA)-ergic input to dLGN relay cells, which represents about one third of all inputs to cat dLGN relay cells (Erisir et al., 1998), come from local (i.e., intrageniculate) interneurons and from neurons of the thalamic reticular formation (nRT; nucleus reticularis thalami), which includes the perigeniculate nucleus (PGN; Mitrofanis, 1994). In the ferret, the dorsocaudal region of the nRT gives rise to the PGN by P0 (Mitrofanis, 1994). Intrageniculate interneurons each receive retinotopic input from one or two RGCs and they are thought to provide spatially precise, feed-forward inhibition to dLGN relay cells (Dubin and Cleland, 1977). In contrast, PGN interneurons receive binocular retinal inputs, peribrachial input, and collaterals from geniculocortical and corticogeniculate fibers; they are thought to provide a more diffuse inhibition of dLGN relay cell activity (Dubin and Cleland, 1977; Hu et al., 1989a). PGN interneurons are also involved in the generation of so-called spindle waves, synchronous oscillations which appear as brief (1-3 second) periods of low-frequency (6-14 Hz) activity in electroencephalograms (EEGs); dLGN relay neurons also exhibit spindle wave activity (von Krosigk et al., 1993).

Extrathalamic Inputs

Retinal ganglion cell input is exclusively glutamatergic and represents about 20% of the total input to dLGN relay cells (in the A layers); in addition, relay cells receive major inputs from two extrathalamic sources: a second glutamatergic input from pyramidal neurons in layer 6 of striate and extrastriate visual cortices (Brodmann's areas 17,18 and 19 in ferret; Clasca et al., 1995), roughly 25% of the total input; and cholinergic input from the pedunculopontine tegmental and laterodorsal tegmental nuclei (PPTg and LDTg, respectively) of the midbrain reticular formation, roughly 25% of the total input (percentages in cat; Erisir et al., 1997b and 1998). In both rat and ferret, the brainstem cholinergic input to the dLGN comes primarily from the PPTg and the LDTg provides only a minor input (Henderson and Sherriff, 1991). The dLGN also receives a variety of minor extrathalamic inputs, which include a serotonergic projection from the raphe nuclei (Mize and Payne, 1987), a noradrenergic projection from the locus coeruleus (Papadopoulos and Parnavelas, 1990), a histaminergic projection from the posterior ventral region of the hypothalamus (Uhlrich et al., 1993), a GABAergic projection from the pretectum (Cucchiaro et al., 1991), and a presumably glutamatergic (Jeon et al., 1997) projection from the superior colliculus (Sutton and Brunso-Bechtold, 1988).

Influence of the Major Extraretinal Inputs on Retinal Afferent Segregation

Research into mechanisms of retinal afferent segregation in the dLGN has focused on the role of activity in the retinogeniculate pathway; however, given that cortical and brainstem inputs represent approximately half of the total input to dLGN relay cells (Erisir et al., 1997b and 1998), these extraretinal inputs may significantly influence retinogeniculate segregation.

Corticogeniculate Inputs

Primary visual cortex (Brodmann's area 17; striate cortex), which receives relay cell input via layer 4, also sends output from layer 6 to dLGN relay cells between P9 and P17 in the ferret (Clasca et al., 1995). However, much cortical input to dLGN comes first from layer 5 neurons in dorsal occipital cortex (presumptive areas 18 and 19; extrastriate cortex), which send a descending projection to dLGN around P5; these axons are collaterals of a projection from layer 5 to brainstem, the majority of which are not removed until after P21 (Clasca et al., 1995). Such collateral pruning without the death of the entire neuron is a widespread phenomenon in the developing mammalian brain (O'Leary, 1992). The function of the persistent extrastriate projection to LGN is not known; however, because extrastriate axons arrive by P5, they might play a role in both eye-specific and ON/OFF retinogeniculate segregation. The layer 6 projection to LGN relay cells is a permanent one; however, since it does not arrive in the LGN until around P9, its influence over retinogeniculate segregation is likely restricted to the period of ON/OFF sublamination.

Cholinergic Brainstem Inputs

Anatomy

The dLGN receives a minor cholinergic input from the parabigeminal nucleus (PBG), which is located in the pontine region of the midbrain (Fitzpatrick et al., 1989; Hutchins and Casagrande, 1988; Harting et al., 1991; Turlejski et al., 1994). The PBG also sends a major cholinergic input to the superior colliculus (Tan and Harvey, 1989; Jhiang et al., 1996).

Major cholinergic input to the dLGN and other principal thalamic relay nuclei come from two nuclei,

also located in the pontomesencephalic tegmental region of the brainstem: pedunculopontine tegmental (PPTg) and lateral dorsal tegmental (LDTg; also dorsolateral tegmental) (rat: Woolf and Butcher, 1986; ferret: Henderson, 1987a; Fitzpatrick et al., 1989; Henderson and Sherriff, 1991; cat: de Lima et al., 1985; de Lima and Singer, 1987; see Semba and Fibiger, 1989 for review). Mesulam et al. (1983) have suggested a standardized nomenclature for the six major cholinergic nuclei of the rat brain (Ch1 through Ch6); Ch5 includes cholinergic neurons in the PPTg, cuneiform nucleus and parabrachial area and Ch6 is comprised solely of cholinergic neurons in the LDTg. Unfortunately, a plethora of less well--defined terms are routinely used to describe this brainstem region and its subregions.

Moruzzi and Magoun (1949) first identified the region as the "brain stem reticular activating system", a term which refers to its ability to switch EEG patterns from the synchronized state which occurs during sleep to the desynchronized state which occurs when the animal is awake. Francesconi et al. (1988) describe this region as the midbrain reticular formation, which includes mainly cholinergic (Shute and Lewis, 1967; de Lima and Singer, 1987; Henderson, 1987b; Fitzpatrick et al., 1989), but also noradrenergic (de Lima and Singer, 1987; Henderson, 1987b; Fitzpatrick et al., 1989) and glycinergic neurons (Fort et al., 1993). In the cat, this region is sometimes called the peribrachial region (PBR; e.g., in Hartveit and Heggelund, 1994), which includes mainly cholinergic, but also noradrenergic and serotonergic, neurons which occupy the periphery of the brachium of the inferior colliculus (BIC); the term more often used in cat is the parabrachial (PB) region, which refers to the more caudal portions of the brainstem cholinergic region (e.g., in Erisir et al., 1997a). The PB contains mostly cholinergic neurons, concentrated along the ventromedial extent of the BIC (Uhlrich et al., 1988). De Lima and Singer (1987) use a closely related name– the medial nucleus of the brachium conjunctivum, which also

describes the anatomical location of the region.

Electron microscopy has shown that cholinergic inputs synapse directly onto both relay cells and interneurons in the dLGN as well as PGN interneurons (Raczkowski and Fitzpatrick, 1989; Beaulieu and Cynader, 1992; Erisir et al., 1997a). In addition, autoradiographic analysis has revealed that relay cells of the dLGN possess both nicotinic (Parkinson et al., 1988) and muscarinic M2 (Spencer et al., 1986) types of acetylcholine (ACh) receptors.

Physiology

Relay cells have two basic response modes: burst firing and tonic firing. Burst firing occurs during spindle waves (6-15 Hz), which occur during slow-wave sleep (McCarley et al., 1983) and absence seizures (see review in Kostopoulos et al., 1981a, b). Burst firing occurs when a dLGN relay cell's resting membrane potential is relatively hyperpolarized (below -60 mV), which deinactivates a low-threshold calcium current (called LT or I_T); depolarization of a relay cell under these conditions, via retinal input, for instance, causes a long LT calcium spike (i.e., depolarization) upon which 2-7 action potentials ride (Dossi et al., 1992; McCormick and Pape, 1990). Burst firing reflects a non-linear transformation of incoming retinal signals (Lu et al., 1993; Guido et al., 1995); however, recent evidence from Guido and Sherman (1998) indicates that signal detection in relay cells is actually improved during burst firing (versus tonic firing).

The second response mode of dLGN relay cells is the tonic, or relay, mode. Tonic firing occurs during REM sleep and the waking state, when spindle waves are absent, and reflects an increased transfer ratio

(i.e., relay cell action potentials are fired more reliably in response to excitatory inputs), a more linear transformation of retinal signals and an increase in the signal to noise ratio (Livingstone and Hubel, 1981; Sawai et al., 1988; Lu et al., 1993; Guido et al., 1995). A relay cell will fire in the tonic mode when its resting membrane potential is above -60 mV, so that I_T is inactivated and there is no LT calcium spike in response to depolarizing inputs (Dossi et al., 1992; McCormick and Pape, 1990).

Response properties of dLGN relay cells can be switched from bursting to tonic (as during the switch from slow-wave sleep to arousal) by stimulation of brainstem regions which send cholinergic input to the dLGN (Francesconi et al., 1988; Hu et al., 1989a; Lu et al., 1993; Hartveit and Heggelund, 1995; Uhlrich et al., 1995) or direct application of ACh to relay cells (Sillito et al., 1983; McCormick and Prince, 1987; Francesconi et al., 1988; McCormick, 1992). These changes are effected by both nicotinic (Hu et al., 1988, 1989a and 1989b; McCormick, 1992; Zhu and Uhlrich, 1997) and muscarinic (Francesconi et al., 1988; Marks and Roffwarg, 1989; McCormick and Prince, 1987; McCormick, 1992) ACh receptors on dLGN relay cells. *In vivo* microdialysis in the rat has shown that ACh release in the thalamus is correlated with behavioral arousal states, so that extracellular ACh levels are elevated during the waking state and REM sleep and significantly attenuated during slow-wave sleep (Williams et al., 1994).

Intrageniculate and perigeniculate (PGN) interneurons which send inhibitory GABAergic inputs to dLGN relay cells also receive cholinergic brainstem inputs. Acetylcholine inhibits both types of interneurons via muscarinic receptors (McCormick and Prince, 1986; McCormick and Pape, 1988; Pape and McCormick, 1995); intrageniculate interneurons are also inhibited via nicotinic receptors (Lee and McCormick, 1995; Zhu and Uhlrich, 1997). Thus, ascending cholinergic brainstem inputs to dLGN relay

neurons excite them not only directly (via nicotinic and muscarinic ACh receptor-mediated depolarization), but also indirectly (via nicotinic and muscarinic ACh receptor-mediated inhibition of inhibitory inputs from both intrageniculate and PGN interneurons).

Putative Role for Cholinergic Inputs to dLGN in Retinogeniculate Segregation

Brainstem cholinergic inputs might influence retinogeniculate segregation by altering the excitability of developing relay neurons, though perhaps not by switching them from bursting to tonic firing. Ramoa and McCormick (1994) have shown that the development of the low-threshold calcium current (I_T) is protracted in the ferret dLGN; I_T is not fully functional until P19. Despite this, cholinergic inputs to dLGN relay neurons do increase relay cell excitability, both by direct excitation of relay cells and by indirect inhibition of inhibitory inputs to relay cells, and might simply enhance the reliability of relay cell responses to retinal inputs (i.e., increase the transfer ratio) during retinal afferent segregation.

A second possibility is that cholinergic brainstem inputs might enhance synaptic plasticity during activity-dependent retinal afferent segregation. Zhu and Uhlrich (1997) recently provided evidence that nicotinic acetylcholine receptors (nAChRs) on dLGN relay cells conduct not only sodium and potassium, but also calcium cations. Recall that the NMDA receptor is thought to play a critical role in neuronal plasticity because, in response to sufficient depolarization, it conducts calcium cations into the postsynaptic neuron. If nAChRs can also conduct calcium cations, perhaps even in the absence of "sufficient" postsynaptic depolarization, cholinergic inputs might induce in dLGN relay cells a state of heightened synaptic plasticity during retinal afferent segregation. Because ON/OFF segregation is both pre- and postsynaptic activity-dependent and because I_T is not fully functional until P19, brainstem cholinergic

inputs might be more likely to exert this type of an influence during ON/OFF segregation than during eye-specific segregation.

Finally, cholinergic afferents might enhance activation of the ON channel in the dLGN. Henderson (1987a) has shown that ON sublaminae in the adult ferret dLGN receive more cholinergic afferents than do OFF sublaminae, suggesting that cholinergic input might have a stronger excitatory influence in the ON relay cells. Physiological evidence for a developmental difference in activation of ON and OFF channels comes from Wong and Oakley (1996), who showed that ON RGCs do not burst as often as do OFF RGCs during ON/OFF segregation. In addition, Kageyama and Wong-Riley (1984) showed that OFF channels are more metabolically active than are ON channels in cat and ferret (both nocturnal animals; this pattern is reversed in diurnal primates); this suggests that overall levels of activity, perhaps even in the developing system, might be lower in the ON sublaminae. If a minimal level of RGC activity is required for ON/OFF segregation to proceed normally, cholinergic inputs may be critical to development of ON sublaminae. Thus, I have examined the development of cholinergic innervation of the ferret dLGN in order to compare the time course of its development to the known stages of eye-specific segregation and ON/OFF sublamination.

MATERIALS AND METHODS

Twenty-two pigmented ferrets (*Mustela putorius furo*) between the ages of P7 and adult were used in this study; 20 were neonates (P0 to P42) and two were adults (>P180). Two ferrets were used for DiI tracing, two for *in vitro* tracing and 18 for immunocytochemistry and/or histochemistry. Ferret

jills were obtained from Marshall Farms (North Rose, NY); jills and kits were housed in our breeding colony. Experiments were performed under a protocol approved by MIT's Animal Care and Use Committee and every effort was made to minimize discomfort, distress and pain to the animals at all times. Ferrets were given an overdose of the barbituate anesthetic sodium pentobarbitol (Nembutal; Abbott Laboratories, North Chicago, IL; 50 mg/ml); the doses were 250 mg/kg body weight for neonates, 125 mg/kg for subadults and 100 mg/kg for adults. Depth of anesthesia was judged to be adequate when there was absolutely no response to a strong pinch between the toes of a hindfoot. In cases where this depth of anethesia was not reached within ten minutes after the initial dose, a supplemental dose (in a few cases, multiple doses) was given until an adequate depth of anesthesia was reached.

Choline Acetyltransferase (ChAT) Immunocytochemistry

Preparation of Tissue Sections

After an adequate depth of anesthesia was reached, each ferret was transcardially perfused first with room temperature (RT) 0.9% saline just until the perfusate from the right atrium became essentially free of blood, immediately followed with ice-cold (0-4° C) 4% pre-filtered paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The volume of paraformaldehyde was scaled according to the size of each ferret (e.g., a 300 g neonate would require 500 ml and an adult, weighing 1 kg, would require 1 liter); paraformaldehyde perfusion was only stopped when the ferret was completely rigid. Brief (1-4 hours) postfixation was sometimes used in neonates when the brain was not sufficiently hardened by the perfusion; it consisted of either 2% paraformaldehyde in PB plus 15% sucrose in 0.01M phosphate-buffered saline (PBS, pH 7.4) or 4% paraformalehyde in PB. Two chunks of

tissue (thalamus and midbrain tegmentum) were manually dissected out of the brain and the meninges were removed. Neonatal tissue was always sectioned on a Frigocut-E 2800 cryostat (Reichert-Jung, Germany); adult tissue was sectioned on either the cryostat or a Vibratome (Ted Pella, Redding, CA). Cryosectioned tissue was cryoprotected in a two-step process: the tissue was placed in 15% sucrose in PBS on a shaker table at RT until it sunk, at which point 30% sucrose was exchanged for the 15% and the tissue was left to sink in the 30% sucrose at 4° C overnight. Vibratome-sectioned tissue was not cryoprotected. 50 μm thick sections were collected in PBS and kept at 4° C until immunocytochemical processing was begun (optimal staining was achieved when processing began within 48 hours of sectioning). Thalamic tissue was sectioned in the horizontal plane in order to optimize viewing of A and A1 laminae and ON and OFF sublaminae; midbrain tissue was sectioned in the coronal plane in order to optimize viewing of PPTg and LDTg.

Immunocytochemistry

Several immunocytochemical processing methods were tried, with very little success, until we adopted both the antibody (monoclonal anti-choline-acetyltransferase made from rat-mouse hybrid cell line; Boehringer-Mannheim, Indianapolis, IN) and method used in the lab of ME Bickford (see Patel and Bickford, 1997 for original protocol), which was altered slightly to optimize ChAT staining in ferret tissue. All incubations and rinses were done on a shaker table. Sections were preincubated in 10% normal goat serum (NGS) in 0.05% Triton-X-100-PBS (PBSX) for 2 hours at RT and then incubated in primary antibody (1:50 anti-ChAT) plus 1% NGS in PBSX for 34-48 hours, also at RT. Omission of the primary antibody prevented immunostaining, indicating that the anti-ChAT antibody binds specifically to ChAT in the ferret brain. Sections were rinsed 3 x 15 min

in PBS and then incubated in secondary antibody (1:100 biotinylated goat-anti-rat IgG; Vector Laboratories, Burlingame, CA) plus 1% NGS in PBS at RT, followed by another 3 x 10 min PBS rinse, one hour in 1:100 Vectastain Elite ABC kit (Vector), 2 x 10 min PBS rinse, 2 x 10 min tris-buffered saline (TBS, pH 8.4) rinse. Sections were developed in 3, 3'-diaminobenzidine (DAB substrate kit for peroxidase, Vector), prepared according to the kit directions, for 6 to 30 minutes (until the reaction product was judged to be dark enough) and then the reaction was stopped by removal of DAB and addition of excess TBS. Unfortunately, since DAB development times were variable, it is not possible to quantify staining intensities across ages; instead, we only discuss the staining pattern. ChAT immunoreactive (ChAT+) fiber staining in the dLGN was optimal when tissue was left in TBS at 4° C for 7-10 days; this may allow the DAB reaction to continue. Sections were mounted from 0.01 M PB onto gelatin-subbed glass slides and allowed to air-dry; within 24-72 hours, the mounted sections were dehydated through graded alcohols (1-2 min in each: 70%, 95%, 95%, 100%, 100% EtOH), defatted by 3 x 2 min immersions in Histoclear, a xylene substitute (National Diagnostics, Atlanta, GA) and coverslipped with Permount (Fisher, Springfield, NJ).

Acetylcholinesterase (AChE) Histochemistry

For AChE histochemistry by a modified Jensen-Blackstad method, we used the same methods of perfusion through sectioning as detailed above. All incubations and rinses were performed on a shaker table at RT. Sections were rinsed once briefly in dH_2O and incubated for 2 hours in incubation medium [0.068 g Parsidol (Parke-Davis, Morris Plains, NJ), 0.3 g acetylthiocholine iodide, 0.225 g glycine, 0.15 g copper sulfate (hydrate), 1.23 g sodium acetate (hydrate) in 300 ml dH_2O, plus glacial acetic acid to reach pH 5]. Sections were developed as follows: incubation

medium was removed, sections rinsed once in dH_2O, incubation in 1.25% sodium sulfite for one minute, 2 x dH_2O rinses, incubation in 1% silver nitrate 1-3 min, 2 x dH_2O rinses, incubation in sodium thiosulfate 5 min, 3 x 5 min 0.9% saline rinses. Sections were mounted within 48 hours and dehydrated and defatted as above for ChAT immunocytochemistry. AChE sections were coverslipped with DPX (Electron Microscopy Sciences, Ft. Washington, PA) because the use of Permount was specifically discouraged in the protocol.

Labeling of Brainstem Projections to dLGN with DiI

Two neonatal ferrets (P7 and P18) were perfused as above; brains were removed and postfixed in 4% paraformaldehyde at 4° C for 48 hours. Each brain was pinned in a dissecting dish and the left cerebral hemisphere was gently lifted at its caudal end to expose the left LGN. A needle was used to embed 1, 1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanine perchlorate (DiI; Molecular Probes, Eugene, OR) crystals just below the surface of the LGN and the brains were returned to individual vials of 4% paraformaldehyde. The vials were wrapped in foil to prevent photobleaching of the DiI and left in a 37° C oven for two weeks to accelerate the movement of DiI through the cell membranes; after two weeks, they were moved to RT. The P7 brain was sectioned (100 μm) in the coronal plane on a Vibratome after 16 weeks of total tracing time; the P18 brain was sectioned at 100 μm after 25 weeks. The P18 sections were counterstained with bis-benzimide (0.001%, filtered before use; 0.1M PB rinse, 10 min bis-benziminde incubation, 0.1M PB rinse). Sections were only dried long enough to fix them on glass slides and mounted with Vectashield mounting medium (Vector) to protect against photobleaching. Sections were kept in a light-excluding box at 4° C and photographed on a Leitz Diaplan (Wild Leitz, Wetzlar, West Germany) within 24 hours.

Labeling of Brainstem Projections to dLGN *In Vitro*

Two ferrets (P7 and P23) were used for in vitro tracing. After the ferrets were adequately anesthetized, brains were carefully removed from the skull and kept continuously submerged, except during transfer to Sylgard (gift of Dow Corning, Midland, MI) dish, in ice-cold artificial cerebrospinal fluid (ACSF, containing, in mM: 126 sucrose, 3 KCl, 2 $MgSO_4$, 25 $NaHCO_3$, 1 $NaHPO_4$, 2.5 $CaCl_2$, 10 dextrose, and 0.5 kynurenic acid) bubbled with carbogen gas (95% O_2/5% CO_2) until the end of the procedure. Cortex and cerebellum were dissected away to leave a thalamus-midbrain chunk. Meninges were gently removed, brains were submerged and pinned through rostromedial thalamus and caudomedial medulla in a Sylgard-lined dissection dish at RT. The P7 brain was labelled with 3% biocytin (Sigma, St. Louis, MO) in 0.05M Tris buffer (pH 8); the P23 brain was labelled with 10% biotin dextran amine, MW 3000 (BDA; Molecular Probes), also in Tris buffer. A few crystals of pontamine sky blue (Sigma) were added to each solution to allow easy visualization of the injection sites. Sharp electrodes were made from thin-walled glass capillary tubes (WPI, Sarasota, FL) on a Flaming Brown pipette puller (Sutter, San Rafael, CA); the tips were broken against a piece of AccuWipe tissue (Fort Howard, Green Bay, WI) and the electrodes were back-filled with tracing solutions. Tracer solutions were pressure injected through the head stage of Axoclamp 2A amplifier (Axon Instruments, Foster City, CA) into both dLGNs; each dLGN received between three and six injections along its dorsocaudal extent. Tracing time was 10 hours for the P7 brain and 11 hours for the P23 brain. At the end of tracing, brains were removed to 4% paraformaldehyde and kept at 4° C overnight for fixation. Tissue was cryoprotected and cut at 50 μm on the cryostat, as described above. Sections were processed as follows: incubated overnight

in 1:100 ABC Elite at RT, rinsed 2 x 5 min in PBS, rinsed 2 x 5 min in TBS and developed in DAB until a dark purple reaction product could be seen in the dLGNs. Sections were mounted from 0.01 M PB onto gelatin-subbed glass slides in rostrocaudal order. After air-drying overnight, sections were counterstained with Neutral Red (Sigma) as follows: 10 sec in 5% acetate buffer (pH 3.3), 20 sec in filtered 0.5% Neutral Red in acetate buffer, 3 x 20 sec in 5% acetate buffer, and then air-dried again. Sections were dehydrated, defatted and mounted as described above for ChAT immunocytochemistry, except that the 70% EtOH step was skipped.

RESULTS

Choline acetyltransferase (ChAT) is the synthesizing enzyme for acetylcholine and, as such, is a specific marker for cholinergic cells; acetylcholinesterase (AChE), on the other hand, is the degradative enzyme for acetylcholine and it is produced by both cholinergic and cholinoceptive cells (Eckenstein and Sofroniew, 1983). We examined the early postnatal development of both enzymes in the developing ferret dorsal lateral geniculate nucleus (dLGN) to determine: whether the arrival of cholinergic inputs in the dLGN is temporally correlated with ON/OFF retinal afferent segregation; whether cholinergic inputs are associated with specific dLGN subregions (e.g., laminar or interlaminar zones) during ON/OFF segregation; and whether the pattern of ChAT immunostaining in the dLGN changes during ON/OFF retinal afferent segregation. We examined ferret brains for ChAT and AChE staining at P8, P13 and P14 (ChAT and AChE, respectively; taken as one timepoint), P17, P20, P23, P26, P32, and adult. In addition, we retrogradely labeled projections to the dLGN at P7, P18 and P23 to compare with the date of arrival of cholinergic

inputs derived from the histological data and to confirm that these inputs do indeed originate in cholinergic nuclei of the brainstem.

Thalamic nuclei receive input from six cholinergic nuclei, four located in basal forebrain and two in upper brainstem; the upper brainstem nuclei provide the major cholinergic input to thalamus (Mesulam et al., 1983). Of the brainstem nuclei, the PPTg provides the main cholinergic input to the dLGN (and, generally, to all sensory and motor relay nuclei of the thalamus), while LDTg targets limbic, associational and intralaminar nuclei (Hallanger et al., 1987). Thus, our discussion will be focused on the development of ChAT immunostaining and AChE histochemistry in dLGN and PPTg.

Ontogenetic Trends in Cholinergic Labeling in Thalamus and Brainstem

ChAT immunoreactivity is not seen in brainstem or dLGN until P13, though there is some light staining in other thalamic regions by P8 (Figs. 1A, B). The LDTg and PPTg also lack ChAT label at P8 (Fig. 1B), suggesting that the ChAT label seen in extrageniculate thalamic regions does not originate in the brainstem, but instead is derived from basal forebrain sources of acetylcholine (Mesulam et al., 1983). From P13 to P23, ChAT staining increases throughout both thalamus and brainstem (e.g., P17: Figs. 1C, D). Between P26 (Fig. 1E, F) and P32 (Figs. 1G, H), there is a profound decrease in ChAT immunoreactivity throughout the thalamus, though ChAT-immunoreactive (ChAT+) somata and fibers are more intensely labeled in the brainstem at these ages than at any other (compare Figs. 1E and 1G with Figs. 1F and 1H, respectively). Because ChAT immunoreactivity is so intense in the brainstem at these ages, it is highly unlikely that the

decrease in thalamic labeling is a methodological artifact. Between P32 and adulthood (Figs. 1I, J), the pattern and intensity of ChAT immunoreactivity changes in both thalamus (note the dramatic increase in ChAT labeling which develops in the dLGN) and brainstem.

AChE labeling appears before ChAT immunoreactivity, appearing by P8 in both brainstem (specifically PPTg and LDTg) and thalamus; it is more intense and more diffuse than ChAT label at every age examined (Figs. 1A-H), except in the adult in which the intensity of the two labels is roughly equivalent (Figs. 1I, J; also, compare Figs. 2 and 3). Generally, AChE labeling increases throughout the thalamus (including the dLGN) between P8 and adulthood (Figs. 1A, C, E, G, I). AChE labeling in the PPTg and LDTg increases in intensity between P8 and P26, when label in the PPTg is maximal (Fig. 1F). [In contrast, LDTg labeling appears to reach its maximum during adulthood (Fig. 1J)]. By P32, the diffuse AChE label in the PPTg decreases slightly, though more individual labeled somata are discernible, both in PPTg and LDTg (Fig. 1H). In the adult, AChE label in the LDTg is still diffuse, while PPTg label is localized to somata and fibers (Fig. 1J). The diffuse and localized AChE label may reflect the two forms of AChE (the soluble and membrane-bound forms, respectively) present in both developing and mature mammalian central nervous systems (Inestroza and Ruiz, 1985; Gorenstein et al., 1991).

Postnatal Development of ChAT Labeling in dLGN

At P8, eye-specific segregation has defined the A and A1 laminae within the dLGN and cytoarchitectonic movement has begun to establish an interlaminar zone between them (Linden et

al., 1981; Cucchiaro and Guillery, 1984; Hahm, 1991b. At this age, there is almost no ChAT immunoreactivity in the dLGN (Fig. 2A). At P13, just before ON/OFF sublamination begins, ChAT+ somata have become visible in the PPTg (not shown) and the dLGN begins to show low-level ChAT immunoreactivity in the the A, A1 and C laminae (Fig. 2B). Thus, it seems that cholinergic inputs from the PPTg are not present during most of the period of eye-specific segregation but that they do indeed arrive in time to influence ON/OFF sublamination in the ferret dLGN.

At P17, when eye-specific segregation is nearly complete in the dLGN and ON/OFF segregation is ongoing, ChAT label is more intense within the neuropil of the A and C laminae and seems to be absent from interlaminar zones (Fig. 2C). At P20, ChAT immunostaining reveals pale interlaminar zones between A and A1 as well as between ON and OFF sublaminae (Fig. 2D). ChAT label is thus restricted to the C laminae and sublaminar zones within both A and A1. Also at P20, individual ChAT+ fibers, including fibers leaving the optic tract to invade the dLGN, have become plainly visible and their dense pattern of termination around the somata of presumptive (based on soma size; Guillery, 1966) relay cells is discernible even by light microscopy (Fig. 4A). By P23, laminar staining seems to be at its peak intensity (though this could not be quantified for methodological reasons discussed above) and ChAT fibers remain visible (Fig. 2E). At P26, however, ChAT labeling intensity decreases dramatically in the entire thalamus (Fig. 1E), including the dLGN, where laminar borders between A and A1 are no longer visible (Fig. 2F). However, ChAT immunoreactivity peaks in the brainstem at this age, indicating that the decrease in ChAT is localized to the thalamus and is not the result of a methodological problem (compare again Figs. 1E

and F). This is not the first report of a developmental decrease in ChAT immunostaining: McHaffie et al. (1991) reported that ChAT immunoreactivity undergoes a developmental decrease throughout the thalamus between the third and fourth postnatal weeks in cat; in addition, Ibanez et al. (1991) reported that ChAT mRNA levels drop throughout the entire rat central nervous system during the third postnatal week. In each of these reports, including this study, the attenuation in ChAT (or ChAT messenger RNA) levels is transient.

At P32, after ON/OFF sublamination is complete, the pattern of ChAT staining reverses, so that laminar zones become very pale and interlaminar zones are darkly stained (Fig. 2G). Individual ChAT+ fibers are visible coursing both through and across interlaminar zones (Fig. 4C). The higher density of ChAT+ innervation in the ON sublaminae (versus OFF sublaminae) of adult ferret dLGN which was reported by Henderson (1987a) is not apparent in our tissue at P32; sublaminar differences were still not discernible even in the adult dLGN (Fig. 2H). Also in the adult dLGN, inter-sublaminar zones are no longer clearly defined by ChAT labeling, though the interlaminar zone between A and A1 is still evident (Fig. 2H). Interestingly, ChAT labeling in the optic tract, present throughout ON/OFF sublamination and still present even at P32 (Figs. 2A-G), almost completely disappears in the adult dLGN (Fig. 2H). The disappearance of ChAT immunoreactivity in the tract might indicate that some portion of the total cholinergic input to the ferret dLGN innervates it via the optic tract during development, but is later removed.

Postnatal Development of AChE Labeling in dLGN

At P8, AChE label is most visible in the C layers of the dLGN, though pale labeling can also be

discerned in the A laminae, which are separated by an already visible interlaminar zone (Fig. 3A). AChE might be expected to appear earlier in the C layers since they are the first to show signs of cytological maturity (Linden et al., 1981) and several studies have demonstrated a temporal correlation between AChE expression and cytological maturation of neocortical neurons (Stitchel and Singer, 1987; de Carlos et al., 1995). AChE labeling remains more intense in the C layers than in the A laminae through P32 (Fig. 3), though label does intensify within the A laminae so that the interlaminar zone between them is easily identified by P17 (Fig. 3C) and the inter-sublaminar zone between the ON and OFF leaflets of the A lamina is visible by P23 (Fig. 3F). AChE labeling of interlaminar zones increases between P20 and P26 so that the interlaminar zone separating A and A1 is not easily identified at P26 (Figs. 3D-F). Between P26 and P32 (i.e., at the end of ON/OFF sublamination), the pattern of AChE labeling reverses so that AChE labeling becomes most dense within interlaminar zones, versus laminar zones, just as the pattern of ChAT labeling does (compare Figs. 2F and G with Figs. 3F and G, respectively). The pattern of AChE labeling reverses again, just as the pattern of ChAT labeling does, between P32 and adulthood (compare Figs. 2G and H with Figs. 3G and H, respectively), so that in the adult, laminar zones (both A and C) are much more densely labeled than are interlaminar zones. It is interesting that AChE labeling is diffuse and roughly localized to the somata of geniculate cells from P8 to P32 (see Figs. 4B and D for a higher-power view of AChE labeling at P20 and P32), but that it apparently becomes less associated with somata and more localized to fibers in adulthood (compare Figs. 3A-G with Fig. 3H).

Optic tract labeling for AChE completely disappears after P32 (recall that ChAT labeling also disappears after P32; see again Fig. 2H), so that the tract is not visible at all with AChE labeling in

the adult (Fig. 3H). Hutchins and Casagrande (1988) have also observed a disappearance of AChE labeling in the optic tract of tree shrews which occurs just after eye opening; they traced fibers within the optic tract to both PBg and vLGN. This might mean there is a transient cholinergic projection to the dLGN from the PBg and that this projection disappears after retinogeniculate segregation is complete. An alternative, and more likely, explanation for the disappearance of both ChAT and AChE labeling in the optic tract is an increase in myelination, which may prevent penetration of immunocytochemical and histochemical reagents, between P32 and adulthood (internal capsule labeling also disappears between these ages; compare Figs. 1G and 1I). Moore et al. (1976) found that in the cat optic tract, only 23% of fibers are myelinated by the end of the second postnatal week, which is roughly equivalent to the end of the fourth postnatal week in the ferret, but 100% of the fibers are myelinated in the adult.

Retrograde Labeling of PPTg and LDTg

There was no labeling of brainstem cholinergic nuclei by either DiI or biocytin at P7. The P7 brain, retrogradely labeled *in vitro* with biocytin, contained a central core of tissue which did not histologically react as robustly as the outer portions of the tissue; we attribute this poor staining to anoxia. The P23 brain contained a much larger core of non-reactive tissue and, in addition, the injection sites were not limited to the dLGN, so data from the P23 brain are omitted from this discussion. In the P7 brainstem, however, the raphe nuclei contained a very pale, diffuse DAB reaction product (not shown). This indicates that the time allowed for retrograde transport of label– 10 hours– was adequate for the tracer to reach the PPTg, LDTg and PBg, which lie roughly adjacent to the raphe nuclei in the brainstem. We consider this data, together with the lack of retrograde DiI labeling in the brainstem and the lack of

ChAT immunostaining at P8, to be reasonable evidence that the brainstem cholinergic inputs to dLGN do not arrive before P8. Retrograde labeling from dLGN with DiI at P18 reveals projections from three cholinergic brainstem nucei, PPTg, LDTg and PBg. The majority of retrogradely labeled somata were observed in PBg (Fig. 5E); fewer somata were labeled in PPTg (Fig. 5D); and, in LDTg, only an occasional isolated neuron was detected (Fig. 5F). In addition, the superior colliculus contained a large number of retrogradely labeled cells (Fig. 5C) and the dorsomedial periaqueductal gray (PAG) contained a few labeled neurons with extremely spiny apical processes (not shown). The presence of a projection from the PPTg, LDTg and PBg at P18 is consistent with the upregulation of ChAT and AChE in the dLGN observed at P17 in immunocytochemical and histochemical material, and supports the likelihood that these brainstem nuclei provide the cholinergic input in the dLGN.

DISCUSSION

Ontogeny of Cholinergic Inputs to Ferret dLGN and Methodological Considerations

We have shown, using both choline acetyltransferase (ChAT) immunocytochemistry and retrograde labeling, that appreciable cholinergic inputs (from three brainstem nuclei: PPTg, LDTg and PBg) to the ferret dLGN are not present during the main period of eye-specific segregation (P0 to P8), but that they arrive by P17/P18 (during ON/OFF sublamination). ChAT labeling indicates that cholinergic input arrives in the dLGN by P13, but additional retrograde labeling is required to confirm this result. Interestingly, ChAT immunoreactivity was also not detected in the brainstem cholinergic nuclei before P13. This may indicate that there is little or no acetylcholine synthesis in the brainstem nuclei before this age; alternatively, our detection threshold for ChAT might be

relatively high, since the anti-ChAT antibody we used does not have any previously reported reactivity in ferret tissue and, initially, we had considerable methodological problems with the ChAT immunocytochemistry in the neonatal brain tissue. [The most serious of these problems concerned the use of Triton: sections processed without Triton only showed very pale staining in somata (i.e., no fiber staining whatsoever) whereas sections processed with greater than 0.1% Triton lost structural integrity and showed nonspecific ChAT staining (e.g., within geniculate cells). The second problem concerned the temperature at which the primary antibody incubation took place: sections incubated in primary antibody at 4° C, even for as long as 72 hours, never exhibited the same intensity of ChAT staining as sections incubated at RT for much shorter time periods.]

If cholinergic inputs are to influence ON/OFF sublamination by altering dLGN relay cell excitability, they might be expected to contact relay cells directly. ChAT labeling is concentrated in laminar zones (i.e., cell-rich zones) from its initial appearance at P13 until P26 (Fig. 2), when ChAT labeling intensity drops throughout the dLGN and the thalamus in general (though brainstem labeling is very intense at this age). In addition, dense ChAT+ labeling surrounds the somata of large geniculate cells (presumably relay cells, since interneurons tend to be much smaller in size; Guillery, 1966) during ON/OFF sublamination (Fig. 4A) but cease to form such networks after sublamination is complete (Fig. 4C). [Interestingly, the pattern of AChE labeling around geniculate somata does not exhibit a parallel change (Figs. 4B, D)]. Thus, cholinergic brainstem inputs are in the right place at the right time to influence ON/OFF sublamination, though they arrive too late to influence eye-specific segregation.

Besides influencing ON/OFF sublamination by generally increasing relay cell excitability and, perhaps, plasticity during development, cholinergic brainstem inputs might also differentially regulate the excitability of ON versus OFF relay cells. An anatomical investigation by Henderson (1987a) demonstrated that ChAT+ fiber innervation of the ON sublamina was greater than innervation of the OFF sublamina in the adult ferret dLGN. We did not find any differential ChAT labeling in neonates or in the adult; however, two methodological differences exist between Henderson's study and our own which might explain this discrepancy. First, Henderson exclusively used ferrets under one year of age, while the adult ferret used in this study was 25 months old, and it is possible that remodeling of cholinergic afferents continues after the first year of life. Secondly, the DAB reaction product in our adult dLGN tissue was exceedingly dark which may have obscured subtle differences in the densities of cholinergic innervation of ON and OFF sublaminae; inter-sublaminar zones may not be visible in the adult dLGN for the same reason (Fig. 2H).

It must be noted that immunocytochemical detection of ChAT is not a measure of ChAT activity. Two developmental studies of ChAT activity in cat visual system have shown an extremely protracted development of ChAT activity in dLGN. Potempska et al. (1979) showed that ChAT activity levels in the dLGN rise rapidly during the first five postnatal weeks, though they do not approach adult levels until approximately the sixteenth postnatal week; this is in contrast to ChAT activity in visual cortex, which maintains a relatively consistent level of activity, but shows a transient developmental increase which peaks around the twelfth postnatal week before returning to baseline levels. Fosse et al. (1989) also showed that ChAT activity levels do not reach adult levels until the seventeenth postnatal week in cat dLGN. We also observed a profound increase in ChAT

labeling between P32 and adulthood (Fig. 2); this increase might underlie increases in ChAT activity. If the intensity of ChAT immunolabeling is indeed well-correlated with ChAT activity, the difficulty we experienced in immunolabeling neonatal tissue for ChAT might simply be explained by a paucity of ChAT at those ages and not by an incompatibility between the ferret enzyme and the anti-ChAT antibody. However, in at least one other developmental system, ChAT immunolabeling and ChAT activity are not well-correlated: Sakaguchi and Saito (1991) have shown, in a study of the male zebra finch song-control system, that ChAT activity transiently and significantly increases during the critical period for song learning, while ChAT immunolabeling remains relatively constant.

Brainstem inputs from PPTg and LDTg to dLGN are not exlusively cholinergic. Noradrenergic neurons (de Lima and Singer, 1987; Henderson, 1987b; Fitzpatrick et al., 1989), glycinergic neurons (Fort et al., 1993), and glutamatergic neurons (Lavoie and Parent, 1994) are also present in both nuclei. In addition, individual cholinergic parabrachial neurons in the cat colocalize reduced nicotinamide adenine dinucleotide phosphate-diaphorase (NADPHd), which regulates nitric oxide synthesis (Bickford et al., 1993); in squirrel monkey, cholinergic PPTg and LDTg neurons colocalize glutamate (Lavoie and Parent, 1994); and in rat, cholinergic LDTg neurons colocalize substance P (Vincent et al., 1983; Sutin and Jacobowitz, 1990). Development of NADPHd staining has been studied in ferret (Cramer et al., 1995) and cat (Guido et al., 1997) dLGN. In both cases, there is an initial paucity of NADPHd labeling followed by a rise in both the intensity of the label and in the number of dLGN neurons expressing NADPHd. This increase peaks around P28 and then falls to adult levels (when very few geniculate somata are stained) by P41 or P42. Because NADPHd expression in the dLGN is endogenous during development, but almost exclusively exogenous

(from brainstem cholinergic inputs) in adulthood, Guido et al. (1997) have suggested two roles for nitric oxide, a retrograde messenger function during developmental plasticity and an orthograde amplifier function on retinogeniculate transmission during adult plasticity. Recently, Williams et al. (1997) confirmed that activation of the LDTg causes an increase in nitric oxide concentrations within the rat thalamus. Because NADPHd labeling is present in the neuropil of the ferret dLGN by P0 (Cramer et al., 1995) and because cholinergic brainstem inputs arrive in time to influence ON/OFF sublamination, we propose that nitric oxide might also act in an orthograde manner during development and that its effects would redouble the excitatory effects that cholinergic inputs have on relay cells.

Ontogeny of AChE Labeling in Ferret dLGN

Acetylcholinesterase (AChE) histochemistry worked quite reliably in both neonatal and adult ferret brain tissue, but since AChE is not a specific marker for cholinergic cells (i.e., it can be produced by non-cholinergic cells; see Eckenstein and Sofroniew, 1983), it does not provide unambiguous information about the ontogeny of cholinergic innervation of the dLGN. Thus, the source(s) of AChE in the ferret dLGN may include, besides cholinergic inputs from PPTg, LDTg and PBg, afferents from the retina, visual cortex, thalamus and non-cholinergic brainstem nuclei; in addition, AChE may be produced by geniculate neurons themselves. In addition, AChE may be released from neuronal somata, axon terminals and dendrites (Greenfield et al., 1983a; Greenfield et al., 1983b). Unless the source(s) of AChE within the dLGN can be identified, the ontogeny of AChE labeling cannot reasonably be linked to the ontogeny of cholinergic innervation of the dLGN.

There is, perhaps, even greater ambiguity in the role(s) of AChE, especially in the developing brain. AChE, besides hydrolyzing acetylcholine, seems to perform several other functions in the brain, including hydrolysis of other neurochemicals, such as substance P (known to colocalize with tegmental cholinergic neurons; see Vincent et al., 1983) and met- and leu-enkephalins (Chubb et al., 1980); more pertinently here, it may have both morphogenic and synaptogenic roles in developing neural systems (Kristt, 1979; Harvey and MacDonald, 1985; Robertson, 1987; Robertson et al., 1987; Robertson et al., 1988a; Robertson et al., 1988b; Robertson et al., 1989; Layer, 1990). In developing thalamocortical systems of rodents, AChE expression in primary sensory nuclei of the thalamus, including the dLGN, coincides with the period during which thalamocortical axons are elongating and making synapses in their respective cortical targets (Robertson et al., 1989). Interestingly, AChE is also transiently expressed in developing rat visual cortex during the period of geniculocortical synaptogenesis and its expression can be dramatically reduced by blockade of retinogeniculate activity or by lesions which disrupt the geniculocortical projection, suggesting that AChE expression there is activity-dependent (Robertson et al., 1987; Robertson et al., 1988a; Robertson et al., 1988b; Robertson et al., 1989). ChAT activity in developing rat visual cortex is not altered by a similar activity blockade (Robertson et al., 1988b). Perhaps, then, AChE labeling in the developing ferret dLGN is related to the morphological and synaptic changes which characterize its early postnatal development; however, it must be noted that AChE expression in the ferret dLGN is not transient as is AChE expression in the rodent dLGN and visual cortex. Nevertheless, it would be interesting to determine whether activity blockade, often used to probe mechanisms of retinogeniculate development, might also influence AChE expression in the dLGN.

Role of Cholinergic Inputs in Developmental and Adult Neural Plasticity

The relationship between cholinergic inputs and mechanisms of neural plasticity, both physiological and anatomical, have been intensely examined in many brain regions. Since brainstem cholinergic inputs arrive in the ferret dLGN in time to influence ON/OFF sublamination, we suggest that they play at least a permissive role in the anatomical plasticity of the retinogeniculate projection during this developmental period. Further anatomical and physiological investigation is required before any stronger statement can be made regarding the specific role of cholinergic inputs in ON/OFF sublamination in the ferret dLGN; however, there is an extensive body of evidence suggesting that cholinergic inputs can influence both anatomical and physiological plasticity in other neural systems. The retinotectal system of submammalian vertebrates undergoes retinal axon segregation whose mechanisms are very similar to retinogeniculate segregation in mammals (see Constantine-Paton et al., 1990; Goodman and Shatz, 1993 for reviews). Schmidt (1995) has demonstrated that extrinsic cholinergic inputs to the optic tectum are required for retinotopic sharpening of the glutamatergic retinotectal projection after optic nerve crush in the goldfish.

Investigations of developmental and adult plasticity in mammalian sensory cortical areas has provided much evidence to support the notion that modulatory cholinergic inputs significantly influence cortical plasticity; some of these studies have also elucidated mechanisms by which acetylcholine produces its effects. In kitten visual cortex, Gu and Singer (1989) have shown that blockade of muscarinic, but not nicotinic, acetylcholine receptors prevents ocular dominance plasticity induced by monocular deprivation. In developing mouse sensorimotor cortex, cholinergic deafferentation at birth causes delays and alterations in cytoarchitectonic differentiation, disorganization of the thalamocortical projection (i.e.,

barrel formation is prevented), and disruption of boundary formation between layers V and VI and between layers III and IV (Hohmann et al., 1988; Hohmann et al, 1991a, 1991b). In adult cat somatosensory cortex, Juliano et al. (1991) have shown that cholinergic depletion prevents an increase in metabolic activity in regions adjacent to an area deprived of afferent activity by digit removal, suggesting that cholinergic inputs are involved in maintenance and plasticity of somatotopic maps. Also in cat somatosensory cortex, Metherate et al. (1988) found that acetylcholine, when delivered concurrently with glutamate or sensory stimulation, produces a marked enhancement in subsequent neuronal responses to either glutamate or sensory stimuli. In adult rat auditory cortex, Aramakis et al. (1997) used a muscarinic agonist to produce a dose-dependent enhancement of NMDA (but not AMPA) receptor-mediated neuronal responses to glutamate; in addition, they provided evidence that muscarinic agonists cause this long-lasting enhancement of NMDA receptor function via a G-protein. Considered together, these anatomical and physiological studies provide strong evidence that modulatory cholinergic inputs can influence anatomical and physiological plasticity both in developing and adult neural systems.

CONCLUSION

Thus, it is reasonable to postulate that, since brainstem cholinergic inputs arrive in the dLGN near the beginning of the ON/OFF sublamination period, they might influence the activity-, NMDA receptor- and NO-dependent developmental plasticity which underlies retinogeniculate sublamination in the ferret dLGN. We suggest two possible mechanisms (see also INTRODUCTION) by which brainstem cholinergic inputs might play such a role: they might increase the responsiveness of relay cells so that reponses to retinal inputs are extremely reliable (perhaps especially in the ON sublamina); and/or they might facilitate

synaptic strengthening (which may be correlated with synaptic stabilization) by enhancing NMDA receptor function (hence enhancing downstream effects of NMDA receptor activation, including, e.g., NO production) at developing retinogeniculate synapses.

REFERENCES

Apfelbach, R and Wester, U. 1977. The quantitative effect of visual and tactile stimuli on the prey-catching behavior of ferrets (*Putorius furo* L.). Behavioural Processes (2): 187-200.

Aramakis, VB, Bandrowski, AE and Ashe, JH. 1997. Activation of muscarinic receptors modulates NMDA receptor-mediated responses in auditory cortex. Exp. Brain Res. (113): 484-496.

Beaulieu, C and Cynader, M. 1992. Preferential innervation of immunoreactive choline acetyltransferase synapses on relay cells of the cat's lateral geniculate nucleus: a double-labelling study. Neurosci. (47): 33-44.

Bickford, ME, Gunluk, AE, Guido, W and Sherman, SM. 1993. Evidence that cholinergic axons from the parabrachial region of the brainstem are the exclusive source of nitric oxide in the lateral geniculate nucleus of the cat. J. Comp. Neurol. (334): 410-430.

Bliss, TV and Collingridge, GL. 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature (361): 31-39.

Campbell, G and Shatz, CJ. 1992. Synapses formed by identified retinogeniculate axons during the segregation of eye input. Neurosci. (12): 1847-1858.

Chen, SJ, Sweatt, JD and Klann, E. 1997. Enhanced phosphorylation of the postsynaptic protein kinase C substrate RC3/neurogranin during long-term potentiation. Brain Res. (749): 181-187.

Chubb, IW, Hodgson, AJ and White, GH. 1980. Acetylcholinesterase hydrolyzes substance P. Neuroscience (5): 2065-2072.

Clasca, F., Angelucci, A. and Sur, M. 1995. Layer-specific programs of development in neocortical projection neurons. Proc. Natl. Acad. Sci. USA (92): 11145-11149.

Constantine-Paton, M, Cline, HT and Debski, E. 1990. Patterned activity, synaptic convergence and the NMDA receptor in developing visual pathways. Annu. Rev. Neurosci. (13): 129-154.

Cook, PM, Prusky, G and Ramoa, AS. 1996. Role of spontaneous retinal activity in reorganization of retinogeniculate connections during development. Soc. Neurosci. Abstr. (22): 304.4.

Cramer, KS, Angelucci, A, Hahm, JO, Bogdanov, MB and Sur, M. 1996. A role for nitric oxide in the development of the ferret retinogeniculate projection. J Neurosci (16): 7995-8004.

Cramer, KS, Moore, CI and Sur, M. 1995. Transient expression of NADPH-diaphorase in the lateral geniculate nucleus of the ferret during early postnatal development. J. Comp. Neurol. (353): 306-316.

Cramer, KS and Sur, M. 1996. The role of NMDA receptors and nitric oxide in retinogeniculate

development. Prog. Brain Res. (108): 235-244.

Cramer, KS and Sur, M. 1997. Blockade of afferent impulse activity disrupts on/off sublamination in the ferret lateral geniculate nucleus. Dev. Brain Res. (98): 287-290.

Cucchiaro, JB, Bickford, ME and Sherman, SM. 1991. A GABAergic projection from the pretectum to the dorsal lateral geniculate nucleus in the cat. Neurosci. (41): 213-226.

Cucchiaro, J and Guillery, RW. 1984. The development of the retinogeniculate pathways in normal and albino ferrets. Proc. R. Soc. Lond. (B: 223): 141-164.

de Carlos, JA, Schlaggar, BL and O'Leary, DD. 1995. Development of acetylcholinesterase-positive thalamic and basal forebrain afferents to embryonic rat neocortex. Exp. Brain Res. (104): 385-401.

de Lima, AD, Montero, VM and Singer, W. 1985. The cholinergic innervation of the visual thalamus: an EM immunocytochemical study. Exp. Brain Res. (59): 206-212.

de Lima, AD and Singer, W. 1987. The brainstem projection to the lateral geniculate nucleus in the cat: identification of cholinergic and monoaminergic elements. J. Comp. Neurol. (259): 92-121.

Dossi, RC, Nunez, A and Steriade, M. 1992. Electrophysiology of a slow (0.5-4 Hz) intrinsic oscillation of cat thalamocortical neurones *in vivo*. J. Physiol. (447): 215-234.

Dubin, MW and Cleland, BG. 1977. Organization of visual inputs to interneurons of lateral geniculate nucleus of the cat. J. Neurophysiol. (40): 410-427.

Eckenstein, F and Sofroniew, MV. 1983. Identification of central cholinergic neurons containing both choline acetyltransferase and acetylcholinesterase and of central neurons containing only acetylcholinesterase. J. Neurosci. (3): 2286-2291.

Erisir, A, Van Horn, SC, Bickford, ME and Sherman, SM. 1997a. Immunocytochemistry and distribution of parabrachial terminals in the lateral geniculate nucleus of the cat: a comparison with corticogeniculate terminals. J. Comp. Neurol. (377): 535-549.

Erisir, A, Van Horn, SC and Sherman, SM. 1997b. Relative numbers of cortical and brainstem inputs to the lateral geniculate nucleus. Proc. Natl. Acad. Sci. USA (94): 1517-1520.

Erisir, A, Van Horn, SC and Sherman, SM. 1998. Distribution of synapses in the lateral geniculate nucleus of the cat: differences between laminae A and A1 and between relay cells and interneurons. J. Comp. Neurol. (390): 247-255.

Esguerra, M, Kwon, YH and Sur, M. 1992. Retinogeniculate EPSPs recorded intracellularly in the ferret lateral geniculate nucleus in vitro: role of NMDA receptors.
Vis. Neurosci. (8): 545-555.

Fitzpatrick, D, Diamond, IT and Raczkowski, D. 1989. Cholinergic and monoaminergic innervation of the cat's thalamus: comparison of the lateral geniculate nucleus with other principal sensory nuclei. J. Comp. Neurol. 1(288): 647-675.

Fort, P, Luppi, PH and Jouvet, M. 1993. Glycine-immunoreactive neurones in the cat brain stem reticular formation. Neuroreport (4): 1123-1126.

Fosse, VM, Heggelund, P and Fonnum, F. 1989. Postnatal development of glutamatergic, GABAergic, and cholinergic neurotransmitter phenotypes in the visual cortex, lateral geniculate nucleus, pulvinar, and superior colliculus in cats. J. Neurosci. (9): 426-435.

Francesconi, W, Muller, CM and Singer, W. 1988. Cholinergic mechanisms in the reticular control of transmission in the cat lateral geniculate nucleus. J. Neurophysiol. (59): 1690-1718.

Galli, L and Maffei, L. 1988. Spontaneous impulse activity of rat retinal ganglion cells in prenatal life. Science (242): 90-91.

Gilbert, CD and Kelly, JP. 1975. The projections of cells in different layers of the cat's visual cortex. J. Comp. Neurol. (163): 81-105.

Godement, P, Wang, LC and Mason, CA. 1994. Retinal axon divergence in the optic chiasm: dynamics of growth cone behavior at the midline. J. Neurosci. (11): 7024-7039.

Godwin, DW, Vaughan, JW and Sherman, SM. 1996. Metabotropic glutamate receptors switch visual response mode of lateral geniculate nucleus cells from burst to tonic. J. neurophysiol. (76): 1800-1816.

Goodman, CS and Shatz, CJ. 1993. Developmental mechanisms that generate precise patterns of neuronal connectivity. Cell (72 Suppl.): 77-98.

Gorenstein, C, Gallardo, KA and Robertson, RT. 1991. Molecular forms of acetylcholinesterase in cerebral cortex and dorsal thalamus of developing rats. Brain Res. Dev. Brain Res. (61): 271-276.

Greenfield, S. 1984. Acetylcholinesterase may have novel functions in the brain. TINS (7): 364-368.

Greenfield, SA, Cheramy, A and Glowinski, J. 1983a. Evoked release of proteins from central neurons in vivo. J. Neurochem. (40): 1048-1057.

Greenfield, SA, Grunewald, RA, Foley, P, Shaw, SG. 1983b. Origin of various enzymes released from the substantia nigra and caudate nucleus: effects of 6-hydroxydopamine lesions of the nigro-striatal pathway. J. Comp. Neurol. (214): 87-92.

Greiner, JV and Weidman, TA. 1981. Histogenesis of the ferret retina. Exper. Eye Res. (33): 315-322.

Gu, Q and Singer, W. 1989. The role of muscarinic acetylcholine receptors in ocular dominance plasticity. EXS (57): 305-314.

Gu Q, Singer W Guido, W, Lu, SM, Vaughan, JW, Godwin, DW and Sherman, SM. 1995. Receiver operating characteristic (ROC) analysis of neurons in the cat's lateral geniculate nucleus during tonic and burst response mode. Vis. Neurosci. (12) 723-741.

Guido, W, Scheiner, CA, Mize, RR and Kratz, KE. 1997. Developmental changes in the pattern of NADPH-diaphorase staining in the cat's lateral geniculate nucleus. Vis. Neurosci. (14): 1167-1173.

Guido, W and Sherman, SM. 1998. Response latencies of cells in the cat's lateral geniculate nucleus are less variable during burst than tonic firing. Vis. Neurosci. (15): 231-237.

Guillery, RW. 1966. A study of Golgi preparations from the dorsal lateral geniculate nucleus of the adult cat. J.Comp. Neurol. (128): 21-50.

Hahm, JO, Langdon, RB and Sur, M. 1991a. Disruption of retinogeniculate afferent segregation by antagonists to NMDA receptors. Nature (351): 568-570.

Hahm, JO. 1991b. Influence of the target on development of the ferret retinogeniculate projection. Doctoral Dissertation, Dept. of Brain and Cognitive Sciences, Massachusetts Institute of Technology.

Hallanger, AE, Levey, AI, Lee, HJ, Rye, DB and Wainer, BH. 1987. The origins of cholinergic and other subcortical afferents to the thalamus in the rat. J. Comp. Neurol. (262): 105-124.

Harrington, ME. 1997. The ventral lateral geniculate nucleus and the intergeniculate leaflet: interrelated structures in the visual and circadian systems. Neurosci. Biobehav. Rev. (21): 705-727.

Harting, JK, Van Lieshout, DP, Hashikawa, T and Weber, JT. 1991. The parabigeminogeniculate projection: connectional studies in eight mammals. J. Comp. Neurol. (305): 559-581.

Hartveit, E and Heggelund, P. 1994. Response variability of single cells in the dorsal lateral geniculate nucleus of the cat. Comparison with retinal input and effect of brainstem stimulation. J. Neurophysiol. (72): 1278-1289.

Hartveit, E and Heggelund, P. 1995. Brainstem modulation of signal transmission through the cat dorsal lateral geniculate nucleus. Exp. Brain Res. (103): 372-384.

Harvey, AR and Mac Donald, AM. 1985. The development of acetylcholinesterase activity in normal and transplanted superior colliculus in rats. J. Comp. Neurol. (240): 117-127.

Hebb, DO. 1949. The organization of behavior. New York (Wiley).

Heggelund, P and Hartveit, E. 1990. Neurotransmitter receptors mediating excitatory input to cells in the cat lateral geniculate nucleus. I. Lagged cells. J. Neurophysiol. (63): 1347-1360.

Henderson, Z. 1987a. Cholinergic innervation of ferret visual system. Neuroscience (20): 503-518.

Henderson, Z. 1987b. Overlap in the distribution of cholinergic and catecholaminergic neurons in the upper brainstem of the ferret. J. Comp. Neurol. (265): 581-592.

Henderson, Z. 1987c. Source of cholinergic input to ferret visual cortex. Brain Res. (412): 261-268.

Henderson, Z and Sherriff, FE. 1991. Distribution of choline acetyltransferase immunoreactive axons and terminals in the rat and ferret brainstem. J. Comp. Neurol. (314): 147-163.

Hendrickson, AE, Wilson, JR and Ogren, MP. 1978. The neuroanatomical organization of pathways between the dorsal lateral geniculate nucleus and visual cortex in Old World and New World primates. J. Comp Neurol. (182): 123-136.

Hohmann, CF, Brooks, AR and Coyle, JT. 1988. Neonatal lesions of the basal forebrain cholinergic neurons result in abnormal cortical development. Brain Res. (470): 253-264.

Hohmann, CF, Kwiterovich, KK, Oster-Granite, ML and Coyle, JT. 1991a. Newborn basal forebrain lesions disrupt cortical cytodifferentiation as visualized by rapid Golgi staining. Cereb. Cortex (1): 143-157.

Hohmann, CF, Wilson, L and Coyle, JT. 1991. Efferent and afferent connections of mouse sensory-motor cortex following cholinergic deafferentation at birth. Cereb. Cortex (1): 158-172.

Hu, B, Bouhassira, D, Steriade, M and Deschenes, M. 1988. The blockage of ponto-geniculo-occipital waves in the cat lateral geniculate nucleus by nicotinic antagonists. Brain Res. (473): 394-397.

Hu, B, Steriade, M and Deschenes, M. 1989a. The effects of brainstem peribrachial stimulation on neurons of the lateral geniculate nucleus. Neurosci. (31): 13-24.

Hutchins, JB and Casagrande, VA. 1988. Development of acetylcholinesterase activity in the lateral geniculate nucleus. J. Comp. Neurol. (275): 241-253.

Hutchins, JB and Casagrande, VA. 1990. Development of the lateral geniculate nucleus: interactions between retinal afferent, cytoarchitectonic, and glial cell process lamination in ferrets and tree shrews. J. Comp. Neurol. (298): 113-128.

Ibanez, CF, Ernfors, P, and Persson, H. 1991. Developmental and regional expression of choline acetyltransferase mRNA in the rat central nervous system. J. Neurosci. Res. (29): 163-171.

Inestroza, NC and Ruiz, G. 1985. Membrane-bound form of acetylcholinesterase activated during

postnatal development of the rat somatosensory cortex. Dev. Neurosci. (7): 120-132.

Jeon, CJ, Gurski, MR and Mize, RR. 1997. Glutamate containing neurons in the cat superior colliculus revealed by immunocytochemistry. Vis. Neurosci. (14): 387-393.

Jiang, ZD, King, AJ and Moore, DR. 1996. Topographic organization of projection from the parabigeminal nucleus to the superior colliculus in the ferret revealed with fluorescent latex microspheres. Brain Res. (743): 217-232.

Johnson, JK and Casagrande, VA. 1993. Prenatal development of axon outgrowth and connectivity in the ferret visual system. Visual Neuroscience (10): 117-130.

Juliano, SL, Ma, W and Eslin, D. 1991. Cholinergic depletion prevents expansion of topographic maps in somatosensory cortex. Proc. Natl. Acad. Sci. USA (88): 780-784.

Kostopoulos, G, Gloor P, Pellegrini, A and Gotman, J. 1981a. A study of the transition from spindles to spike and wave discharge in feline generalized penicillin epilepsy: microphysiological features. Exp. Neurol. (73): 55-77.

Kostopoulos, G, Gloor, P, Pellegrini, A, and Siatitsas, I. 1981b. A study of the transition from spindles to spike and wave discharge in feline generalized penicillin epilepsy: EEG features. Exp. Neurol. (73): 43-54.

Kristt, DA. 1979. Development of neocortical circuitry: histochemical localization of acetylcholinesterase in relation to the cell layers of rat somatosensory cortex. J. Comp. Neurol. (186): 1-15.

Kwon, YH, Nelson, SB, Toth, LJ and Sur, M. 1992. Effect of stimulus contrast and size on NMDA receptor activity in cat lateral geniculate nucleus. J. Neurophysiol. (68): 182-196.

Lavoie, B and Parent, A. 1994. Pedunculopontine nucleus in the squirrel monkey: distribution of cholinergic and monoaminergic neurons in the mesopontine tegmentum with evidence for the presence of glutamate in cholinergic neurons. J. Comp. Neurol. (344): 190-209.

Layer, PG. 1990. Cholinesterases preceding major tracts in vertebrate neurogenesis. Bioessays (12): 415-420.

Lee, KH and McCormick, DA. 1995. Acetylcholine excites GABAergic neurons of the ferret perigeniculate nucleus through nicotinic receptors. J. Neurophysiol. (73): 2123-2128.

Linden, DC, Guillery, RW and Cucchiaro, J. 1981. The dorsal lateral geniculate nucleus of the normal ferret and its postnatal development. J. Comp. Neurol (203): 189-211.

Livingstone, MS and Hubel, DH. 1981. Effects of sleep and arousal on the processing of visual information in the cat. Nature (291): 554-561.

Lu, SM, Guido, W and Sherman, SM. 1992. Effects of membrane voltage on receptive field properties of lateral geniculate neurons in the cat: contributions of the low-threshold Ca2+ conductance. J. Neurophysiol. (68): 2185-2198.

Lu, SM, Guido, W and Sherman, SM. 1993. The brain-stem parabrachial region controls mode of response to visual stimulation of neurons in the cat's lateral geniculate nucleus. Vis. Neurosci. (10): 631-642.

Maffei, L and Galli-Resta, L. 1990. Correlations in the discharges of of neighboring rat retinal ganglion cells during prenatal life. Proc. Natl. Acad. Sci. USA (87): 2861-2864.

Marks, GA and Roffwarg, HP. 1989. The cholinergic influence upon rat dorsal lateral geniculate nucleus is dependent on state of arousal. Brain Res. (494): 294-306.

McCarley, RW, Benoit, O and Barrionuevo, G. 1983. Lateral geniculate nucleus unitary discharge in sleep and waking: state- and rate-specific aspects. J. Neurophysiol. (50): 798-818.

McCormick, DA. 1992. Cellular mechanisms underlying cholinergic and noradrenergic modulation of neuronal firing mode in the cat and guinea pig dorsal lateral geniculate nucleus. J. Neurosci. (12): 278-289.

McCormick, DA and Bal, T. 1997. Sleep and arousal: thalamocortical mechanisms. Annu. Rev. Neurosci. (20): 185-215.

McCormick, DA and Pape, HC. 1990. Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones. J. Physiol. (431): 291-318.

McCormick, DA and Prince, DA. 1987. Actions of acetylcholine in the guinea-pig and cat medial and lateral geniculate nuclei, in vitro. J. Physiol. Lond. (392): 147-165.

McCormick, DA, Trent, F and Ramoa, AS. 1995. Postnatal development of synchronized network oscillations in the ferret dorsal lateral geniculate and perigeniculate nuclei. J. Neurosci. (15): 5739-5752.

McCormick, DA and von Krosigk, M. 1992. Corticothalamic activation modulates thalamic firing through glutamate "metabotropic" receptors. Proc. Natl. Acad. Sci. USA (89): 2774-2778.

McHaffie, JG, Beninato, M, Stein, BE and Spencer, RF. 1991. Postnatal development of acetylcholinesterase in, and cholinergic projections to, the cat superior colliculus. J. Comp. Neurol. (313): 113-131.

Meister, M, Wong, ROL, Baylor, DA and Shatz, CJ. 1991. Synchronous bursts of action potentials in ganglion cells of the developing mammalian retina. Science (252): 939-943.

Mesulam, MM, Mufson EJ, Wainer, BH and Levey, AI. 1983. Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). Neurosci. (10): 1185-1201.

Metherate, R, Tremblay, N and Dykes, RW. 1988. Transient and prolonged effects of acetylcholine on responsiveness of cat somatosensory cortical neurons. J. Neurophysiol. (59): 1253-1276.

Mitrofanis, J. 1994. Development of the thalamic reticular nucleus in ferrets with special reference to the perigeniculate and perireticular cell groups. Eur. J. Neurosci. (6): 253-263.

Mize, RR and Payne, MP. 1987. The innervation density of serotonergic (5-HT) fibers varies in different subdivisions of the cat lateral geniculate nucleus complex. Neurosci. Lett. (82): 133-139.

Mooney, R, Madison, DV and Shatz, CJ. 1993. Enhancement of transmission at the developing retinogeniculate synapse. Neuron (10): 815-825.

Moore, CL, Kalil, R and Richards, W. 1976. Development of myelination in optic tract of the cat. J. Comp. Neurol. (165): 125-136.

Moruzzi, G and Magoun, HW. 1949. Brain stem reticular formation and the activation of the EEG. Electroencephalogr. Clin. Neurophysiol. (1): 455-473.

Nelson, R, Famiglietti, EV Jr and Kolb, H. 1978. Intracellular staining reveals different levels of stratification for on- and off-center ganglion cells in cat retina. J. Neurophysiol. (41): 472-483.

O'Leary, DM. 1992. Development of connectional diversity and specificity in the mammalian brain by the pruning of collateral projections. Curr. Opin. Neurobiol. (2): 70-77.

Papadopoulos, GC and Parnavelas, JG. 1990. Distribution and synaptic organization of serotoninergic and noradrenergic axons in the lateral geniculate nucleus of the rat. J. Comp. Neurol. (294): 345-355.

Parkinson, D, Kratz, KE and Daw, NW. 1988. Evidence for a nicotinic component to the actions of acetylcholine in cat visual cortex. Exp. Brain Res. (73) 553-568.

Patel, NC and Bickford, ME. 1997. Synaptic targets of cholinergic terminals in the pulvinar nucleus of the cat. J. Comp. Neurol. (387): 266-278.

Penn, AA, Riquelme, PA, Feller, MB and Shatz, CJ. 1998. Competition in retinogeniculate patterning driven by spontaneous activity. Science (279): 2108-2112.

Potempska, A, Skangiel-Kramska, J and Kossut, M. 1979. Development of cholinergic enzymes and adenosine-triphosphatase activity of optic system of cat in normal and restricted visual input conditions. Dev. Neurosci. (2): 38-45.

Raczkowski, D and Fitzpatrick, D. 1989. Organization of cholinergic synapses in the cat's dorsal lateral

geniculate and perigeniculate nuclei. J. Comp. Neurol. (288): 676-690.

Ramoa, AS and McCormick, DA. 1994a. Developmental changes in electrophysiological properties of LGNd neurons during reorganization of retinogeniculate connections.
J. Neurosci. (14): 2089-2097.

Ramoa, AS and McCormick, DA. 1994b. Enhanced activation of NMDA receptor responses at the immature retinogeniculate synapse. J. Neurosci. (14): 2098-2105.

Reiner, PB and Vincent, SR. 1987. Topographic relations of cholinergic and noradrenergic neurons in the feline pontomesencephalic tegmentum: an immunohistochemical study. Brain Res. Bull. (19): 705-714.

Robertson, RT. 1987. A morphogenic role for transiently expressed acetycholinesterase in developing thalamocortical systems? Neurosci. Lett. (75): 259-264.

Robertson, R., Ambe, RK and Yu, J. 1989. Intraocular injections of tetrodotoxin reduce transiently expressed acetylcholinesterase activity in developing rat visual cortex. Brain Res. Dev. Brain Res. (46): 69-84.

Robertson, RT, Fogolin, RP, Tijerina, AA and Yu, J. 1987. Effects of neonatal monocular and binocular enucleation on transient acetylcholinesterase activity in developing rat visual cortex. Brain. Res. (430): 185-197.

Robertson, RT, Hanes, MA and Yu, J. 1988a. Investigations of the origins of transient acetylcholinesterase activity in developing rat visual cortex. Brain Res. (469): 1-23.

Robertson, RT, Hohmann, CF, Bruce, JL and Coyle, JT. 1988b. Neonatal enucleations reduce specific activity of acetylcholinesterase but not choline acetyltransferase in developing rat visual cortex. Dev. Brain Res. (39): 298-302.

Robertson, RT, Poon, HK, Mirrafati, SJ and Yu, J. 1989. Transient patterns of acetylcholinesterase activity in developing thalamus: a comparative study in rodents. Brain Res. Dev. Brain Res. (48): 309-315.

Sakaguchi, H and Saito, N. 1991. Developmental change of cholinergic activity in the forebrain of the zebra finch during song learning. Brain Res. Dev. Brain Res. (62): 223-228.

Sawai H, Morigiwa, K and Fukuda, Y. 1988. Effects of EEG synchronization on visual responses of the cat's geniculate relay cells: a comparison among Y, X and W cells. Brain Res. (455): 394-400.

Schuman, EM and Madison, DV. 1991. A requirement for the intercellular messenger nitric oxide in long-term potentiation. Science (254): 1503-1506.

Semba, K and Fibiger, HC. 1989. Organization of central cholinergic systems. Prog. in Brain Res. (79):

37-63.

Shatz, CJ. 1983. The prenatal development of the cat's retinogeniculate pathway. J. Neurosci. (4): 1378-1397.

Shatz, CJ. 1990. Competitive interactions between retinal ganglion cells during prenatal development. J. Neurobiol. (21): 197-211.

Shatz, CJ and Kirkwood, PA. 1984. Prenatal development of functional connections in the cat's retinogeniculate pathway. J. Neurosci. (4): 1378-1397.

Shatz, CJ and Stryker, M. 1988. Prenatal tetrodotoxin infusion blocks segregation of retinogeniculate afferents. Science (242): 87-89.

Sherman, SM. 1996. Dual response modes in lateral geniculate neurons: mechanisms and functions. Vis. Neurosci. (13): 205-213.

Sherman, SM and Koch, C. 1986. The control of retinogeniculate transmission in the mammalian lateral geniculate nucleus. Exp Brain Res (63): 1-20.

Shute, CC and Lewis, PR. 1967. The ascending cholinergic reticular system: neocortical, olfactory and subcortical projections. Brain (90):497-520.

Sillito AM, Kemp, JA and Berardi, N. 1983. The cholinergic influence on the function of the cat dorsal lateral geniculate nucleus (dLGN). Brain Res. (280): 299-307.

Sillito, AM, Murphy, PC, Salt, TE and Moody, CI. 1990. Dependence of retinogeniculate transmission in cat on NMDA receptors. J. Neurophysiol. (63): 347-355.

Spencer, DG, Horvath, E and Traber, J. 1986. Direct autoradiographic determination of M1 and M2 muscarinic acetylcholine receptor distribution in the rat brain: relation to cholinergic nuclei and projections. Brain Res. (380): 59-68.

Stent, GS. 1973. A physiological mechanism for Hebb's postulate of learning. Proc. Natl. Acad. Sci. USA (70): 997-1001.

Stitchel, CC and Singer, W. 1987. Quantitative analysis of the choline acetyltransferase-immunoreactive axonal network in the cat primary visual cortex: II. Pre- and postnatal development. J. Comp. Neurol. (258): 99-111.

Sretavan, DW and Reichardt, LF. 1993. Time-lapse video analysis of retinal ganglion cell axon pathfinding at the mammalian optic chiasm: growth cone guidance using intrinsic chiasm cues. Neuron (10): 761-777.

Stryker, MP and Zahs, KR. 1983. On and off sublaminae in the lateral geniculate nucleus of the ferret. J. Neurosci. (10): 1943-1951.

Sutin, EL and Jacobowitz, DM. 1990. Localization of substance P mRNA in cholinergic cells of the rat laterodorsal tegmental nucleus: in situ hybridization histochemistry and immunocytochemistry. Cell Mol. Neurobiol. (10): 19-31.

Sutton, JK and Brunso-Bechtold, JK. 1988. The roles of specificity and competition in the formation of a laminated colliculogeniculate projection. J. Neurosci. (8): 435-444.

Tan, MM and Harvey, AR. 1989. The cholinergic innervation of normal and transplanted superior colliculus in the rat: an immunohistochemical study. Neuroscience (32): 511-520.

Turlejski, K, Djavadian, RL and Dreher, B. 1994. Extent of bilateral collateralization among pontomesencephalic tegmental afferents to dorsal lateral geniculate nuclei of pigmented and albino rats. Neurosci. (60): 521-535.

Uhlrich, DJ, Cucchiaro, JB and Sherman, SM. 1988. The projection of individual axons from the parabrachial region of the brainstem to the dorsal lateral geniculate nucleus in the cat. J. Neurosci. (8): 4565-4575.

Uhlrich, DJ, Manning, KA and Pienkowski, TP. 1993. The histaminergic innervation of the lateral geniculate complex in the cat.Vis Neurosci (10): 225-235.

Uhlrich, DJ, Tamamaki, N, Murphy, PC and Sherman, SM. 1995. Effects of brain stem parabrachial activation on receptive field properties of cells in the cat's lateral geniculate nucleus. J. Neurophysiol. (73): 2428-2447.

Vincent, SR, Satoh, K, Armstrong, DM and Fibiger, HC. 1983. Substance P in the ascending cholinergic reticular system. Nature (306): 688-691.

von Krosigk, M, Bal, T and McCormick, DA. 1993. Cellular mechanisms of a synchronized oscillation in the thalamus. Science (261): 361-364.

White, CA and Sur, M. 1992. Membrane and synaptic properties of developing lateral geniculate nucleus neurons during retinogeniculate axon segregation. PNAS (89): 9850-9854.

Wilkemeyer, MF and Angelides, KJJ. 1996. Addition of tetrodotoxin alters the morphology of thalamocortical axons in organotypic cocultures. Neurosci. Res. (43): 707-718.

Williams, JA, Comisarow, J, Day, J, Fibiger, HC and Reiner, PB. 1994. State-dependent release of acetylcholine in rat thalamus measured by *in vivo* microdialysis. J. Neurosci. (14): 5236-5242.

Williams, JA, Vincent, SR and Reiner, PB. 1997. Nitric oxide production in rat thalamus changes with

behavioral state, local depolarization, and brainstem stimulation. J. Neurosci. (17): 420-427.

Wong, ROL, Meister, M and Shatz, CJ. 1993. Transient period of correlated bursting activity during development of the mammalian retina. Neuron (11): 923-938.

Wong, ROL and Oakley, DM. 1996. Changing patterns of spontaneous bursting activity of on and off retinal ganglion cells during development. Neuron (16): 1087-1095.

Woolf, NJ and Butcher, LL. 1986. Cholinergic systems in the rat brain: III. Projections from the pontomesencephalic tegmentum to the thalamus, tectum, basal ganglia, and basal forebrain. Brain Res. Bull. (16): 603-637.

Zhu, JJ and Uhlrich, DJ. 1997. Nicotinic receptor-mediated responses in relay cells and interneurons in the rat lateral geniculate nucleus. Neurosci. (80): 191-202.

FIGURE LEGENDS

Figure 1. Overview of the main events in postnatal development of cholinergic inputs to the ferret dLGN. Each image is a composite which includes, on the left, ChAT immunolabeling, and on the right, AChE histochemical labeling. For each age, two low-power images are shown: a horizontal section through dorsal thalamus at the level of the dLGN on the left and a coronal section through pontomesencephalic brainstem at the level of the PPTg and LDTg on the right. Arrows indicate the position of the dLGN in thalamic sections; PPTg and LDTg are labeled in (B). (A) Thalamus and (B) brainstem at P8. (C) Thalamus and (D) brainstem at P17. (E) Thalamus and (F) brainstem at P26; note the decrease in thalamic ChAT labeling, though ChAT labeling has peaked in the brainstem, notably in the PPTg. (G) Thalamus and (H) brainstem at P32; note the return of ChAT labeling to the thalamus and its altered pattern (i.e., visible in discrete fibers which are located in interlaminar rather than laminar zones). In the brainstem, PPTg and LDTg somata are highly visible. (I) Thalamus and (J) brainstem in the adult; note that the ChAT immunolabeling pattern is most intense at this age and that it has again reversed (i.e., most dense labeling is in laminar rather than interlaminar zones). PPTg: pedunculopontine tegmental nucleus; LDTg; laterodorsal tegmental nucleus; R: rostral; D: dorsal; M: medial. Scale bars = 500 μm.

Figure 2. Higher-power images of ChAT ontogeny in the ferret dLGN. Arrowheads indicate the position of the interlaminar zone between A and A1; arrows indicate the position of lamina A. (A) P8; note that the dLGN is less ChAT-ir than surrounding thalamic regions and no laminae can be distinguished within the dLGN. (B) P13 (i.e., just before the onset of ON/OFF sublamination); ChAT labeling remains low within the dLGN, though very pale interlaminar zones can be seen between slightly darker A, A1 and C laminae. (C) P17; the PGN has become distinct and the division between PGN and dLGN, as well as interlaminar zones within the dLGN, are readily apparent. (D) P20; inner and outer sublaminae are visible within A and A1. (E) P23; interlaminar and laminar zones are even more easily distinguishable. (F) P26 (when ON/OFF sublamination is complete); the division between PGN and dLGN is still distinct, but interlaminar zones within dLGN are much less so. (G) P32; note the reversal in the ChAT labeling pattern so that the interlaminar and inter-sublaminar zones are the most densely labeled areas. (H) Adult; in dLGN, the ChAT labeling pattern has reversed once again and its intensity has increased dramatically, while the PGN appears pale. The division between ON and OFF sublaminae is not visible and ChAT label in the optic tract, seen consistently at younger ages, has completely disappeared. PGN: perigeniculate nucleus; Ai and Ao: inner and outer sublamina of lamina A, respectively; A1i and A1o: inner and outer sublaminae of lamina A1; C: all four C laminae; R: rostral; M: medial. Scale bar = 250 μm.

Figure 3. Higher-power images of AChE ontogeny in the ferret dLGN. Arrowheads indicate the position of the interlaminar zone between A and A1; arrows indicate the position of lamina A. (A) P8; labeling appears dark in the C laminae and pale in the A laminae. (B) P13; labeling in the C laminae has dramatically increased. (C) P17; labeling has increased in the A laminae and the interlaminar zone between them is readily apparent. Some label appears in the PGN. (D) P20; no change from P17. (E) P23; labeling intensity has increased in the A laminae and sublaminae can be identified within lamina A. (F) P26; labeling has decreased throughout the A and C laminae but increased in interlaminar zones so that they are much less distinct than at P23. (G) P32; AChE labeling has decreased in laminar zones and

increased in interlaminar zones, mirroring the changes in ChAT labeling seen at this age. (H) Adult; AChE labeling has reversed its pattern once again so that the most intense label appears in laminar zones. Note the appearance of distinct AChE+ fibers and the more intense labeling of sublaminae Ai and A1i (ON) versus Ao and A1o (OFF) and also the disappearance of label from the optic tract (OT). Abbreviations are the same as in Fig. 2. Scale bar = 250 μm.

Figure 4. High-power images of ChAT and AChE labeling in the dLGN during and after ON/OFF sublamination. Arrowheads indicate presumptive relay cell somata; arrows indicate ChAT+ or AChE+ fibers. (A) At P20, during ON/OFF sublamination, ChAT-ir fibers (not individually distinguishable) are visibly concentrated around somata (indicated by bracket), whereas (C) at P32 (after sublamination is complete), individual ChAT+ fibers can be identified, but concentrations of fibers can no longer be seen surrounding somata. Thus, it seems ChAT+ inputs are targeted to somata during ON/OFF sublamination but alter their pattern of termination after sublamination is complete. In contrast, AChE labeling at both (B) P20 and (D) P32 appears as a diffuse, granular reaction product concentrated around somata but also present throughout the neuropil. There is no apparent change in the pattern of AChE labeling after sublamination is complete, though AChE+ fibers become more visible at that time. Scale bar = 10 μm.

Figure 5. High-power images of brainstem neurons retrogradely labeled by DiI injections in the ferret dLGN at P18. Low-power images of an AChE-labeled coronal section through (A) rostral and (B) caudal pontomesencephalic brainstem of a P17 ferret, indicating the positions of the three cholinergic brainstem nuclei and the superior colliculus, which each send ascending projections to dLGN. (C) A large group of DiI labeled neurons in the superior colliculus (SC). (D) DiI labeled neurons in the parabigeminal nucleus (PBg). (E) DiI labeled neuron in the laterodorsal tegmental nucleus (LDTg); only a few neurons were labeled in the LDTg. (F) DiI labeled neurons in the pedunculopontine tegmental nucleus (PPTg); several neurons were retrogradely labeled in the PPTg. Scale bars, AChE sections (A, B) = 1.5 mm. Scale bars, DiI images (C-F) = 50 μm.

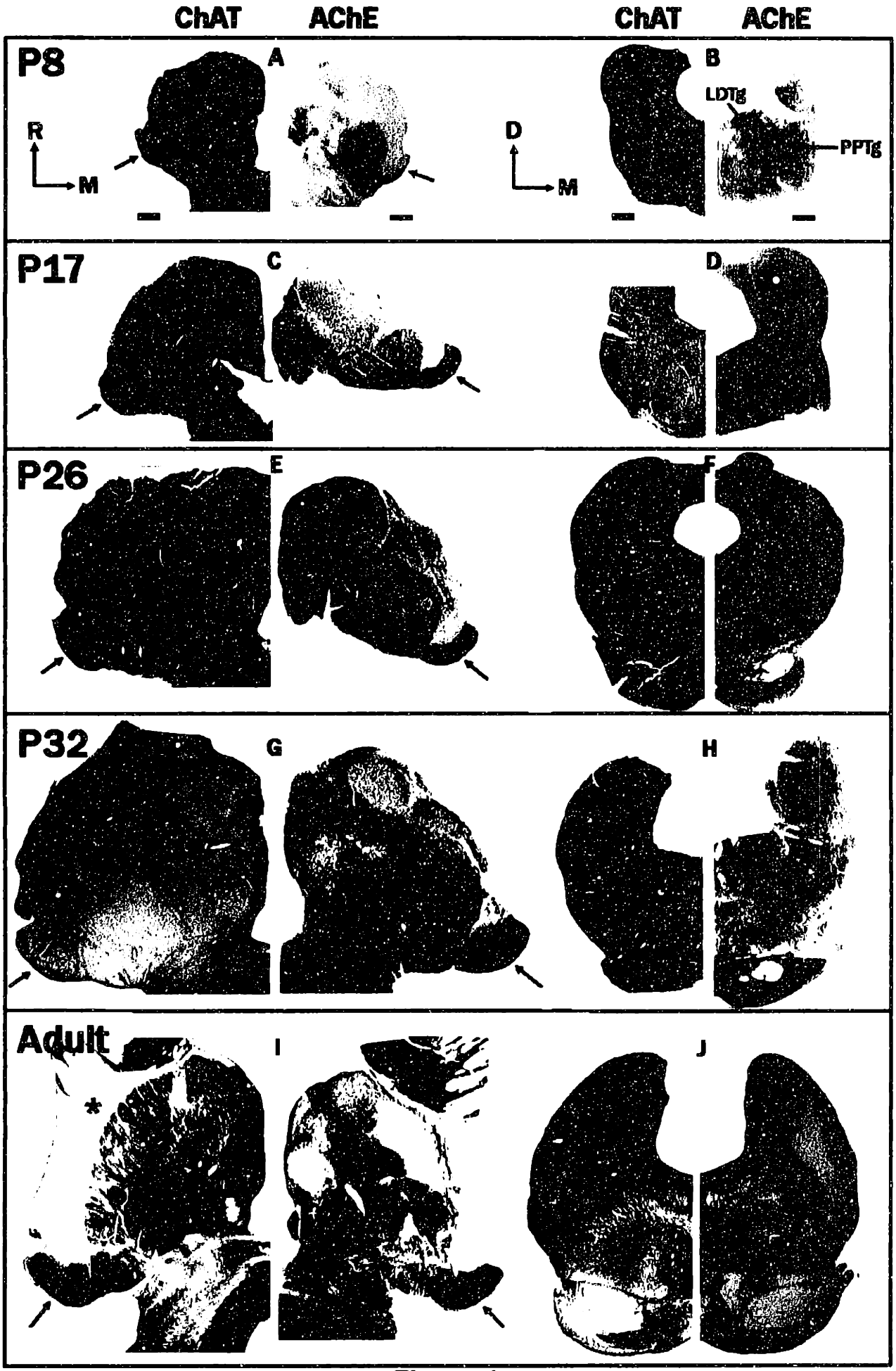
ChAT
AChE
ChAT
AChE
P8
A
B
LDTg
PPTg
R
M
D
M
P17
C
D
P26
E
F
P32
G
H
Adult
I
J
*

Figure 1

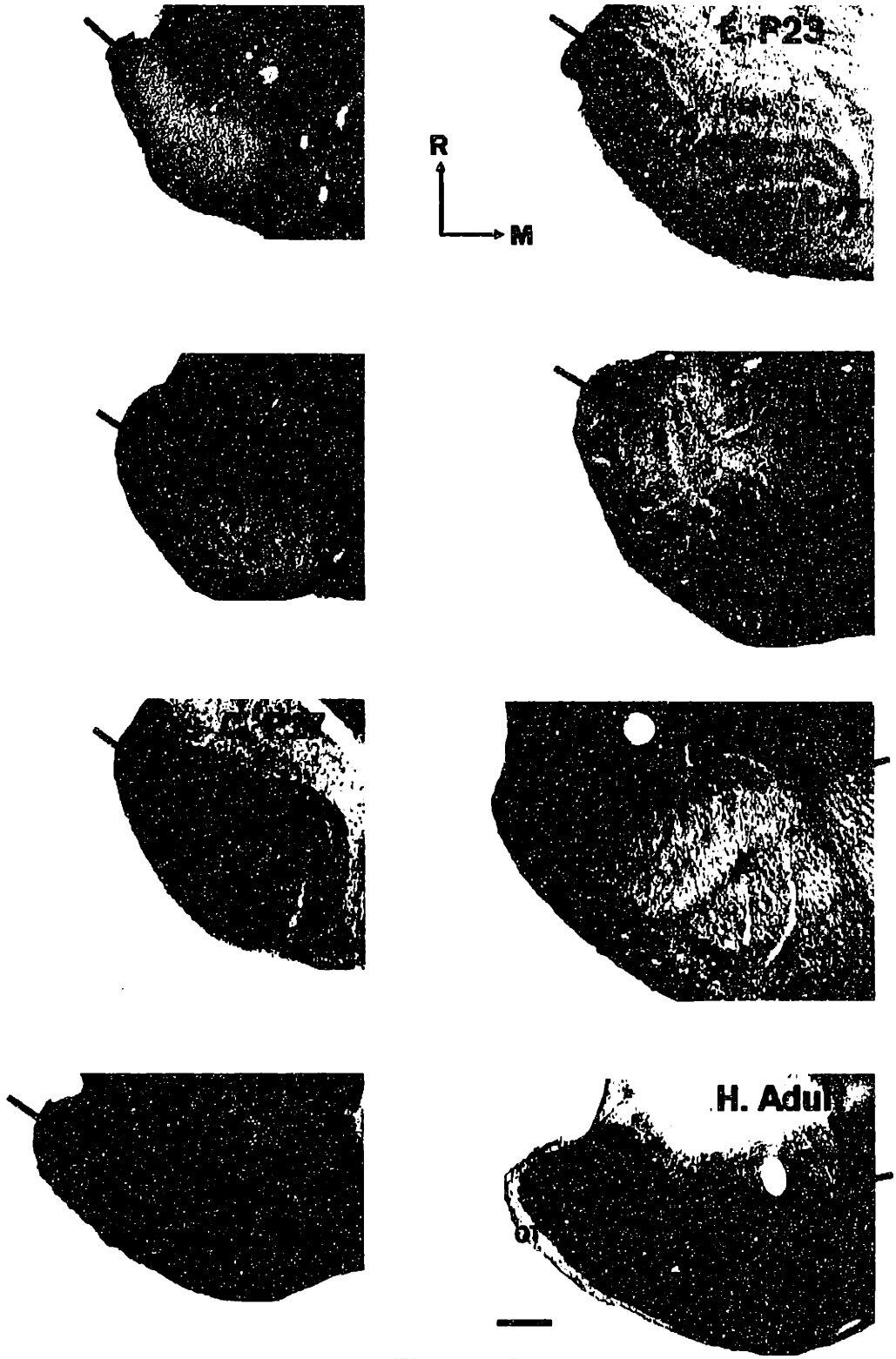

Figure 2

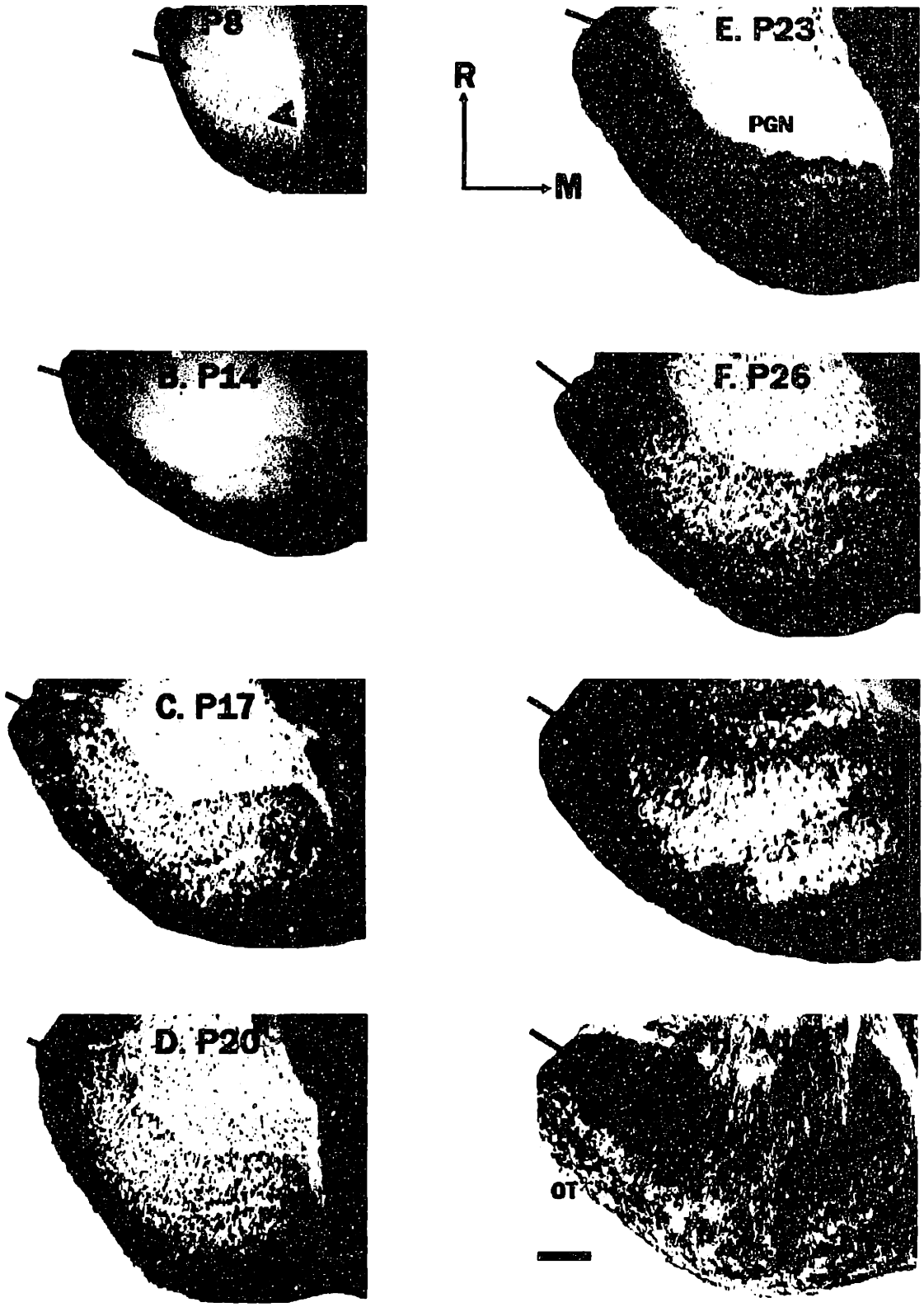

Figure 3

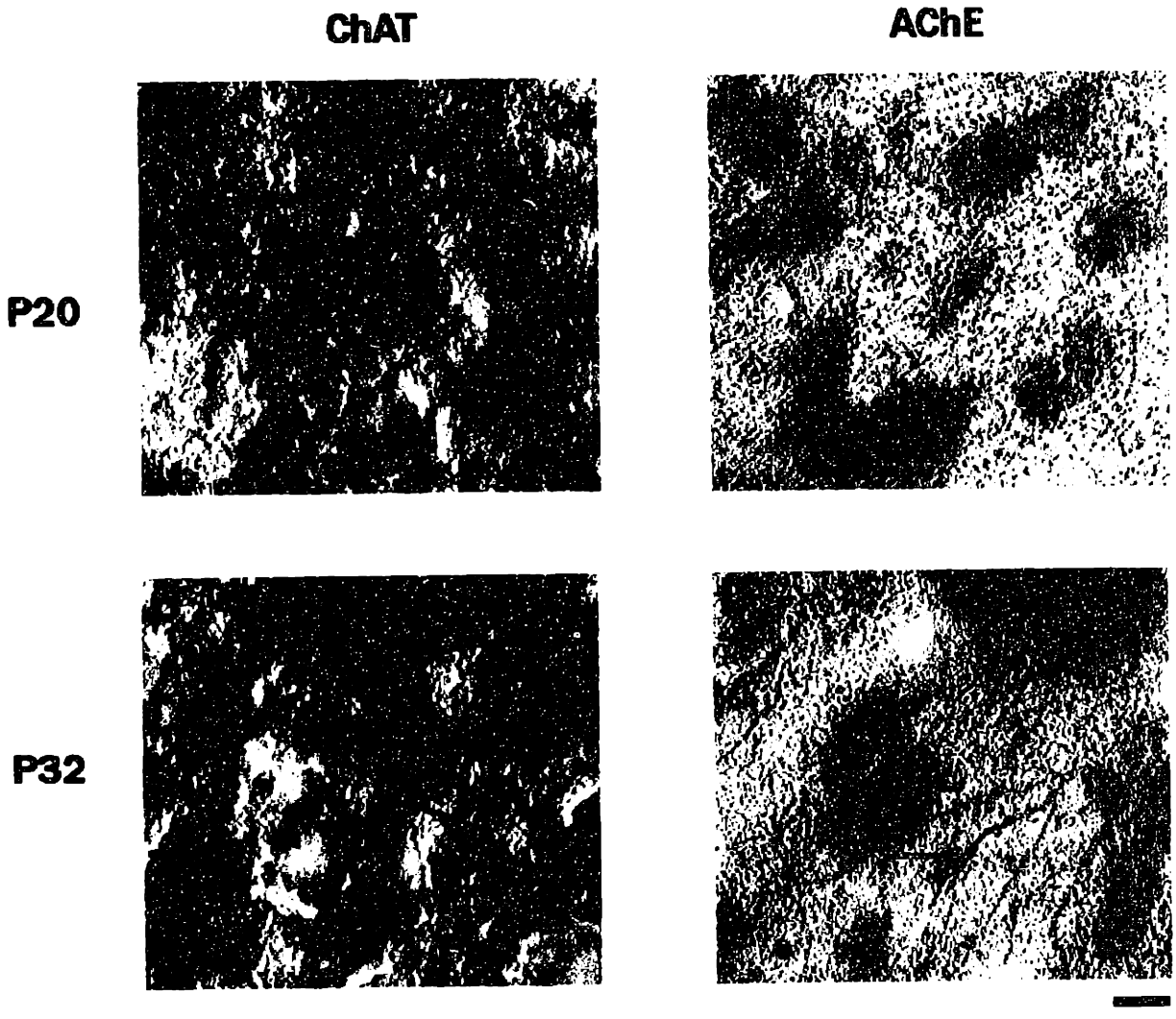

Figure 4

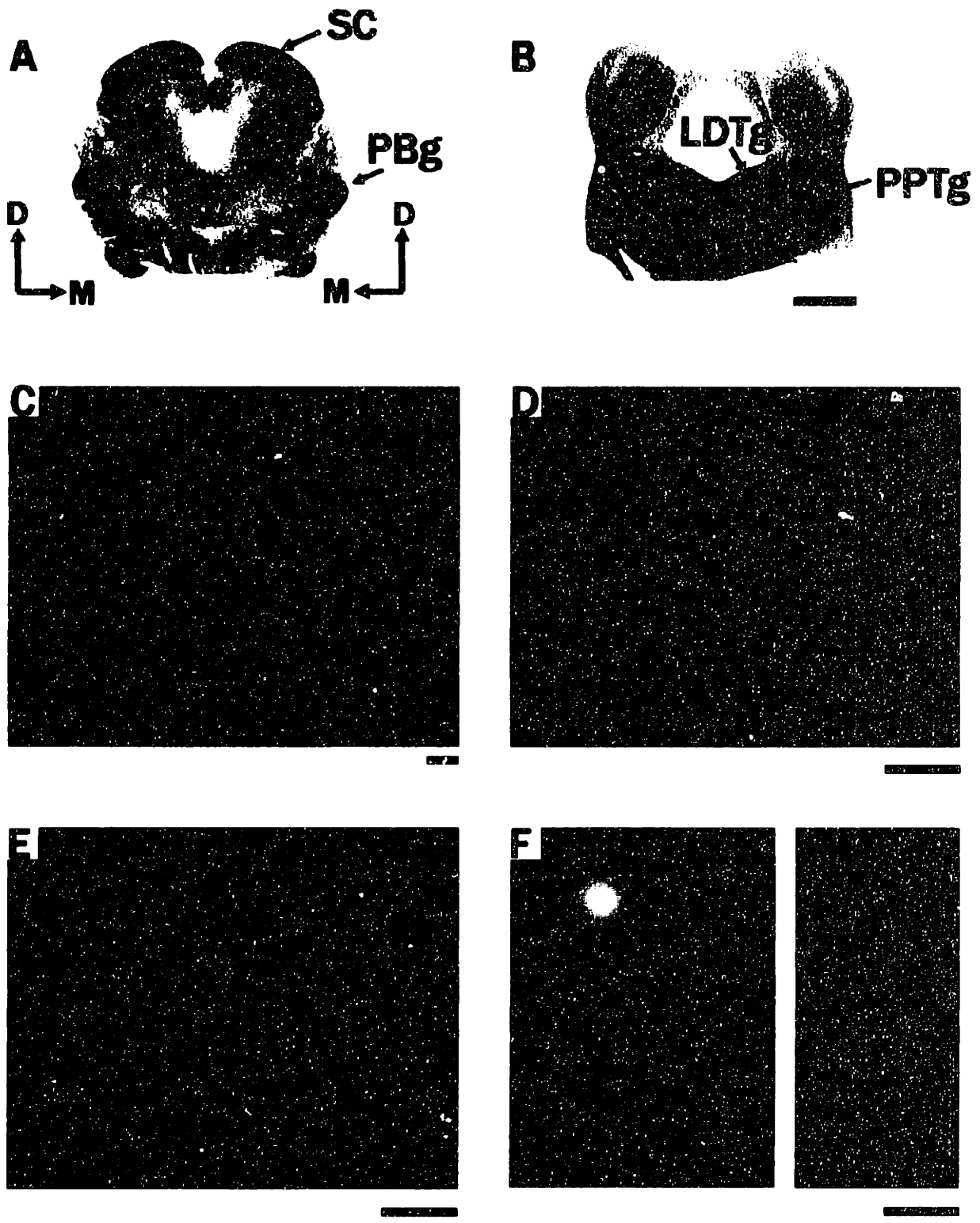

Figure 5