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Research Report

# Brainstem nitrergic innervation of the mouse visual thalamus

*Anita K. McCauley*[a], *Steven T. Frank*[b], *Dwayne W. Godwin*[b,*]

[a]*Department of Biology, Wake Forest University, Winston-Salem, NC 27106, USA*
[b]*Department of Neurobiology and Anatomy, Wake Forest University Health Sciences, Winston-Salem, NC 27157, USA*

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ABSTRACT

Ascending sensory information flowing through the visual thalamus is dynamically regulated by a number of modulatory influences. An important part of this ascending modulation is a cholinergic projection from the parabrachial region of the brainstem (PBR). In addition to containing cholinergic fibers, this projection has been identified in some species as the exclusive extrinsic source of fibers containing the neuronal form of nitric oxide synthase (nNOS). In this study, we examined the nitrergic innervation to the adult mouse visual thalamus. Retrograde tract-tracing with fluorescent microspheres was combined with nNOS and choline acetyltransferase (ChAT) immunocytochemistry, and nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) histochemistry to identify the source of nitrergic innervation. Double-labeling revealed that only two regions of the mouse brain contained nitrergic neurons that projected to the visual thalamus: the pedunculopontine tegmentum and, to a lesser extent, the lateral dorsal tegmentum (LDT). Though the LDT projected heavily to the mouse visual thalamus, very few of the retrogradely labeled neurons in that region colocalized NADPH-d. These observations suggest that the parabrachial brainstem region is the primary source of nitrergic fibers in the mouse visual thalamus, similar to that found in cat and monkey. Such similarity suggests that the presence of nNOS in presynaptic terminal fields of the visual thalamus is an important conserved property of thalamic physiology and that the mouse is a valid model for studies of nNOS functions in the brain.



## 1. Introduction

Nitric oxide (NO) is a gaseous signaling molecule produced by calcium-dependent activation of nitric oxide synthase (NOS; Bredt and Snyder, 1990). The diversity of function and conservation among species supports the importance of NO as a neuromodulator (Dawson et al., 1991; East et al., 1991; Snyder and Bredt, 1991; Bredt and Snyder, 1992; Garthwaite and Boulton, 1995; Cudeiro and Rivadulla, 1999; Choi and Lipton, 2000; Choi et al., 2000). Through cGMP, PKG, and s-nitrosylation pathways, NO may regulate several phenomena that govern neurotransmission, including LTP in the hippocampus and LTD in the cerebellum (Bredt and Snyder, 1989; East and Garthwaite, 1991; O'Dell et al., 1991; Shibuki and Okada, 1991; Schuman and Madison, 1991; Lipton et al., 1998; Hardingham and Fox, 2006; Ikeda et al., 2006; Nugent et al., 2007; Sjöström et al., 2007). Though the role of NO was initially controversial, evidence now suggests that NO is a common

* *Corresponding author*. Department of Neurobiology and Anatomy and the Neuroscience Program, Wake Forest University Health Sciences, 1 Medical Center Blvd., Winston-Salem, NC 27157-1010, USA. Fax: +1 336 716 4534.
E-mail address: dgodwin@wfubmc.edu (D.W. Godwin).

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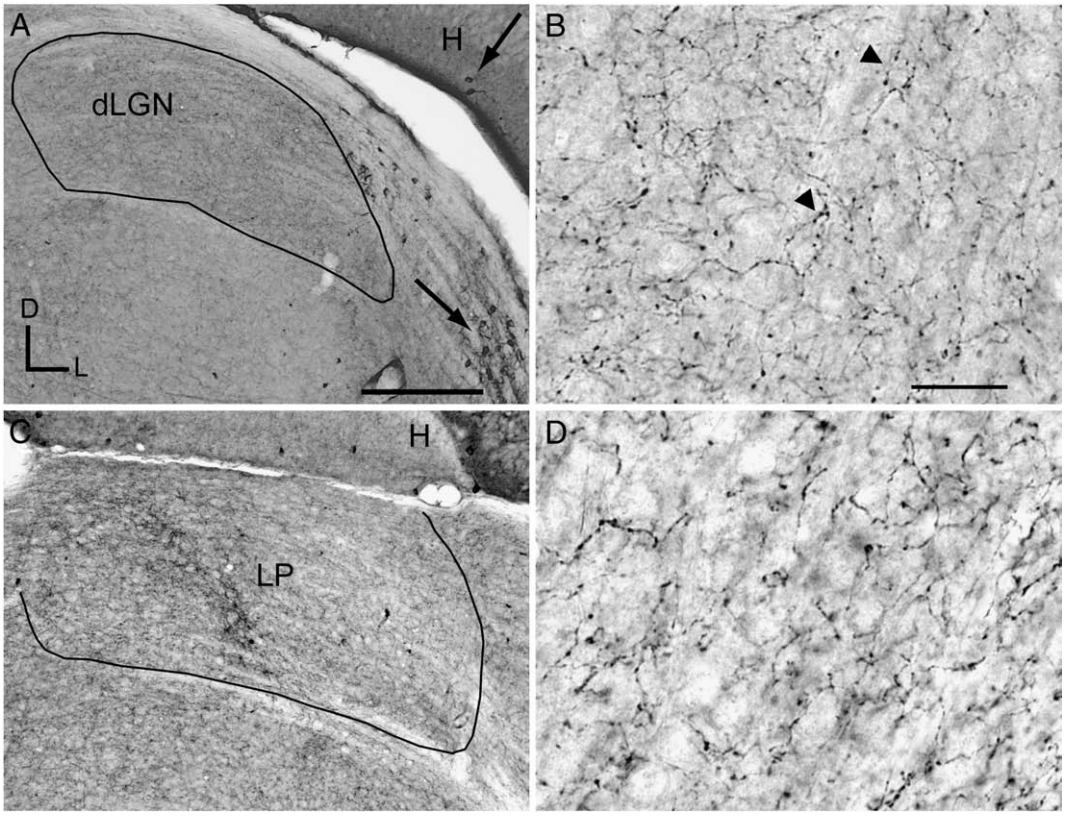


**Fig. 1 – NOS immunohistochemical staining in the mouse visual thalamus. All label is with the nNOS antibody. (A) The dorsal LGN (dLGN) contains a lattice of labeled fibers, but no somatodendritic labeling is present. Somatodendritic labeling with nNOS is observed in the adjacent ventral LGN and the hippocampus (H; indicated by arrows). (B) A high magnification view shows a dense meshwork of nitrergic fibers in the dLGN. The fine structure of nitrergic fibers includes many areas with thick, bead-like swellings (arrowheads). (C) NOS label in the lateral posterior nucleus (LP) reveals a pattern of staining similar to the dLGN. No somatodendritic label was observed in LP, though labeled cells could be seen in the hippocampus. (D) The nitrergic fibers in the LP are similar to those in the dLGN, filling the entire nucleus and containing many areas with thick, bead-like swellings. d, dorsal; l, lateral; scale bar in A: 200 μm, applies to C; scale in B: 25 μm, applies to D. Lines trace the approximate boundaries of the dLGN and LP.**

element in some forms of LTP (Nelson and Turrigiano, 2008). Data also suggest that NO is important for developmental plasticity involved in axon refinement. Studies in ferret (Cramer et al., 1996), mouse (Wu et al., 2000a,b), rat (Vercelli et al., 2000; Campello-Costa et al., 2000) and chick (Ernst et al., 1999, 2000; Wu et al., 2001) suggest that NO acts as a retrograde messenger necessary for the elimination of topographically incorrect projections.

NOS expression has been described in many brain regions and is often found in cholinergic or GABAergic cell groups (Bickford et al., 1999; McCauley et al., 2003; Guirado et al., 2003). Two major sites of NOS and acetylcholine colocalization are the parabrachial brainstem and the basal forebrain, both of which contain large projection neurons that are involved in modulating activity in other brain regions (Pasqualotto and Vincent, 1991; Kimura and Vincent, 1992; Bickford et al., 1993; Bickford et al., 2000; Centonze et al., 2003). Cholinergic cell groups do not always colocalize NOS, including the parabigeminal and oculomotor nuclei of the brainstem (Bickford et al., 1993). Within the superior colliculus (SC), cells positive for choline acetyltransferase (ChAT) or acetylcholinesterase (AChE) do not appear to colocalize NOS (Scheiner et al., 2000). Interestingly however, cholinergic fibers within the SC which arise from the parabrachial region of the brainstem do colocalize NOS.

In this paper, we focus on NOS expression and projections from the parabrachial region of the brainstem (PBR). The PBR is defined from previous anatomical studies as the region of the midbrain surrounding the brachium conjunctivum including the central tegmental field, and the pedunculopontine and lateral dorsal tegmental nuclei (deLima and Singer, 1987; Bickford et al., 1993; Erisir et al., 1997). The PBR has been associated with shifts in attention, arousal, and behavioral state (Singer 1977; Steriade and Deschenes, 1988; Hu et al., 1989b; Hu et al., 1989c; Gerber et al., 1990; Williams et al., 1997; Datta 1995, 1997), as well as in modulating sensory processing including regulating the flow of visual information in the dLGN (Singer 1977; Burke and Cole, 1978; Sherman and Koch, 1986; Cudeiro et al., 1994a,b, 1996; Alexander et al., 2006). The PBR is not a direct target of ascending sensory information, unlike the SC, tectum, and LGN. Behavioral state-dependent signaling by acetylcholine and nitric oxide provides rich possibilities for the modulation of retinogeniculate, geniculocortical and corticogeniculate relay of visual information (Cudeiro et al., 1994a,b, 1996; Alexander et al., 2006).