Neuroscience 143 (2006) 1021–1030

DOPAMINE INNERVATION OF THE MONKEY MEDIODORSAL THALAMUS: LOCATION OF PROJECTION NEURONS AND ULTRASTRUCTURAL CHARACTERISTICS OF AXON TERMINALS

D. S. MELCHITZKY,[a,c] S. L. ERICKSON[b] AND D. A. LEWIS[a,b]*

[a]*Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA 15213, USA*

[b]*Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15213, USA*

[c]*Department of Biology, Mercyhurst College, Erie, PA 16546, USA*

Abstract—Dopamine (DA) axons and receptors have recently been identified in the primate thalamus, including the mediodorsal thalamic nucleus (MD). In order to determine whether the DA innervation of the primate MD shares the anatomical features of the mesocortical or nigrostriatal DA projections, we performed tract-tracing and immunocytochemistry studies in macaque monkeys (*Macaca fascicularis*) to identify the location of the DA neurons that project to MD and immuno-electron microscopy to determine the distribution of the dopamine transporter (DAT) in axons within the MD. Similar to the mesocortical projection, retrogradely-labeled, tyrosine hydroxylase-containing neurons were present in dorsal tier ventral mesencephalic nuclei, such as the ventral tegmental area and the dorsal portion of the substantia nigra pars compacta. In contrast, no dual-labeled neurons were present in the ventral tier nuclei, the primary origin of the nigrostriatal DA pathway. In addition, like the DA projection to the prefrontal cortex, DAT immunoreactivity was predominantly localized to the pre-terminal portion of axons in the MD, and was infrequently found in association with synaptic vesicles, in contrast to nigrostriatal DA axons. These findings indicate that the DA projection to the MD shares anatomical features with the mesocortical DA system, suggesting that the functional properties of DA neurotransmission in the MD might be more similar to those in the cortex than in the striatum.

Key words: dopamine transporter, tyrosine hydroxylase, prefrontal cortex, ventral mesencephalon.

Dopamine (DA) influences a range of brain functions, including cognitive processes such as working memory. Regions of the primate brain that mediate working memory, such as the prefrontal cortex (Goldman et al., 1971; Alexander and Goldman, 1978) and the mediodorsal thalamic nucleus (MD) (Isseroff et al., 1982; Aggleton and Mishkin, 1983), contain both DA axons (Lewis et al., 1987, 2001; Melchitzky and Lewis, 2001; Sánchez-González et al., 2005) and DA receptors (Suzuki et al., 1998; Gurevich and Joyce, 1999), indicating that DA neurotransmission in these brain regions might regulate working memory functioning. Indeed, either increases or decreases in the level of DA neurotransmission in the prefrontal cortex impair performance on working memory tasks (Brozoski et al., 1979; Roberts et al., 1994; Arnsten et al., 1994). Furthermore, subjects with schizophrenia perform poorly on tests that involve working memory (Park and Holzman, 1992), and interestingly, abnormalities in the DA system have been reported in both the prefrontal cortex (Akil et al., 1999; Abi-Dargham et al., 2002) and the thalamus (Yasuno et al., 2004; Talvik et al., 2003) of individuals with schizophrenia.

The presence of DA axons in the macaque monkey MD has been reported in several studies (Freeman et al., 2001; Melchitzky and Lewis, 2001; Sánchez-González et al., 2005), with a pattern of innervation distinct from that of norepinephrine (Melchitzky and Lewis, 2001). For example, we found that axons immunoreactive for the dopamine transporter (DAT) were heterogeneously distributed within MD, with the highest density located in the parvocellular and multiform subdivisions. Consistent with other studies demonstrating that antibodies against tyrosine hydroxylase (TH) preferentially label DA-containing axons in the primate brain (Lewis et al., 1987; Noack and Lewis, 1989), the density and distribution of TH-immunoreactive (IR) axons were very similar to those of DAT-IR axons. In contrast, axons labeled for DA-β-hydroxylase, a specific marker of norepinephrine-containing structures, were present in higher density and were homogeneously distributed throughout the entire MD.

The DA projections to cortical or subcortical structures arise from neurons in the ventral mesencephalon, which in primates is composed of two main DA-containing cell groups, the dorsal and ventral tier neurons (Haber and Fudge, 1997). The striatum receives projections primarily from DA neurons in the ventral tier, comprised of cells in the substantia nigra pars compacta. In contrast, the cells of the dorsal tier, which consists of the dorsal portion of the substantia nigra pars compacta, the ventral tegmental area and the retrorubral area (Haber and Fudge, 1997), provide DA projections to cortical areas, including the prefrontal cortex. The DA projections to the striatum and prefrontal cortex also differ in the subcellular distribution of DAT. For example, in both monkeys (Lewis et al., 2001) and rodents (Sesack et al., 1998), DAT immunoreactivity is present in

*Correspondence to: D. A. Lewis, Western Psychiatric Institute and Clinic, Biomedical Science Tower, W1651, 3811 O'Hara Street, Pittsburgh, PA 15213, USA. Tel: +1-412-624-3934; fax: +1-412-624-9910.
E-mail address: lewisda@upmc.edu (D. A. Lewis).

Abbreviations: COMT, catechol-O-methyltransferase; CTb, cholera toxin b subunit; DA, dopamine; DAT, dopamine transporter; IR, immunoreactive; MD, mediodorsal thalamic nucleus; PB, phosphate buffer; TH, tyrosine hydroxylase.

0306-4522/06$30.00+0.00 © 2006 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2006.08.056

both pre-terminal and terminal axons in the striatum, whereas DAT labeling appears to be restricted to pre-terminal axons in the prefrontal cortex. Since the functional properties of the nigrostriatal and mesocortical systems differ in many respects (Lewis and Sesack, 1997), these findings suggest that the location of the DA cell bodies of origin and the subcellular distribution of DAT in the axons arising from those neurons define functionally-distinct populations of DA terminal fields.

In this study, we sought to determine if the DA innervation of the parvocellular MD, the subdivision of this nucleus containing the greatest density of DAT- and TH-IR axons, is more like the mesocortical or nigrostriatal DA projections. Thus, we utilized retrograde tracing and dual-label immunocytochemistry to identify the location of the DA-containing neurons that project to parvocellular MD and immuno-electron microscopy to determine the subcellular location of DAT in MD axons.

EXPERIMENTAL PROCEDURES

Retrograde tracing and dual label immunocytochemistry

Animals and surgical procedures. Four adult, male long-tailed macaque monkeys (*Macaca fascicularis*) were used for these studies. All procedures were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and with the guidelines set forth by the University of Pittsburgh's Institutional Animal Care and Use Committee (IACUC). Every effort was made to minimize the number of animals used and their suffering. Detailed information regarding the injection site locations and injection parameters has been previously published (Erickson and Lewis, 2004; Erickson et al., 2004). Briefly, monkeys received either unilateral (CM234 and CM228) or bilateral (CM226 and CM250) iontophoretic (CM226 and CM234) or pressure (CM228 and CM250) injections of 1% (in water) cholera toxin b subunit (CTb; List Biologicals, Campbell, CA, USA) into the MD, resulting in six hemispheres for examination. One injection of CTb was made in each hemisphere. Pressure injections of CTb (0.3 μl) were made using a Hamilton syringe (25 or 32 gauge, Hamilton Company, Reno, NV, USA). Iontophoretic injections were made using glass pipettes with an inner diameter of 10 μm. Positive current was administered in 7-second on–off cycles at 2–5 μA for 10–15 min. In all six hemispheres, the CTb injection site was localized to the parvocellular portion of MD, but at varying rostrocaudal levels (Fig. 1).

Tissue preparation. After a survival time of 13–15 days, animals were perfused with a solution of 4% paraformaldehyde in 0.1 M phosphate buffer (PB) at 4 °C and coronal blocks through the diencephalon were sectioned on a cryostat at 50 μm, as previously described (Erickson et al., 2004). A one in two series of sections from all four animals (six hemispheres) was processed for single label CTb immunocytochemistry visualized with 3,3′-diaminobenzidine (Erickson et al., 2004), and these sections were used to guide the selection of sections for dual-label (i.e. CTb and TH) experiments. For all four animals, sections containing the ventral mesencephalon were processed for dual-label immunocytochemistry. In addition, large numbers of CTb-labeled neurons in the hypothalamus were found in two animals (CM228 and CM250) in the initial single-label series. Thus, sections through the hypothalamus of these animals were also processed for dual-label immunocytochemistry.

Tissue sections were processed for CTb and TH dual-label immunocytochemistry, using a previously described protocol (Lewis et al., 2001; Melchitzky et al., 2005). A polyclonal CTb antibody (goat anti-CTb, 1:20,000, List Biologicals) and a monoclonal TH antibody (mouse anti-TH, 1:10,000, kindly provided by Dr. Greg Kapatos, Wayne State University) were utilized. The CTb antibody was visualized with a donkey anti-goat biotinylated IgG (1:200, Jackson ImmunoChemicals, West Grove, PA, USA) and streptavidin conjugated to indocarbocyanine (1:500, Jackson), and the TH antibody was visualized with a donkey–anti-mouse IgG conjugated to either fluorescein isothiocyanate (1:150, Jackson) or aminomethylcoumarin acetate (1:150, Jackson). Sections were mounted on gel-coated slides, air dried and coverslipped with a 1:1 mixture of 0.1 M PB and glycerol.

In the sections selected for dual-label experiments, the locations of all single (CTb-IR) and dual-labeled (CTb- and TH-IR) neurons were recorded onto camera lucida drawings made from adjacent sections processed only for CTb. A subset of camera lucida drawings was selected and scanned on an Agfa Arcus II scanner (Agfa Corporation, Ridgefield Park, NJ, USA), and the resulting Tiff files were used as templates for creating renditions with Freehand software (Macromedia Inc., San Francisco, CA, USA). Drawings from three rostrocaudal levels for both the ventral mesencephalon (anterior substantia nigra, red nucleus, retrorubral area) and hypothalamus (preoptic, tuberal and posterior hypothalamus) were utilized.

Photomicrographs were taken of selected dual-labeled cells. Fields of CTb- and TH-IR neurons were obtained on a Zeiss Axiophot equipped with a Zeiss AxioCam CCD camera operated with AxioVision software (Carl Zeiss, Thornwood, NY, USA). Images were cropped and lettering was applied using Adobe Photoshop 6.0 software (Adobe Systems, Seattle, WA, USA). The images were not otherwise altered. All photographic images were printed on a Fujix Pictography 3000 digital printer (Fuji Photo, Tokyo, Japan).

Immuno-electron microscopy

Animals and tissue preparation. Two additional adult, male long-tailed monkeys (CM237 and CM240) were perfused as described above, except that the perfusate was 4% paraformaldehyde/0.2% glutaraldehyde in 0.1 M PB at 29 °C (Melchitzky et al., 1999). Small blocks of tissue containing the thalamus were excised from the coronal blocks and sectioned at 50 μm on a Vibratome.

Immunocytochemistry. Tissue sections were processed for immuno-electron microscopy as previously described (Melchitzky et al., 1999, 2005), using either a monoclonal anti-rat DAT antibody (Chemicon, Temecula, CA, USA; diluted 1:3500) or the same mouse monoclonal TH antibody (diluted 1:4000) used in the dual-label fluorescence experiments described above. The DAT and TH antibodies were visualized with 3,3′-diaminobenzidine. The sections were exposed to 2% osmium tetroxide for 1 h, dehydrated and embedded in Epon 812 (Melchitzky et al., 1998).

Electron microscopy. From each animal, one trapezoid block per condition was taken from the ventrolateral region of MD. We have previously demonstrated that this region of MD, which includes portions of both the parvocellular and multiform subdivisions, has the greatest density of DAT-IR axons (Melchitzky and Lewis, 2001). The trapezoid blocks were then sectioned on a Reichert ultramicrotome (Nussloch, Germany) and ultrathin sections were collected on individual copper grids. The grids were stained with uranyl acetate and lead citrate and examined on a JEOL 100 CX electron microscope (JEOL-USA, Peabody, MA, USA), as previously described (Melchitzky et al., 1999). For each block, two grids were analyzed, with one section per grid arbitrarily chosen as the starting point for examination. Within this section, all DAT- or TH-IR structures were photographed at $\times$19,000.