THE JOURNAL OF COMPARATIVE NEUROLOGY 490:270–294 (2005)

Afferents of the Ventral Tegmental Area in the Rat-Anatomical Substratum for Integrative Functions

STEFANIE GEISLER AND DANIEL S. ZAHM*
Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, St. Louis, Missouri 63104

ABSTRACT

The ventral tegmental area (VTA) is critically important to an organism's capacity to detect rewards and novelty and to enlist appropriate behavioral responses. Although there has been substantial progress concerning information processing at the single cell and molecular levels in the VTA, our knowledge of its overall afferent connections is based principally on the benchmark description by Phillipson ([1979] J. Comp. Neurol. 187:117–144). Given that, since then, the sensitivity of tracing methods and knowledge about the organization of brain structures have increased considerably, we undertook to reevaluate the VTA afferents of the rat. The retrograde tracer Fluoro-Gold was injected into different parts of the VTA, and labeled neurons were visualized by immunocytochemistry. Retrogradely labeled neurons were not confined to nuclei but rather constituted an elongated formation stretching from the prefrontal cortex rostrally to the medulla oblongata caudally. In the case of descending afferents, this formation was centered on the medial forebrain bundle and the fasciculus retroflexus. The input to the VTA in general was bilateral, with a smaller descending and comparable ascending projection from the contralateral side. Injections of the anterograde tracers *Phaseolus vulgaris*-leucoagglutinin or biotinylated dextran amine into selected forebrain structures revealed a surprisingly sparse terminal arborization in the VTA. Furthermore, structures projecting to the VTA innervate other brain areas with similar or greater robustness, which in turn also provide a strong input to the VTA, indicating an anatomical network. Given the importance of the VTA in basic behaviors, this organization might provide a basis for an extraordinary level of afferent integration. J. Comp. Neurol. 490: 270–294, 2005.

Indexing terms: accumbens; connections; dopamine; lateral hypothalamus; network; reward; VTA

A function of the ventral tegmental area (VTA) is to detect primary rewards and reward-predicting stimuli and novelty and to enlist appropriate adaptive behavioral responses (White, 1996; Schultz et al., 1997; Rebec et al., 1997a,b; Schultz, 1998). Insofar as the VTA receives direct inputs from neither the internal milieu nor the external environment (Phillipson, 1979a; Oades and Halliday, 1987), and inasmuch as rewards and reward-predicting stimuli are highly variable in form and content, the VTA might be expected to have enormous capacity to integrate various forms of information in order to extract patterns relevant to its function. The resulting signals are largely encoded by synchronous changes in the firing activity of neurons in the VTA (Schultz et al., 1997, 1998) and consequent changes in dopamine release in its target areas, especially the nucleus accumbens and prefrontal cortex (Overton and Clark, 1997; Rebec et al., 1997a,b; Lewis and O'Donnell, 2000; Floresco et al., 2003; Bamford et al., 2004). VTA signaling is thought to increase an organism's probability of survival and reproduction and to be pathologically altered in drug addiction and schizophrenia (White and Wang, 1983; Jones et al., 2000; Ungless et al., 2001; Saal et al., 2003; for reviews see, e.g., White, 1996; Spanagel and Weiss, 1999; Kelley, 2004) How the signals

Grant sponsor: U.S. Public Health Service; Grant number: DA 15207; Grant number: NS 23805.

*Correspondence to: Daniel S. Zahm, Department of Pharmacological and Physiological Science, St. Louis University School of Medicine, 1411 South Grand Blvd., St. Louis, MO 63104. E-mail: zahmds@slu.edu

Received 23 December 2004; Revised 30 March 2005; Accepted 10 May 2005

DOI 10.1002/cne.20668

Published online in Wiley InterScience (www.interscience.wiley.com).

are generated, what drives and inhibits VTA neurons, and how the VTA integrates information are subjects of intense research.

The function of a brain structure depends on its internal organization and how this organization is affected by extrinsic influences. Although there has been substantial progress concerning information processing (see, e.g., Thomas et al., 2000; Neuhoff et al., 2002, Melis et al., 2004; Paladini and Williams, 2004) and input specificity (see, e.g., Woulfe and Beaudet, 1992; Charara et al., 1996; Carr and Sesack, 2000; Georges and Aston-Jones, 2002) in the VTA at the single cell and molecular levels, our knowledge about the overall organization of afferent connections of the VTA is based principally on the benchmark description by Phillipson (1979a). Since then, "new" brain nuclei have been delineated or brain areas have been found to consist of functionally distinct compartments, leading to new concepts about structure and function of brain areas (see, e.g., Paxinos, 1995). Furthermore, new tracers have become available that are more avidly taken up and more sensitively visualized (Gerfen and Sawchenko, 1984, 1985; Schmued and Fallon, 1986; Luppi et al., 1987, 1990; Chang et al., 1990; Brandt and Apkarian, 1992; Veenman et al., 1992) and less subject to uptake by fibers of passage (Pieribone and Aston-Jones, 1988; Schmued and Heimer, 1990). In view of these developments, we undertook a reexamination of the afferent connections of the VTA.

The location of several of the main fiber bundles of the brain within the VTA posed a major challenge for these studies. Tracer injected into the VTA might be incorporated by axons passing through without establishing synaptic contacts, which would result in false-positive retrograde labeling of neurons. To minimize this problem, we iontophoretically injected the retrograde tracer Fluoro-Gold (FG) with a low, discontinuous current, in order to reduce damage to axons and consequent uptake of tracer by fibers of passage. To confirm the retrograde data, 36 injections of the anterogradely transported tracers *Phaseolus vulgaris*-leucoagglutinin (PHA-L) or biotinylated dextran amine (BDA) were made into 14 different

Abbreviations

AA	anterior amygdaloid area
ac	anterior commissure
Acb	accumbens nucleus
AHA	anterior hypothalamic nucleus
AI	agranular insular cortex
aq	aqueduct
ATg	anterotegmental nucleus
BST	bed nucleus of stria terminalis
cAcb	accumbens nucleus, core
cc	corpus callosum
CG	central gray
Cg	cingulate cortex
CeA	central nucleus of the amygdala
Cl/En	claustrum/endopiriform nucleus
CnF	cuneiform nucleus
cp	cerebral peduncle
CPu	caudate putamen
CS	colliculus superior
DA	dorsal hypothalamic area
DP	dorsal peduncular cortex
DpMe	deep mesencephalic field
DMH	dorsal hypothalamic nucleus
DR	dorsal raphe
DTg	dorsal tegmental nucleus
f	fornix
FG	Fluoro-Gold
fr	fasciculus retroflexus
g7	genu of the nucleus of the 7th cranial nerve
Gi	gigantocellular field of reticular formation
GP	globus pallidus
HDB	diagonal band of Broca, horizontal limb
ic	internal capsula
IL	infralimbic cortex
IO	inferior olive
IP	interpeduncular nucleus
IRt	intermediate field of reticular formation
LDTg	laterodorsal tegmental nucleus
LH	lateral hypothalamic area
LHb	lateral habenula
LPO	lateral preoptic area
LRt	lateral field of reticular formation
LSD	lateral septum, dorsal part
LSI	lateral septum, intermediate part
LSV	lateral septum, ventral part
M	mammillary nucleus
MCPO	magnocellular preoptic area
MeA	medial amygdala
MHb	medial habenula
ml	medial lemniscus
MnPO	median preoptic area
mp	mammillary peduncle
MPA	medial preoptic area
MR	median raphe
MS	medial septum
mt	mammillothalamic tract
MVe	medial vestibular nucleus
n7	7th cranial nerve
opt	optic tract
OT	olfactory tubercle
ox	optic chiasm
Pa	paraventricular nucleus of the hypothalamus
PAG	periaqueductal gray
PB	parabrachial nucleus
pc	posterior commissure
PFC	prefrontal cortex
PH	posterior hypothalamic nucleus
PMR	paramedian raphe
PnC	caudal field of pontine reticular formation
PnO	oral field of pontine reticular formation
PnR	pontine raphe
PPTg	pedunculopontine nucleus
PrL	prelimbic cortex
Pr	prepositus nucleus
PVA	paraventricular nucleus of the thalamus
py	pyramidal tract
R	red nucleus
ROb	raphe obscurus
rpAcb	accumbens rostral pole
RRF	retrorubral field
scp	superior cerebellar peduncle
SFi	septofimbrial nucleus
shAcb	accumbens nucleus, shell
SLSI	sublenticular substantia innominata
sm	stria medullaris
SNc	substantia nigra, pars compacta
SNl	substantia nigra, pars lateralis
SNr	substantia nigra, pars reticularis
SuM	supramammillary nucleus
TC	tuber cinereum
tgx	tegmental decussation
VDB	diagonal band of Broca, vertical limb
VP	ventral pallidum
VTA	ventral tegmental area
VTg	ventral tegmental nucleus
xscp	decussation of the superior cerebellar peduncle
ZI	zona incerta
III	3rd ventricle
4V	fourth ventricle
6	nucleus of the sixth nerve

forebrain structures that contained retrogradely labeled neurons after FG injections into the VTA. All injections of anterogradely transported tracer resulted in labeled fibers with terminal-like arborizations and varicosities in the VTA.

MATERIALS AND METHODS

Tracer injections

All experiments were carried out in accordance with guidelines published in the National Institutes of Health *Guide for the care and use of laboratory animals*. Animals were housed in group cages on a 12-hour light-dark cycle and given food and water ad libitum. If not indicated otherwise, chemicals were purchased from Sigma (St. Louis, MO).

Male Sprague Dawley rats (Harlan, Indianapolis, IN), weighing 220–420 g, were deeply anesthetized by intraperitoneal injections of a cocktail consisting of 45% ketamine (100 mg/ml), 35% xylazine (20 mg/ml), 20% saline at a dose of 0.16 ml/100 g body weight and placed into a Kopf stereotaxic instrument. In the first set of experiments, the retrograde tracer FG (Fluorochrome, Inc., Englewood, CO; 1% in 0.1 M cacodylate buffer, pH 7.4) was injected iontophoretically through 1.0-mm filament containing glass pipettes pulled to tip diameters of 15–20 μm. By using 1 μA positive pulses (7 seconds on and 7 seconds off for 10–15 minutes), FG was delivered into different areas of the VTA as well as into areas adjacent to it serving as controls. In a second set of experiments, the anterograde tracers PHA-L (Vector Laboratories, Burlingame, CA; 2.5% in 0.1 M phosphate buffer, pH 7.4) or BDA (Molecular Probes, Eugene, OR; 10% in 0.01 M phosphate buffer, pH 7.4) were injected into forebrain areas that exhibited substantial amounts of retrogradely transported FG following injections into the VTA. Filament containing glass pipettes with outside diameters of 1 mm were pulled, and the tips were broken back to achieve diameters of 10–12 μm for PHA-L and 18–20 μm for BDA. The tracers were iontophoretically delivered using discontinuous 4 μA (for PHA-L) and 3 μA (for BDA) positive pulses (7 seconds on and 7 seconds off for 15 minutes). The selection of stereotaxic coordinates was guided by the atlas of Paxinos and Watson (1998). After surgery, the rats were kept warm until they had fully recovered from anesthesia.

After survivals of 3–5 days in the case of FG and 10 days in the case of PHA-L and BDA injections, rats were again deeply anesthetized as described above and perfused transaortically with 4% paraformaldehyde and 2.5% sucrose in 0.1 M phosphate buffer (PB), pH 7.4. Brains were removed, placed in fresh fixative for 4 hours, cryoprotected in 25% sucrose overnight, shock frozen in dry ice, and subsequently sectioned in the coronal plane at 50 μm with a cryo-sliding microtome.

Immunocytochemistry

All steps were carried out under gentle agitation on a horizontal rotator (Lab-Line; Fisher, Pittsburgh, PA). Free-floating sections were rinsed in 0.1 M PB (pH 7.4), placed into 1% sodium borohydride for 15 minutes, thoroughly rinsed in 0.1 M PB again, pretreated with 0.1 M PB containing 0.2% Triton X-100, and transferred into solution containing the primary antibody. Dilutions of primary antibodies were as follows: rabbit anti-FG (Chemicon, Temecula, CA) 1:8,000; goat anti PHA-L (Vector Laboratories) 1:5,000, or mouse anti-tyrosine hydroxylase (ImmunoStar, Inc., Hudson, WI) 1:10,000 in 0.1 M PB with 0.2% Triton X-100. On the following day, after being thoroughly rinsed in 0.1 M PB, sections were placed into a solution containing biotinylated antibody against rabbit, goat, or mouse IgGs (Vector Laboratories), accordingly, at a dilution of 1:200 in 0.1 M PB with 0.2% Triton X-100 and left there for 1 hour. Sections were again rinsed in 0.1 M PB with 0.2% Triton X-100 and immersed in a solution containing avidin-biotin-peroxidase complex (ABC; Vector Laboratories; 1:200 in 0.1 M PB containing 0.2% Triton X-100) for another 1 hour. After thorough rinsing in 0.1 M PB, a color reaction was developed by immersing the sections for about 15 minutes in a solution of 0.05 M PB containing 0.05% 3,3′-diaminobenzidine (DAB), 0.04% ammonium chloride, 0.2% beta-D-glucose, and 0.0004% glucose oxidase. Sections containing BDA were rinsed in PB and immersed in the ABC complex (1:200 in 0.1 M PB containing 0.2% Triton X-100), and a color reaction was carried out as described above. Reacted sections were mounted onto gelatin-coated slides, intensified in 0.005% osmium tetroxide and 0.1% thiocarbohydrazide, dehydrated through a graded series of alcohol, transferred into xylene, and coverslipped with Permount (Fisher, Pittsburgh, PA). No staining was observed when the primary or secondary antibodies or ABC reagents were omitted.

Nissl stain

Sections were mounted onto gelatin-coated slides, air dried, de- and rehydrated through a graded series of alcohol, placed in distilled water for 2 minutes, transferred into cresyl violet (0.2% cresyl violet acetate, 20 mM acetic buffer, pH 4.0), and left there for 30 minutes. Another dehydration through a graded series of alcohol concentrations preceded transfer of the sections into xylene and coverslipping with Permount (Fisher).

Analysis

From a large library of cases, 61 were selected for the present study. Sections were analyzed by using a Nikon Eclipse E600 light microscope. Sections from selected series were drawn. Retrogradely labeled neurons were plotted and counted with the aid of the Neurolucida hardware–software platform (MicroBrightField, Inc., Williston, VT). Labeled neurons were counted in all sections that passed through a given structure (section thickness 50 μm, distance between sections 250 μm). Drawings were arranged and finished in Adobe Illustrator 9.0. Images for illustration were acquired with a digital camera (Optronics, Goleta, CA), and minor adjustments of color and contrast were made in Adobe Photoshop 7.0.

RESULTS

In all of the cases with FG deposition in the VTA evaluated in this study, a general principle emerged that retrogradely labeled neurons are not confined to distinct nuclei but, rather, constitute an elongated formation. This formation is centered on the medial forebrain bundle and fasciculus retroflexus in the forebrain and extends throughout the brainstem into the medulla oblongata. Within the confines of this continuous formation, some structures are preferentially enriched with retrogradely

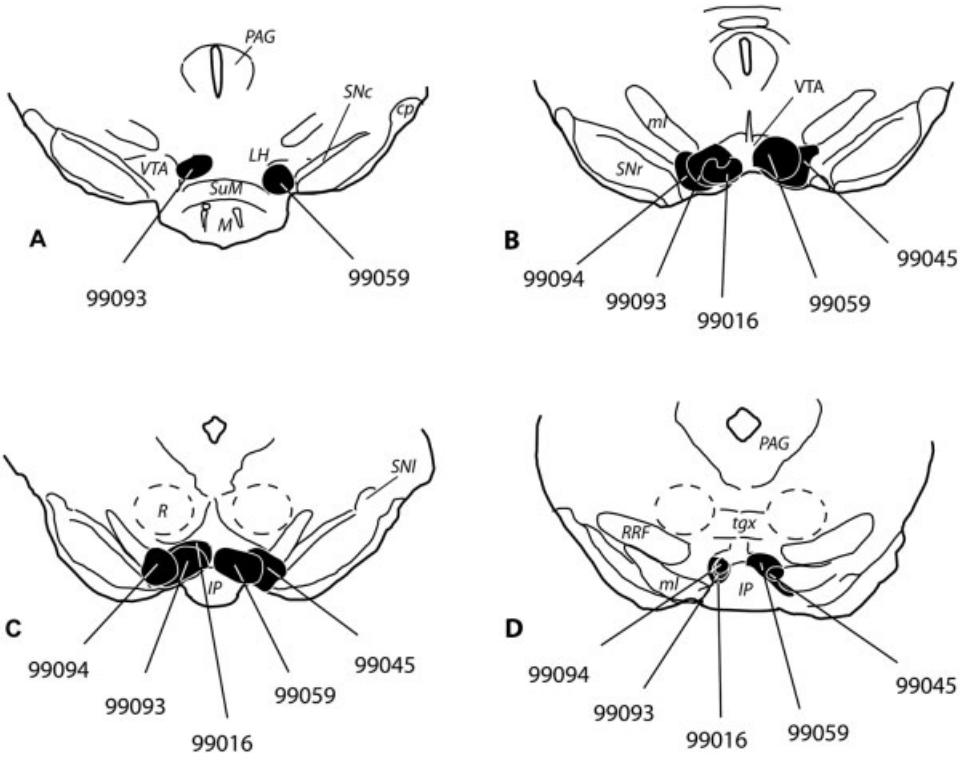

Fig. 1. **A–D:** Schematic representations of Fluoro-Gold injection sites in the VTA. The sections are ordered rostrocaudally, A representing the most rostral. Tyrosine hydroxylase-immunoreacted sections were used as templates to delineate the VTA.

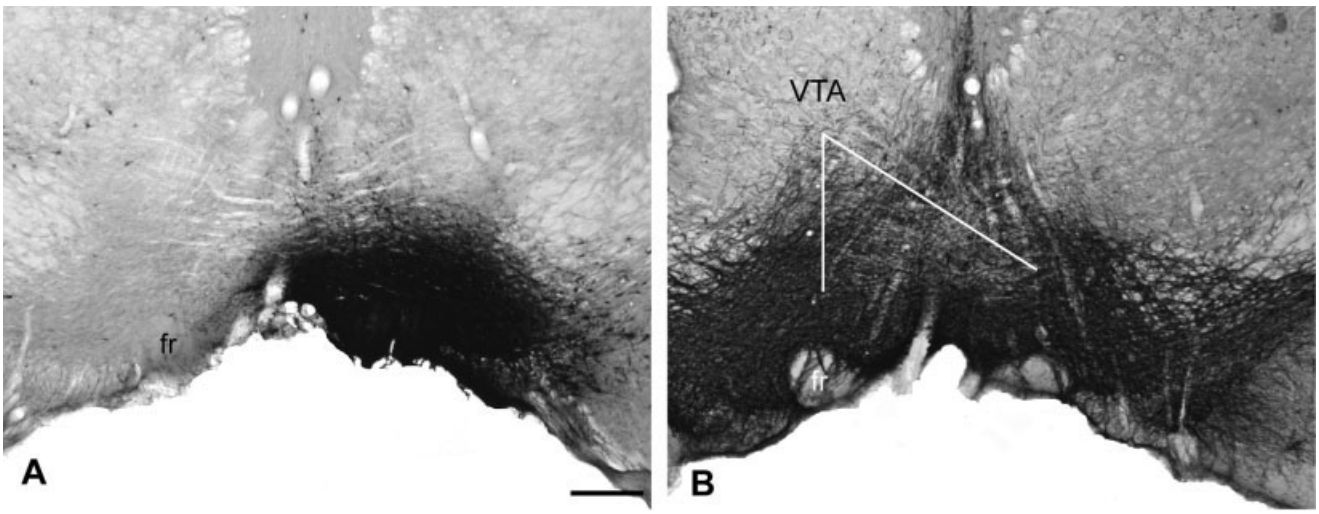

Fig. 2. The injection site in case 99059 is shown at its largest dorsoventral and mediolateral extent (**A**). The VTA was delineated by reference to tyrosine hydroxylase immunoreactivity as shown in **B**. fr, fasciculus retroflexus. Scale bar = 250 μm in A (applies to A,B).

labeled neurons, whereas others appear to be more or less avoided.

Large injections

Among eight cases with tracer deposits involving almost the entire rostrocaudal extent of the VTA on one side of the brain, the injection sites from five are schematically depicted in Figure 1. Among these five, case 99059 is representative and will be described in detail. Spindle-shaped in the rostrocaudal direction, the injection site extends rostrally to the transition area between the VTA and the lateral hypothalamic area and caudally to the

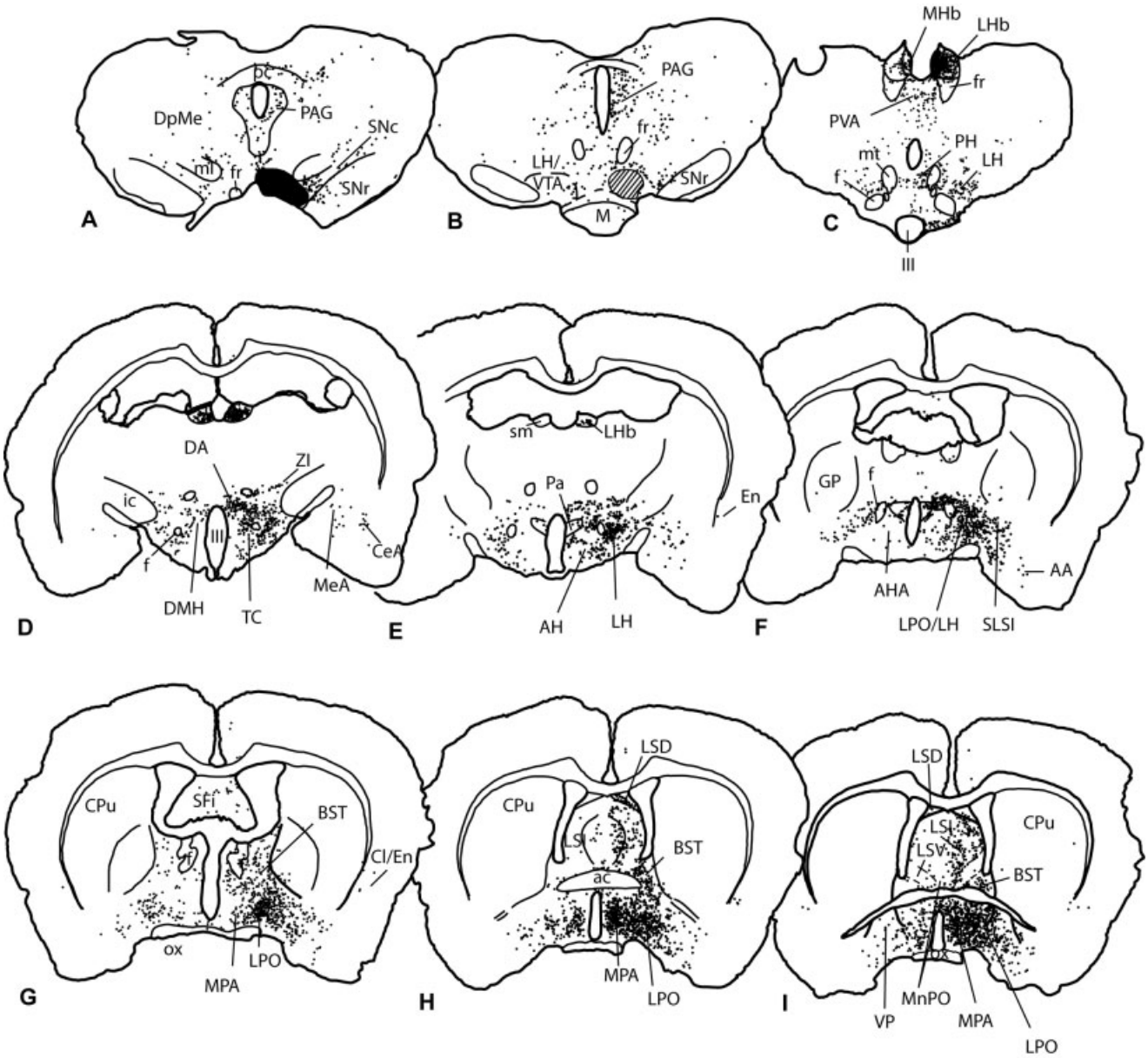

Fig. 3. **A–O:** Schematic representations of retrogradely labeled neurons after a large Fluoro-Gold injection involving almost the entire VTA on one side of the brain (case 99059). Each retrogradely labeled neuron is represented by one dot. Sections are ordered from caudal to rostral starting at the level of the VTA (A). Note that retrogradely labeled neurons are not confined to distinct nuclei but comprise an elongated formation. The main formation is centered on the medial forebrain bundle and extends via the lateral hypothalamic area (C–F) and lateral septum (H–M) into the prefrontal cortex (K–O). A second formation centered on the fasciculus retroflexus and involving the periaqueductal gray, lateral habenular complex, and paraventricular thalamic nucleus lies dorsal and in parallel to the first (A–E). Within the confines of these formations, some brain structures are relatively enriched with retrogradely labeled neurons, whereas others are more or less avoided. Note the lesser input from the contralateral side of the injection side.

caudal limit of the VTA. It fills almost the entire dorsoventral and mediolateral extent of one side of the rostral VTA (Figs. 1, 2A, 3A), whereas the deposition of FG is more concentrated in the ventral part of the VTA at the level of the interpeduncular nucleus (Fig. 1C), into which some spread of tracer is observed. A number of fiber bundles that pass through the VTA, including the fasciculus retroflexus, mammillary peduncle, and medial lemniscus, are involved in this injection site to varying degrees.

The distribution of retrogradely labeled neurons in this case is described in the following sections and charted in Figures 3 and 8. To identify the brain areas containing retrogradely labeled neurons, each section stained with antibodies against FG was compared with the corresponding Nissl-stained sections.

Descending afferents. Proceeding rostralward from the VTA (Fig. 3A), only few retrogradely labeled neurons are scattered in the posterior hypothalamus throughout

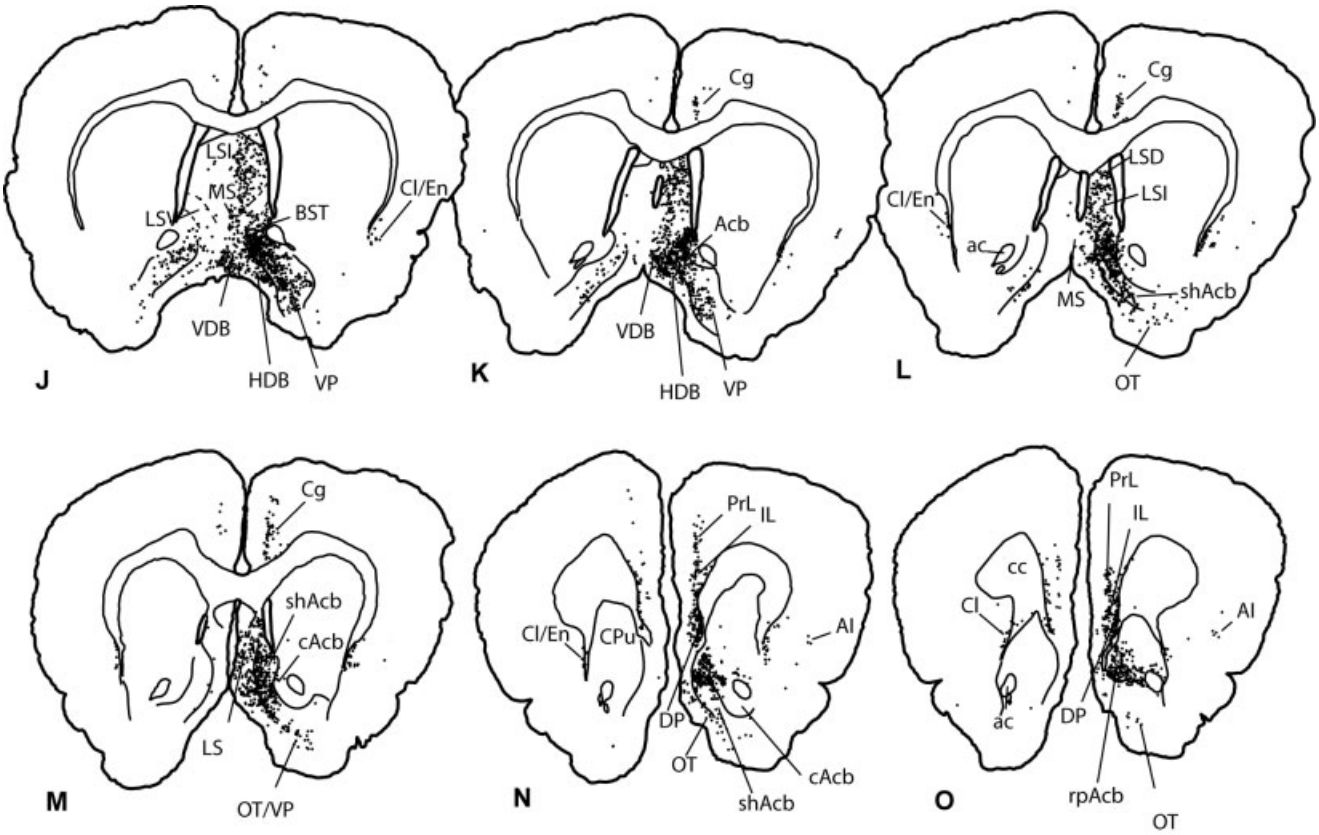

Figure 3 (Continued)

the dorsal premammillary and posterior hypothalamic nucleus (Fig. 3C). Although the injection site involves a transition area between the VTA and the caudal lateral hypothalamic area (Fig. 3B), the caudal lateral hypothalamic area is the only part of the lateral hypothalamus that contains few FG-positive neurons (Fig. 3B,C). Proceeding rostralward, more retrogradely labeled neurons occupy tuberal and anterior hypothalamic levels, mainly in the lateral and to a lesser extent the medial hypothalamus (Fig. 3D–F). The lateral hypothalamic area contains numerous, heavily labeled, large, multipolar neurons emitting relatively thick, long, and sparsely branching labeled dendrites (Fig. 4A,B). These dendrites stretch out in a radiating manner that would be expected to intercept the course of the longitudinally traversing fibers of the medial forebrain bundle. Some of the labeled neurons are situated closer together, forming a cluster, whereas others are loosely scattered around them, together encompassing the entire breadth and height of the lateral hypothalamic area (Figs. 3D–F, 4). Medial to it, fewer and smaller labeled neurons are situated mostly in the dorsal hypothalamic area (Fig. 4A). Some extend into the dorsomedial hypothalamic nucleus, as do a few into the tuberal, ventromedial, and anterior hypothalamic nuclei (Fig. 3D,E). Dorsal to the medial and lateral hypothalamus, some labeled neurons are arranged in a thin mediolateral layer involving the ventral part of the zona incerta and an area medial to it (Fig. 3D,E).

The numbers of labeled cells increase further at the transition between the lateral hypothalamic and the lateral preoptic area. In addition to the numerous FG-positive neurons in the lateral hypothalamic area, labeled cells extend medially and dorsally in a band-like structure that arches over the fornix bundle to surround the paraventricular hypothalamic nucleus (Figs. 3F, 4C,D). An occasional labeled neuron is also present within the paraventricular nucleus. This band of cells is continuous with a group of retrogradely labeled neurons medial and lateral to the ascending limb of the stria medullaris, which merges imperceptibly with a group of FG-positive neurons in the lateral preoptic area (Fig. 3F–I). Here, a multitude of retrogradely labeled neurons similar to (and continuous with) those of the lateral hypothalamic area fills the entire extent of the lateral preoptic area. These neurons are surrounded medially by numerous labeled neurons in the medial preoptic area and laterally by scattered neurons in the bed nucleus of the stria terminalis (Fig. 3G–I).

The number of labeled neurons at this level in turn is exceeded by a multitude of labeled neurons lying ventral to the crossing of the anterior commissure (Figs. 3I, 5). Here, retrogradely labeled cells encompass the territories of the median preoptic nucleus, medial and lateral preoptic area, horizontal limb of the diagonal band of Broca, and bed nuclei of the stria terminalis and extend dorsally to the lateral septum complex (Figs. 3I, 5). Proceeding rostrally, many large, retrogradely labeled neurons occupy the ventral pallidum (Fig. 6A,B). Moderate numbers of retrogradely labeled medium spiny neurons are observed in the nucleus accumbens, these being relatively confined to the shell (Figs. 3L–N, 6C,D) and rostral pole (Figs. 3O,

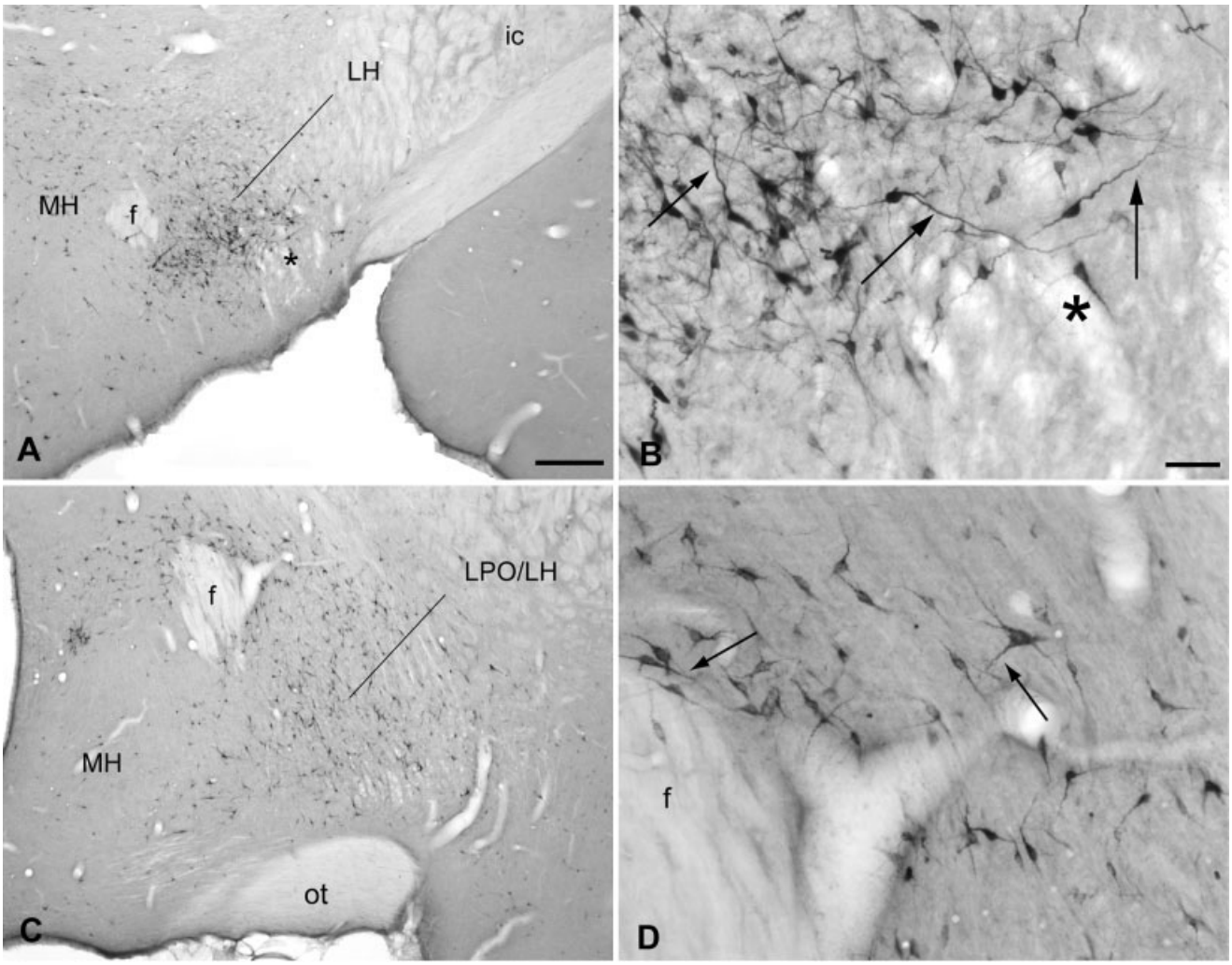

Fig. 4. After Fluoro-Gold injections into the VTA, numerous retrogradely labeled neurons can be observed in the lateral hypothalamic area (LH; **A**). Fewer Fluoro-Gold positive neurons are situated in different nuclei of the medial hypothalamus (MH). At higher magnification (**B**), Fluoro-Gold-positive neurons expressing the features of the typical "reticular" hypothalamic neurons can be readily observed. At the level of the transition between lateral hypothalamic area and lateral preoptic area (LPO/LH; **C**) retrogradely labeled neurons encompass the entire LPO/LH, arching as a band-like structure over the fornix bundle (f) into the dorsal part of the MH. At higher magnification (**D**), the typical morphology of lateral hypothalamic neurons is again readily recognized. Note the long, thick, sparsely branching dendrites of these neurons (arrows, B,D). The fornix serves as a fiducial in C,D. Asterisks mark the same vessel in A and B. ic, internal capsule; ot, optic tract. Scale bars = 250 μm in A (applies to A,C); 50 μm in B (applies to B,D).

7A). Medial to the ventral pallidum, numerous densely packed, retrogradely labeled neurons in the horizontal limb of the diagonal band of Broca continue in decreasing numbers into the vertical limb/medial septum complex (Fig. 3I,J). The lateral septum also contains numerous labeled cells confined largely to the intermediate part, with only a few present in the dorsal part and an occasional FG-positive neuron in the ventral part (Fig. 3H–M).

Moderate numbers of retrogradely labeled neurons are found in different areas of the medial prefrontal cortex (Fig. 7A). These cells are typically triangular and are localized to the deep layers of the cingulate cortex and the prelimbic (Fig. 7A,B) and infralimbic (Fig. 7A,C) cortices (Fig. 3K–O). More densely packed and smaller FG-positive neurons are present in an area that Paxinos and Watson (1998) identified as dorsal peduncular cortex (Figs. 3N,O, 7C). In addition, moderate numbers of retrogradely labeled neurons are observed in the rostral claustrum/endopiriform nucleus complex (Fig. 3G–O). These neurons are arranged as a thin band of labeled cells near the corpus callosum (Fig. 7A,E).

Retrogradely labeled neurons positioned dorsal and in parallel to those centered on the medial forebrain bundle occupy the periaqueductal gray, intralaminar thalamic nuclei, and lateral habenula. Proceeding from the VTA, a band of FG-positive neurons extends dorsally along the third ventricle to involve the periaqueductal gray and thalamic parafascicular nucleus and continues into the lateral habenula (Fig. 3B–D). The lateral habenula is entirely and evenly filled with retrogradely labeled neurons of different sizes. Retrograde labeling is also observed in the medial habenula (Fig. 3C,D). These neurons should be

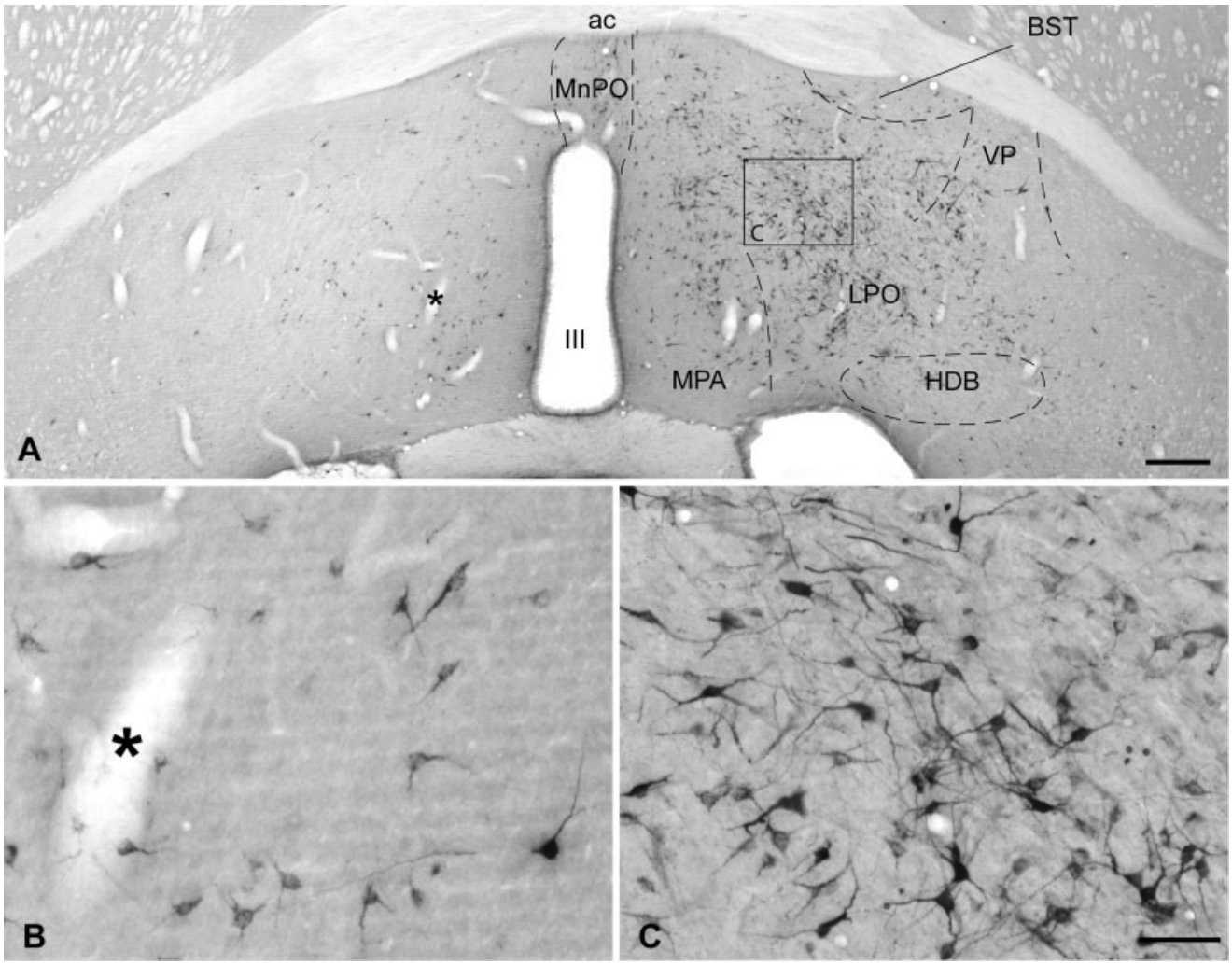

Fig. 5. Ventral to the crossing of the anterior commissure (ac), numerous retrogradely labeled neurons are observed (**A**) ipsilaterally (A, right side, and C) as well as contralaterally (A, left side, and B) to the injection. These neurons occupy a large area of the basal forebrain, are unconfined to nuclei, and display similar morphological characteristics, i.e., long, nonbranching dendrites compared with a small cell body (**B,C**) The same vessel is marked in A and B (asterisks). BST, bed nucleus of stria terminalis; HDB, horizontal limb of diagonal band of Broca; MnPO, median preoptic nucleus; MPA, medial preoptic area; VP, ventral pallidum; III, third ventricle. Scale bars = 250 μm in A; 50 μm in C (applies to B,C).

regarded with caution, however, insofar as the main efferent bundle of the medial habenula, the fasciculus retroflexus, is involved in the injection site and an anterograde tracing study did not find a projection from the medial habenula to the VTA (Herkenham and Nauta, 1979). Ventral to the habenula, FG-positive neurons are scattered throughout the paraventricular thalamic nucleus, preferentially in its caudal part and close to the lateral habenula (Fig. 3C).

Ascending afferents. At levels between the rostrocaudal limits of the VTA itself (Fig. 8A,B), a few retrogradely labeled neurons are scattered throughout the periaqueductal gray and lateralward along the substantia nigra pars compacta and reticulata to the substantia nigra pars lateralis. A few FG-positive neurons can also be seen in the deep layers of the superior colliculus. Proceeding caudally, retrogradely labeled neurons are found largely in two areas: the reticular formation and the periaqueductal/central gray, including the dorsal raphe, laterodorsal tegmental nucleus, and locus coeruleus (Fig. 8).

Moderate numbers of retrogradely labeled neurons are concentrated in the dorsolateral quadrant of the periaqueductal gray (Fig. 8B–D), which otherwise exhibits fewer labeled neurons scattered throughout. Ventrally, numerous FG-positive neurons in the dorsal raphe (Fig. 9A,B) are easily distinguishable from the surrounding labeled periaqueductal gray neurons by their denser packing (Fig. 8D,E). The very rostral dorsal raphe contains small labeled cells, whereas large, multipolar labeled neurons are increasingly recognized more caudally and in the pontine part of the dorsal raphe outnumber the small ones. Large, retrogradely labeled neurons are found lateral to the dorsal raphe, in the laterodorsal and pedunculopontine teg-

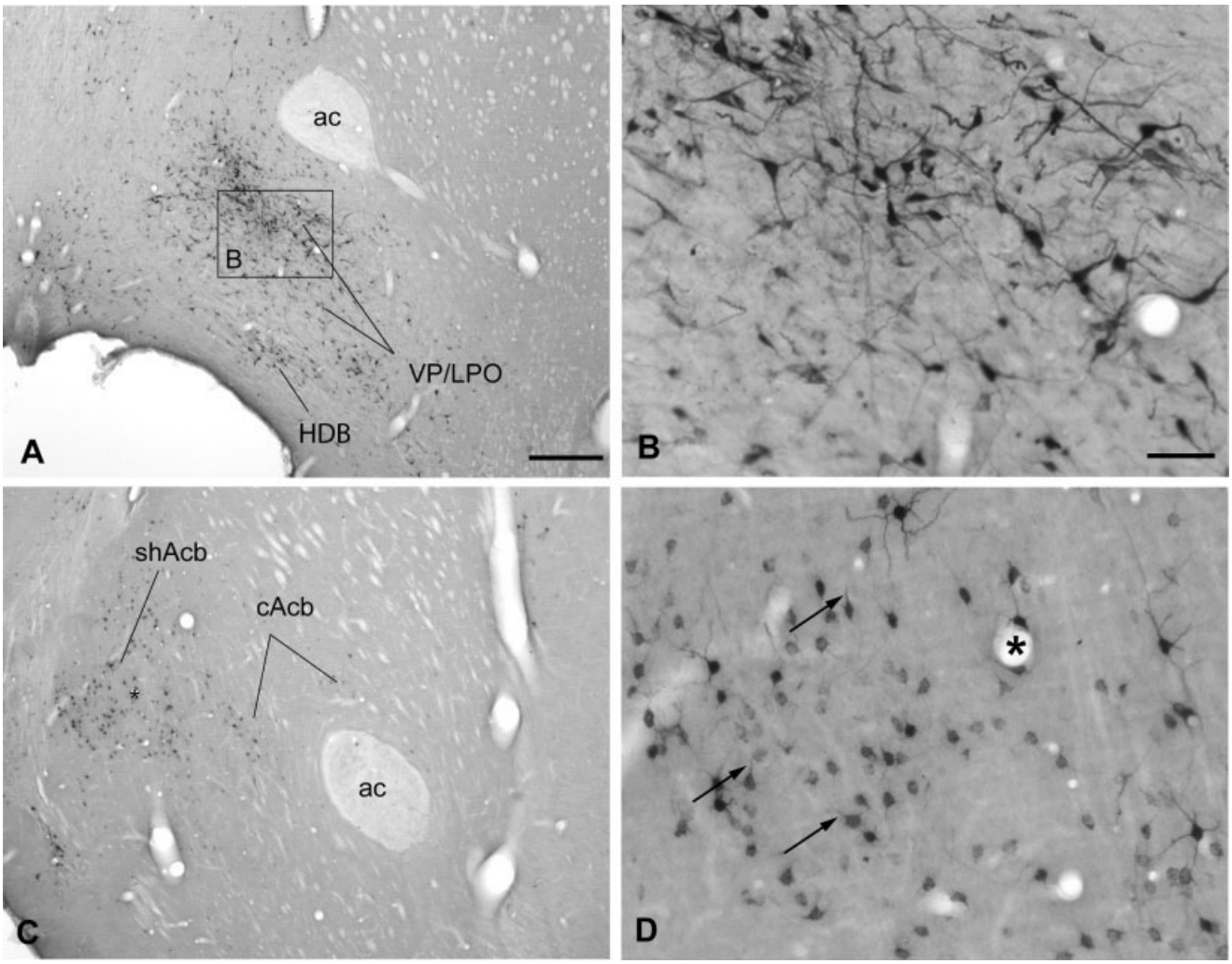

Fig. 6. In the area where ventral pallidum and lateral preoptic area interdigitate (VP/LPO; **A**) densely packed retrogradely labeled neurons are found, all of which express the typical morphology of reticular neurons (**B**). In contrast, smaller neurons with a different morphology are observed in the nucleus accumbens (**C,D**). Here, Fluoro-Gold-positive neurons are largely confined to the shell (shAcb; in case 99059 in its dorsomedial part); a few labeled neurons can also be observed in the core (cAcb; C). As can be seen at higher magnification (D), retrogradely labeled neurons have short, thin, ramifying dendrites (arrows), i.e., a morphology typical of medium spiny neurons, and thus, look very different from those in B and Figures 4 and 5. Asterisks mark the same vessel in C,D. ac, anterior commissure; HDB, horizontal limb of diagonal band of Broca. Scale bars = 250 μm in A (applies to A,C); 50 μm in B (applies to B,D).

mental nuclei (Fig. 8E,F) and, somewhat farther caudally, in the locus coeruleus. Lateral to the locus coeruleus, numerous small retrogradely labeled neurons are observed in the parabrachial nucleus, mainly in its dorsal part. Retrogradely labeled neurons scattered throughout the central gray are observed to the level of the genu of the nucleus of the seventh cranial nerve (Fig. 8G).

In the reticular formation, retrogradely labeled neurons can be roughly described as being in three groups: two more or less distinct columns occupy the midline and paramedian planes, and a third group is seemingly randomly distributed throughout the deep mesencephalic field and pontine reticular formation. Again, these groups of labeled neurons are not strictly segregated but always have retrogradely labeled neurons "connecting" them.

The midline is occupied by scattered, small, oval, labeled neurons located in the median and pontine raphe nuclei (Fig. 8E,F). Caudally to the pontine raphe, large, multipolar, FG-positive neurons with long, thick dendrites are loosely scattered throughout the raphe interpositus in numbers that diminish approaching the root of the seventh cranial nerve (Fig. 8G). Proceeding farther caudally, a few labeled neurons are seen occasionally in the midline of the gigantocellular field of the reticular formation to the level of the prepositus nucleus (Fig. 8H,I).

Laterally to the midline, a moderate number of retrogradely labeled neurons is observed in a paramedian plane. Just caudally to the VTA, labeled neurons are interspersed in the decussation of the superior cerebellar peduncle (Figs. 8C,D, 9C) and, proceeding somewhat farther caudally, spread in the paramedian raphe nucleus (Figs. 8E, 9D), but not caudally beyond it.

Lateral to the labeled neurons in the paramedian plane are numerous FG positive cells scattered throughout dif-

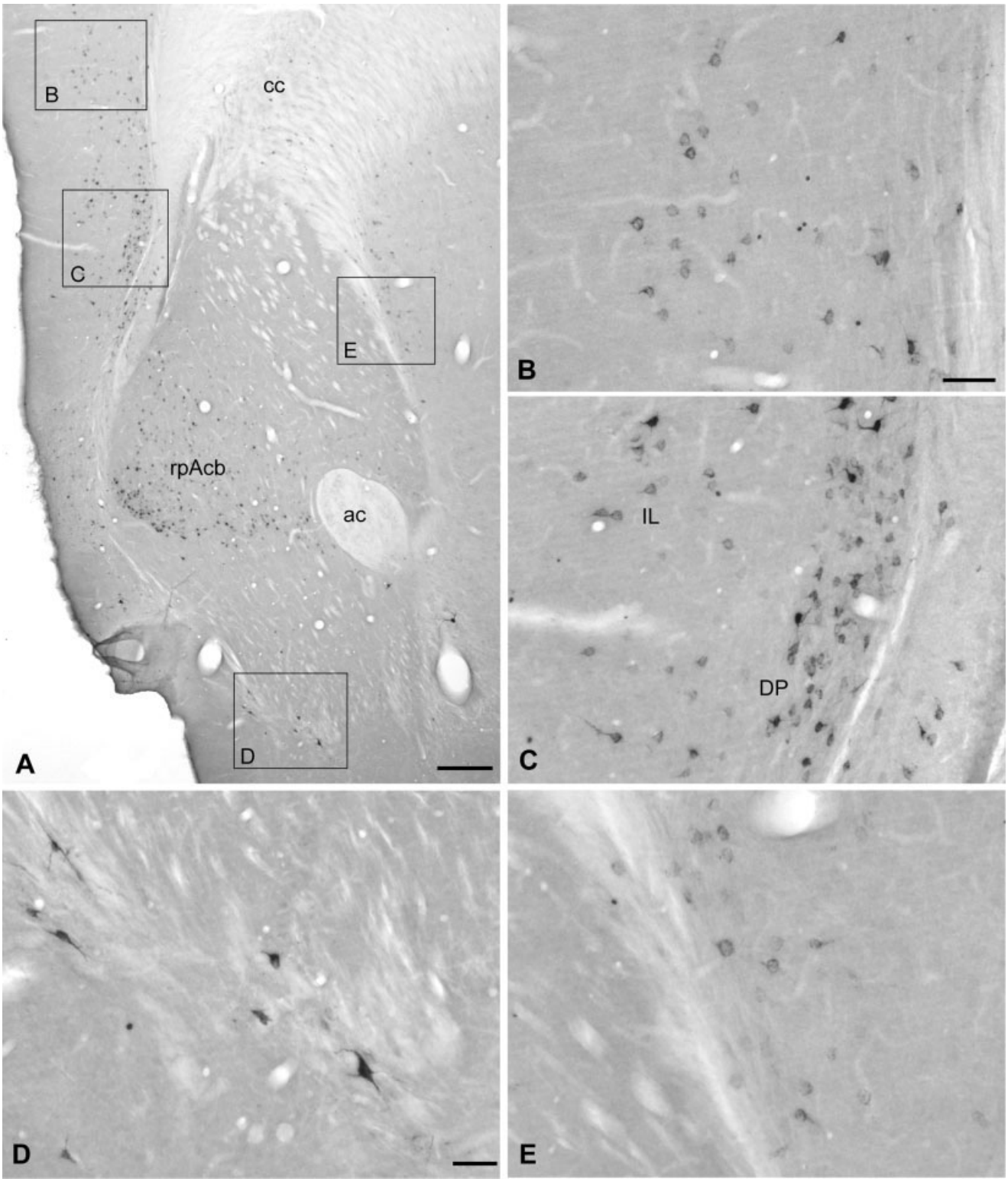

Fig. 7. Micrograph illustrating a section of the rostral pole of nucleus accumbens (rpAcb; **A**). Note the different morphologies of retrogradely labeled neurons in different brain areas: **B**, prelimbic cortex; **C**, infralimbic (IL) and dorsal peduncular cortex (DP); **D**, ventral pallidum; and **E**, a thin band of labeled cells in the claustrum. ac, anterior commissure; cc, corpus callosum. Scale bars = 250 μm in A; 50 μm in B (applies to B,C,E), D.

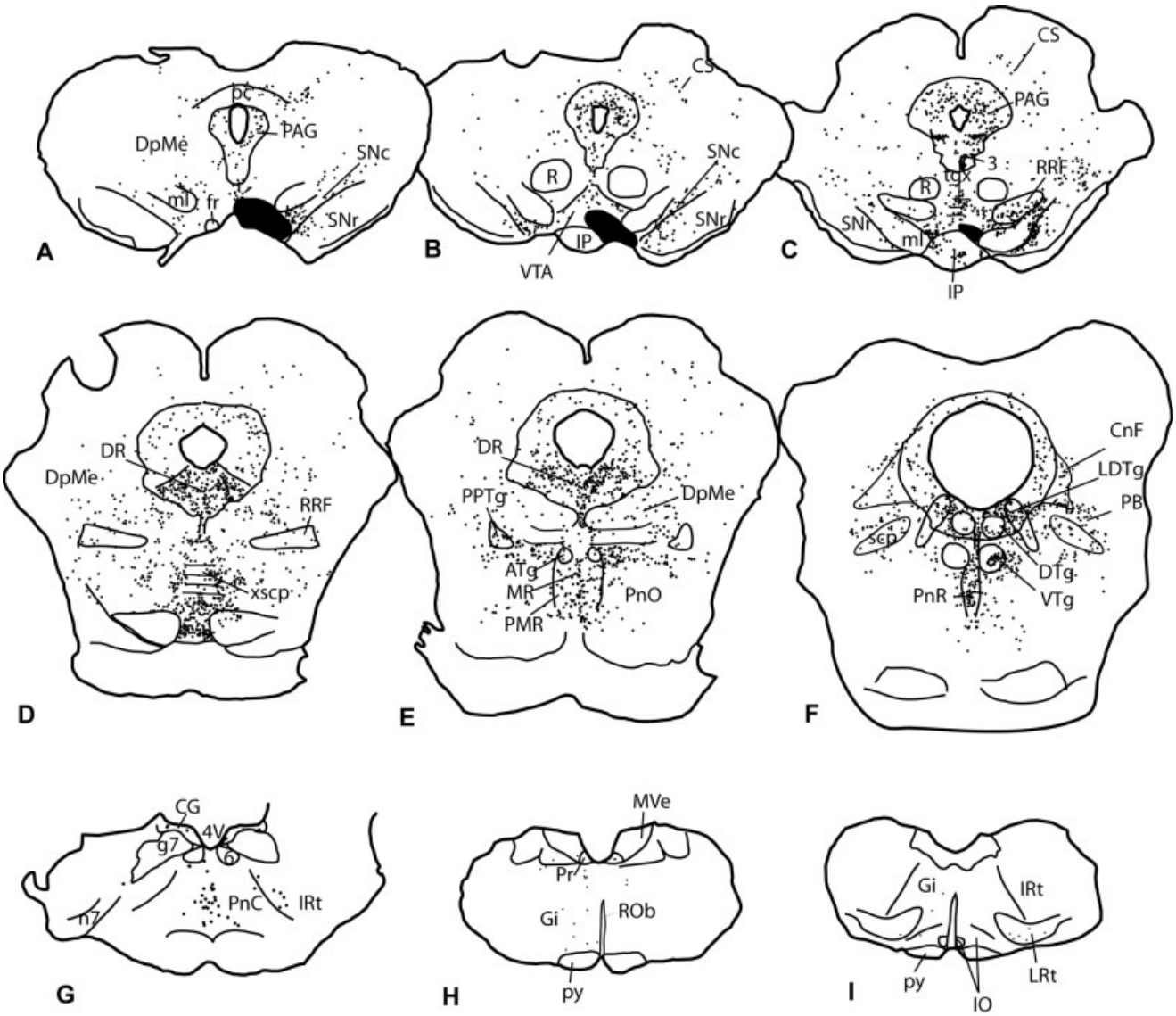

Fig. 8. **A–I:** Schematic representation of retrogradely labeled neurons in the brainstem of case 99059. The injection site is drawn in A–C. Sections are ordered from rostral to caudal, progressing from the level of the VTA (A) to the medulla oblongata (I). Each dot represents one retrogradely labeled neuron. Note the comparable input from the contralateral side of the injection side.

ferent parts of the reticular formation. Although the rostral part of the deep mesencephalic field contains hardly any retrogradely labeled neurons (Fig. 8A,B), FG-positive neurons are more numerous there just caudal to the VTA (Fig. 8C,D). In addition to those seemingly randomly distributed throughout the deep mesencephalic field, labeled neurons also invade the serotoninergic B9 group, dopaminergic retrorubral field (Fig. 8C,D), and cholinergic pedunculopontine nucleus (Fig. 8E). Few relatively small, dorsolaterally positioned labeled neurons are present in and around the cuneiform nucleus (Fig. 8F). At the level of the decussation of the cerebellar peduncle, the deep mesencephalic field moves dorsally, yielding space to the oral field of the pontine reticular formation, where large, multipolar neurons are scattered throughout the area (Fig. 8E,F). The oral pontine field merges with the caudal pontine field, where an occasional large, multipolar FG-positive neuron is observed (Fig. 8G).

Contralateral afferents. It is important to note that almost all structures that provide an input to the VTA do so bilaterally by sending either a lesser (in the case of the descending afferents; Fig. 3) or a fairly comparable (as is the case for the ascending afferents; Fig. 8) input from the contralateral side. In the case of the descending afferents, the relative numbers of retrogradely labeled neurons occupying the various contralateral structures are proportional to what is seen ipsilaterally. Thus, areas with especially numerous retrogradely labeled neurons contralaterally include the lateral hypothalamic area, lateral and medial preoptic area, ventral pallidum, and lateral

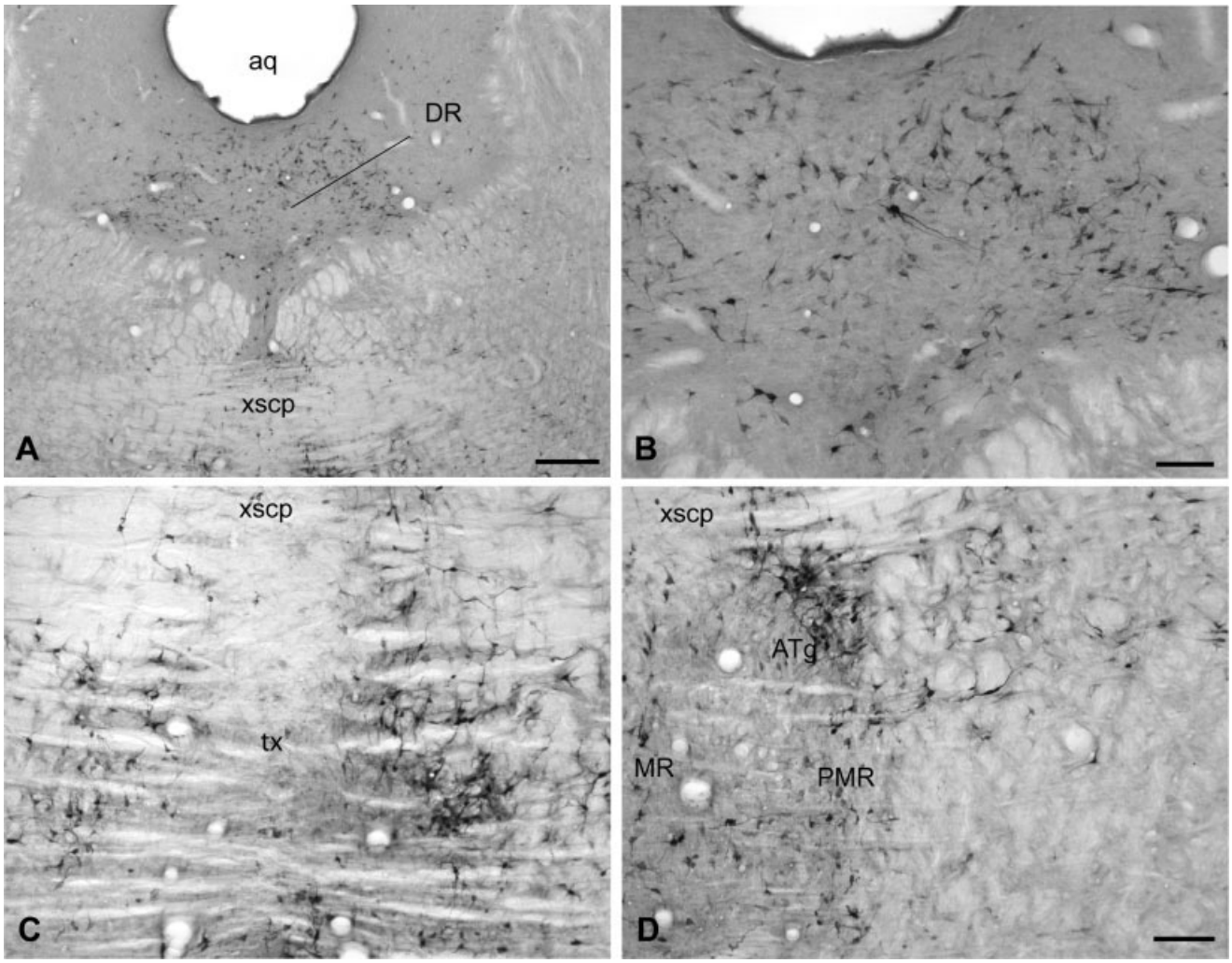

Fig. 9. Micrographs illustrating retrogradely labeled neurons in the brainstem. Densely packed Fluoro-Gold-positive neurons can be observed in the dorsal raphe (DR) at lower (**A**) and higher (**B**) magnifications. Fluoro-Gold-positive neurons are also observed in the median (MR) and paramedian raphe (PMR; **D**) as well as paramedian between the fibers of the tegmental decussation (tx; **C**) and just ventral to the decussation of the superior cerebellar peduncle (xscp; D). aq, aqueduct; ATg: anterotegmental nucleus. Scale bars = 300 μm in A; 100 μm in B (applies to B,C), D.

habenula. The nucleus accumbens is exceptional in this regard, insofar as FG-positive neurons are found there only very sporadically contralateral to the VTA injection side.

Topography of VTA afferents

The large injections, one of which was described in the previous paragraph, give an impression of the overall input to the VTA. The VTA, however, is not thought to be a homogenous structure. Based on differences in cell morphology, it is commonly accepted to divide the VTA into subnuclei (Olszewski and Baxter, 1954; Phillipson 1979b; Halliday and Törk, 1986; Oades and Halliday, 1987). In addition, functional studies suggest differences between rostral and caudal VTA (see, e.g., Ikemoto and Wise, 2002; Bolaños et al., 2003; Rodd et al., 2004). To investigate whether some of these heterogeneities are reflected in differences in the afferent connections of the VTA, in the next set of experiments small deposits of FG were placed into different parts of the VTA, and the distributions of retrogradely labeled neurons were analyzed and compared.

Medial vs. lateral. In the first set of experiments, FG was centered in the medial (cases 05005, 05018), in the lateral (case 99039), or in the far lateral (case 99132) part of the VTA (Figs. 10, 11), the latter including the transition to the medial substantia nigra pars compacta. In cases 99039 and 99132, some tracer also spread into the medial- and dorsalmost substantia nigra pars reticulata.

Some noticeable differences can be observed, which are found mainly in the ventral striatopallidal system. Whereas only few retrogradely labeled neurons in the rostral pole of the accumbens are observed after an injection into the medial part of the VTA (Fig. 11A1), the accumbens rostral pole contains a moderate amount of FG-positive neurons after tracer deposit into the lateral part (Fig. 11B1) and an even higher amount of retro-

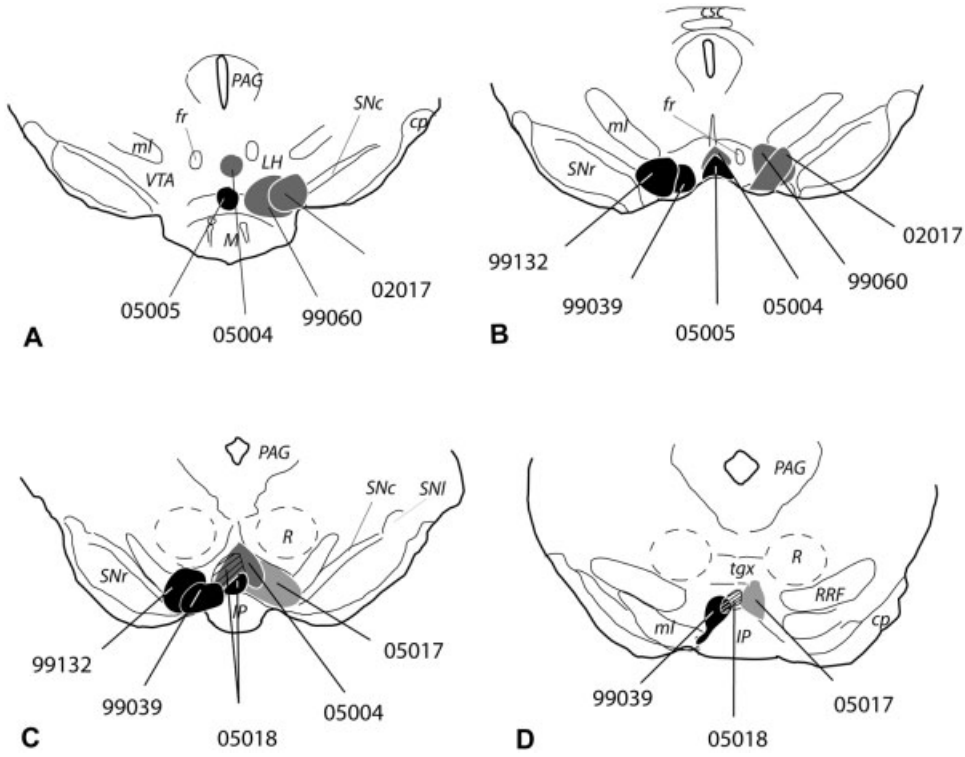

Fig. 10. **A–D:** Schematic representations of injection sites in different parts of the VTA. On the left side of the VTA (drawn in black) are injection sites that were made either in the medial (cases 05005 and 05018) or lateral (cases 99132 and 99039) part of the VTA. On the right side of the VTA are injection sites in the rostral (dark gray; cases 99060, 05004, 05005, 02017) or caudal (light gray; case 05017) part of the VTA. Drawings are ordered from rostral to caudal, A representing the most rostral.

gradely labeled neurons after injections into the far lateral part of the VTA (Fig. 11C1). Tracer deposits into the medial VTA result in FG-positive neurons confined to the dorsal and medial accumbens shell, whereas injections into the lateral and far lateral VTA result in labeling progressively farther ventrally and laterally in the shell (Fig. 11A2,B2,C2). The lateral part of the sub- and postcommissural ventral pallidum contains a multitude of retrogradely labeled neurons only after injections into the lateral and far lateral VTA (Fig. 11A3,B3,C3). In addition to these differences, some minor differences are observed. Case 05018 (medial injection) results in a preferential labeling of the median over the paramedian raphe (Fig. 11A5). In case 99132 (far lateral injection), more retrogradely labeled neurons are within the boundaries of the paraventricular hypothalamic nucleus than in all other cases.

Rostral vs. caudal. In the functional studies cited above, the interpeduncular nucleus was used to divide the VTA into rostral and caudal parts, the rostral VTA being rostral to the interpeduncular nucleus. Therefore, we considered the injections in cases 99060 and 02017 as rostrolateral (Fig. 10A,B), in cases 05004 and 05005 as rostromedial (Fig. 10A–C), and in case 05017 (Fig. 10C,D) as caudal in the VTA. Based on this division, no noticeable differences in retrograde labeling were detected.

Control injections

Injections rostral to the VTA (case 05003), medial substantia nigra pars reticulata (cases 99127, 99114, 9046), lateral interpeduncular nucleus (case 99029), and midbrain tegmentum dorsolateral to the VTA including the deep mesencephalic field and red nucleus (cases 99033, 04135; Fig. 12) resulted in characteristic patterns of retrograde labeling, all of which were distinct from those observed after tracer placements into the VTA. After FG injections into the medial substantia nigra pars reticulata (Figs. 11, column D, 12B), by far the most retrogradely labeled neurons were observed in the medial caudate-putamen (Fig. 11D2,D3), globus pallidus, and subthalamic nucleus. Densely packed labeled neurons were also found in the zona incerta and anterotegmental and ventral and dorsal tegmental nucleus. Some labeled neurons were observed in the periaqueductal and central gray. Structures that project heavily to the VTA, such as lateral preoptic- and lateral hypothalamic area, lateral habenula, and dorsal raphe, contained few and only lightly stained neurons after injections into the substantia nigra pars reticulata. Although the rostral pole of the nucleus accumbens was retrogradely labeled after substantia nigra pars reticulata and VTA injections, tracer deposits into the substantia nigra pars reticulata resulted in densely packed labeled neurons confined to the dorsal part of the rostral pole of the nucleus accumbens (Fig. 11D1). In contrast, injections into the lateral VTA resulted in labeling of loosely scattered neurons predominantly in the ventromedial and central part of the accumbens rostral pole (Fig. 11B1,C1). Thus, the medial substantia nigra pars reticulata and the VTA not only receive a different set of afferents but also differ considerably in the organization of their afferents.

Whereas the VTA receives a wide input from many sources, without a predominant one, the substantia nigra pars reticulata is innervated from a restricted set of nuclei, in which neurons projecting to the substantia nigra pars reticulata are numerous and very densely packed.

Injections placed into the midbrain tegmentum dorsolateral to the VTA (cases 99033, 04135) produced retrograde labeling predominantly in the zona incerta, fields of Forel, substantia nigra pars reticulata, principal sensory trigeminal nucleus, and ventral part of the pontine reticular formation. After an FG injection into the lateral interpeduncular nucleus (case 99029), which involved to a small degree the VTA, crus cerebri, and medial lemniscus, many retrogradely labeled neurons were observed in the dorsal and median raphe nuclei and in the ventral, dorsal, and laterodorsal tegmental nuclei. Furthermore, the medial habenula was heavily labeled, mainly in its dorsal part, in an area that Andres et al. (1999) identified as the superior subnucleus of the medial habenula. This is in accordance with results from a study of Herkenham and Nauta (1977), who showed that the dorsal part of the medial habenula projects to the lateral interpeduncular nucleus. Some retrogradely labeled neurons were found in the horizontal limb of the diagonal band of Broca, lateral preoptic area, lateral habenula, zona incerta, periaqueductal and central gray, raphe magnus, locus coeruleus, and, probably because of the involvement of the medial lemniscus in the injection site, the nucleus cuneatus.

The injection rostral to the VTA was placed ventral to the periaqueductal gray between the fasciculi retroflexus and appeared to extend ventralward with them. Retrograde labeling in this case was very sparse; only few neurons were labeled, and they were situated in the lateral ventral pallidum, magnocellular preoptic nucleus, lateral hypothalamic area, lateral part of lateral habenula, periaqueductal gray, and substantia nigra pars reticulata.

Patterns of terminal arborization in the VTA

The data obtained so far suggest extraordinarily abundant and diverse input to the VTA, but how might such a multifarious innervation of one brain structure be organized? What patterns of terminal arborization allow so many neurons from so many sources to project to the VTA? To gain insight into the patterns of afferent terminations in the VTA, 36 injections of the anterograde tracers PHA-L or BDA were placed in 14 different forebrain areas identified in the previous experiments as sources of descending projections to the VTA (Fig. 13) These experiments, first, provide an important corroboration of the data obtained from retrograde tracing and, second, reveal remarkably uniform patterns of terminations in the VTA irrespective of the brain areas of origin.

PHA-L injections were placed into the lateral hypothalamic area, lateral habenula, lateral preoptic area, horizontal limb of the diagonal band of Broca, sublenticular substantia innominata, dorsomedial ventral pallidum, core of the nucleus accumbens, and prefrontal cortex (Fig. 13). In addition, a PHA-L control injection was placed into the dorsomedial entopeduncular nucleus. BDA was injected into the lateral septum, central nucleus of the amygdala, different parts of the shell or core of the nucleus accumbens, and medial, central, and lateral ventral pallidum (Fig. 13). Except for the control injection into the entopeduncular nucleus, all injections resulted in anterogradely labeled axons in the VTA (Fig. 14). These labeled axons from different sites of origin have a remarkably similar morphology and distribution in the VTA: relatively straight labeled fibers with short collaterals and a poor terminal arborization are distributed throughout the entire mediolateral and dorsoventral VTA ipsilateral to the injection sites. These anterogradely labeled axons possess multiple round varicosities of different sizes (commonly thought to represent synaptic-like specializations, i.e., synapses en passant) separated from each other by intervaricose axon segments of different length (Fig. 14, insets at lower right). Fewer labeled fibers of the same morphology can be observed in the VTA contralateral to the injection sites.

It should be noted that after BDA injections into the central nucleus of the amygdala, which contained only a few retrogradely labeled neurons after tracer deposits into the VTA, most labeled fibers pass through the rostral and lateral VTA without expressing terminal-like specializations. Only an occasional fiber with varicosities could be observed in the VTA.

When the densities of anterogradely labeled axons in the VTA from different sites of origins are compared, it becomes apparent that there is no clear single main afference, but, rather, several brain areas, including the lateral hypothalamic area, lateral preoptic area, ventral pallidum, accumbens shell, and prefrontal cortex, provide comparably strong inputs to the VTA (Fig. 14).

A remarkable and important characteristic of the VTA innervation is that most of the structures observed in the anterograde tracing experiments to project to the VTA also innervate with at least similar and usually greater robustness several other brain structures, each of which in turn also provides an input to the VTA (Figs. 14A′–F′, 15). The nucleus accumbens shell, e.g., in addition to sending a projection to the VTA (Fig. 14E), heavily innervates the ventral pallidum (Fig. 14E′) and lateral preoptic and lateral hypothalamic area, which in turn reciprocate the projection and innervate the VTA. The lateral preoptic and lateral hypothalamic area, in addition to innervating the VTA (Fig. 14A,B), send a comparably dense projections to the lateral habenula (Fig. 14A′), which again also projects to the VTA. The same is true for the other structures analyzed, as schematically depicted in Figure 15. This indicates that the VTA and its descending afferents constitute a neuronal network.

DISCUSSION

The present study reveals more abundant inputs to the VTA than anticipated. An additional finding is that neurons projecting to the VTA are not situated in distinct nuclei but rather constitute an elongated formation stretching from the prefrontal cortex rostrally to the medulla oblongata caudally. Structures containing especially many retrogradely labeled neurons include, in order from rostral to caudal, prefrontal cortex, lateral septum, medial septum-diagonal band complex, accumbens shell, ventral pallidum, medial and lateral preoptic area, lateral hypothalamic area, and lateral habenula in the case of the descending afferents and dorsal raphe, periaqueductal gray, and mesencephalic and pontine reticular formation in the case of the ascending afferents. In addition, this formation of VTA projection neurons extends into the medial hypothalamus, where some retrogradely labeled neurons are found in the tuber cinereum, paraventricular and

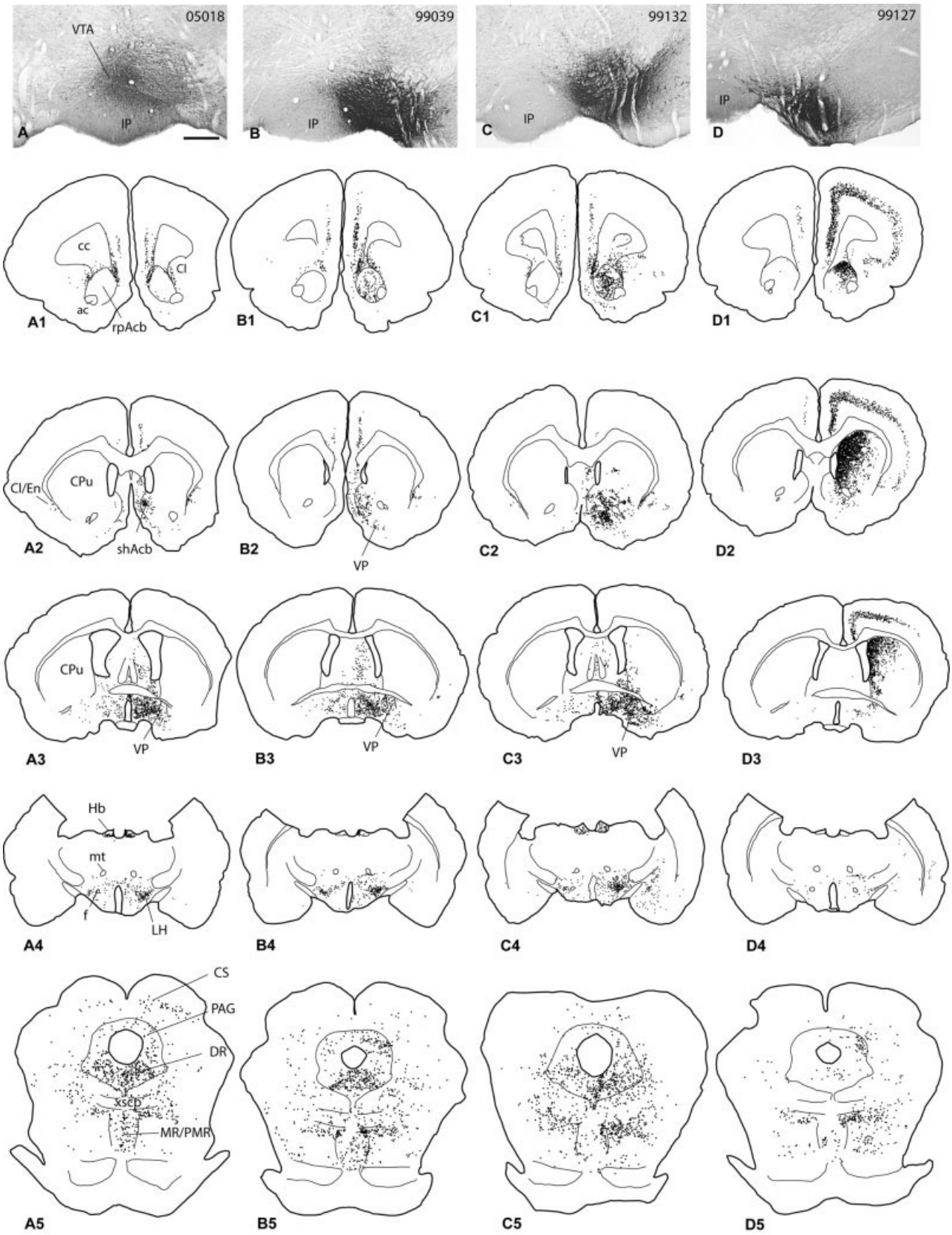
05018
VTA
IP
A
99039
IP
B
99132
IP
C
99127
IP
D
cc
ac
rpAcb
Cl
A1
B1
C1
D1
Cl/En
CPu
shAcb
A2
VP
B2
C2
D2
CPu
VP
A3
B3
C3
D3
Hb
mt
f
LH
A4
B4
C4
D4
CS
PAG
DR
xscp
MR/PMR
A5
B5
C5
D5

Figure 11

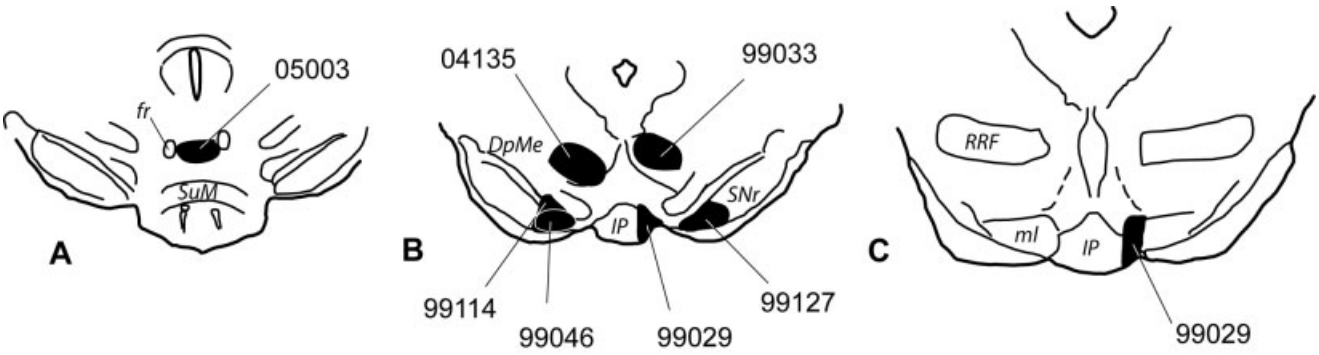

Fig. 12. Schematic representation of control injections placed rostrally, laterally, dorsolaterally, and caudally to the VTA: **A**, between fasciculi retroflexus, case 05003 (rostral); **B**, into substantia nigra pars reticulata (SNr), cases 99114, 99046, 99127, (lateral) and into deep mesencephalic field (DpMe) and red nucleus, cases 04135, 99033 (dorsolateral); and **C**, into the interpeduncular nucleus (IP) and medial lemniscus (ml), case 99029 (caudal).

anterior hypothalamic nuclei, and dorsal hypothalamic area. The anterograde tracing data of the present study not only confirm the result of the FG injections into the VTA but also show that descending afferents of the VTA, in addition to projecting to the VTA, also project at least as robustly to other structures that in turn also project to the VTA, consistent with the presence of an interconnected network of the afferents of the VTA.

Technical considerations

The use of FG as a retrograde tracer has several advantages. FG is easily incorporated by axonal terminals, is quickly transported retrogradely, and can fill soma and dendritic processes extensively up to the fourth and fifth branching order of the dendritic tree, thus providing excellent morphological detail (Schmued and Fallon, 1986; Chang et al., 1990). Although several studies have reported no uptake of FG by undamaged fibers of passage (Schmued and Fallon, 1986; Pieribone and Aston-Jones 1988; Schmued and Heimer, 1990), another study found such an incorporation (Dado et al., 1990). In the study of Dado et al. (1990), little uptake of FG by fibers of passage was observed if no tissue necrosis was visible, so care was taken in the present study to minimize tissue damage. The following steps were taken: iontophoretic application of the tracer (Schmued and Heimer, 1990); a low (1 μA), discontinuous (7 seconds on, 7 seconds off) current to prevent the development of heat at the tips of electrodes and consequent tissue damage; evenly broken-back electrode tips; and, a 1% solution of FG, as opposed to a more concentrated solution, which is shown to cause more tissue damage (Schmued and Fallon, 1986). The retrograde data thus obtained are in good agreement with previous retrograde (Phillipson, 1979a; Simon et al., 1979) and anterograde (see, e.g., Swanson, 1976; Saper et al., 1979; Satoh and Fibiger, 1986; Hallanger and Wainer, 1988; Sesack et al., 1989; Heimer et al., 1991; Groenewegen et al., 1993, 1994; Risold et al., 1994; Zahm et al., 1996, 1999; Vertes et al., 1999; Vertes, 2004) tracing studies. Evidence that in the present study some uptake of FG by fibers of passage did indeed occur is provided by retrogradely labeled neurons in the oculomotor nucleus whose nerve passes through the VTA. Also, retrogradely labeled neurons were observed in the motor and sensory cortex after a control injection into the substantia nigra pars reticulata in which the spread of tracer involved the cerebral peduncle. In addition, the large number of retrogradely labeled neurons observed in the medial habenula should be treated with caution. Anterograde labeling in the VTA was not observed after WGA-HRP injections into the medial habenula (Herkenham and Nauta, 1979). Phillipson (1979a), however, reported retrogradely labeled neurons in the medial habenula exclusively after injections of the retrograde tracer HRP into the interfascicular subnucleus of the VTA. In our hands, extensive retrograde labeling in the medial habenula was observed independently of whether the interfascicular nucleus was involved in the injection site or not. An anterograde tracing study of the medial habenula using PHA-L as tracer is clearly necessary to solve this problem.

Some of the FG injections into the VTA involved to varying degrees the interpeduncular nucleus (Fig. 1). Afferents of the interpeduncular nucleus are well investigated and arise mainly from the medial habenula, dorsal and median raphe, dorsal tegmental nucleus, and nucleus incertus and to a lesser degree from the horizontal limb of the diagonal band of Broca, claustrum, medial and lateral preoptic area, lateral hypothalamic area, ventral- and laterodorsal tegmental nucleus, locus coeruleus, and periaqueductal and central gray (Marchand et al., 1980; Contestabile and Flumerfelt, 1981; Hamill and Jacobowitz, 1984) and, thus, are quite distinct from the afferents of the VTA. Although in the present study the control injection into the interpeduncular nucleus involved only its lateral

Fig. 11. Fluoro-Gold was injected medially (case 05018; **A**), laterally (case 99039; **B**), or far laterally (case 99132; **C**) into the VTA and, for comparison and as a control, into the dorsomedial substantia nigra pars reticulata (case 99127; **D**). After VTA injections, differences in the distribution of retrogradely labeled neurons can be seen predominantly in the basal forebrain (rows 1–3), whereas, farther caudally, retrograde labeling is similar among cases (rows 4, 5). The injection into the substantia nigra pars reticulata reveals a very different distribution of retrogradely labeled neurons (compare column D with columns A–C). Note, that the input to the substantia nigra pars reticulata is much more restricted than that to the VTA. Scale bar = 300 μm in A (applies to A–D).

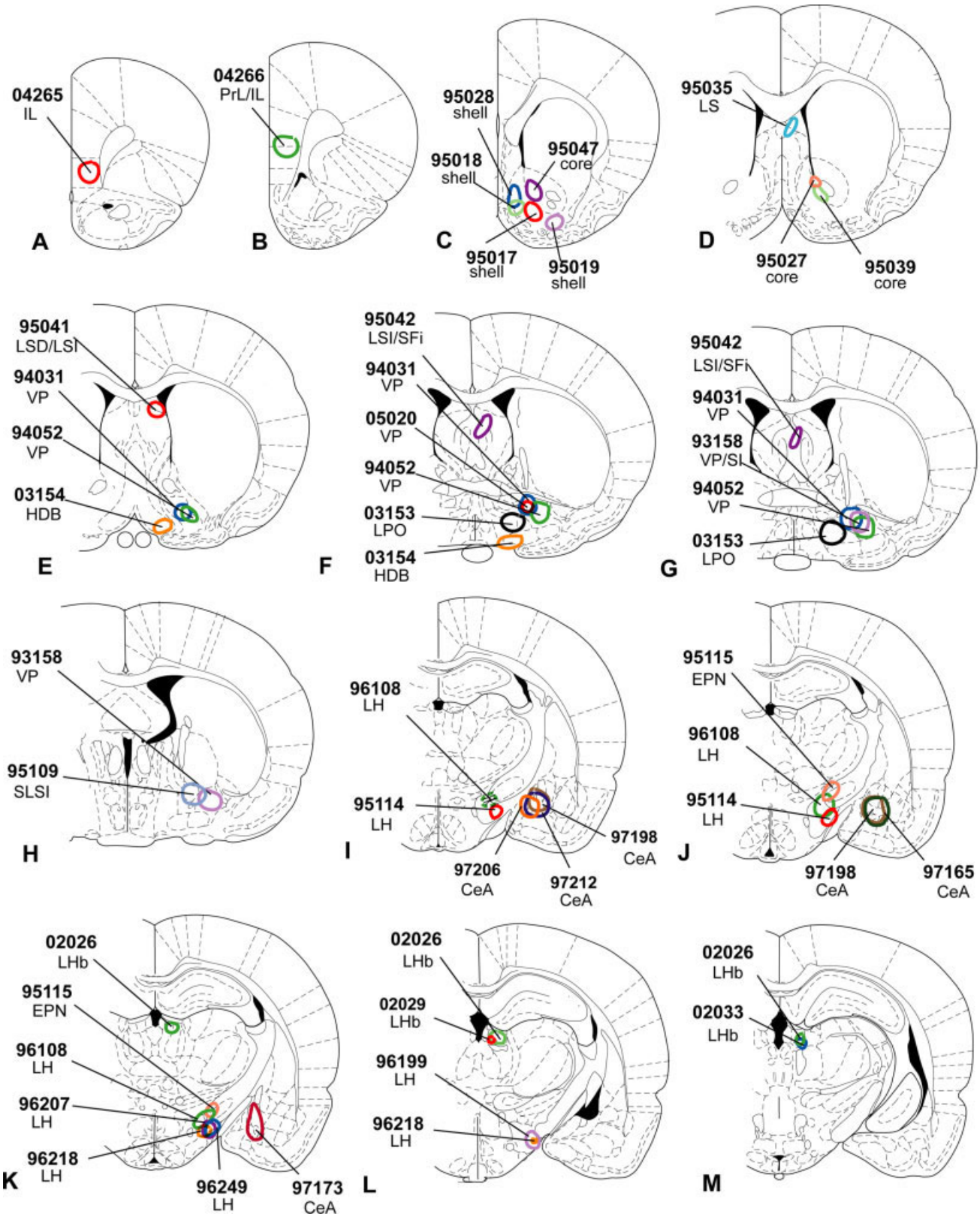

Fig. 13. **A–M:** Schematic representations of injections of the anterograde tracers *Phaseolus vulgaris*-leucoagglutinin (PHA-L) or biotinylated dextran amine (BDA) in several forebrain regions. PHA-L was injected into the prefrontal cortex (ll, Pr/IL; A,B), lateral preoptic area (F,G), ventral pallidum (case 05020; F), horizontal limb of diagonal band of Broca (F), sublenticular substantia innominata (H), lateral hypothalamic area (L–M), and lateral habenula (LHb; K–M) and as a control into the entopeduncular nucleus (EPN; J,K). BDA was injected into accumbens shell (C) and core (D), septum (LS, LSD/LSI, LSI/SFi; E–G), ventral pallidum (E–H) and central nucleus of amygdala (I–K). Templates modified from Paxinos and Watson (1998), reprinted with permission from Elsevier.

part, the same pattern of retrograde labeling as described in the literature could be observed (see Results).

To corroborate the retrograde tracing data, the present study includes 36 cases in which anterograde tracers were injected into 14 different forebrain structures that contained retrograde labeling after FG deposition in the VTA. All of these injections produced substantial numbers of anterogradely labeled fibers in the VTA, with terminal arborizations and varicosities, which, when examined via electron microscopy, are almost invariably found to reflect synaptic specializations. These data indicate that many of the retrogradely labeled structures indeed are likely to have synapses in the VTA. Nevertheless, it cannot be ruled out that some of the retrogradely labeled neurons observed in the present study result from neurons that send fibers through the VTA without synaptically contacting VTA neurons.

The VTA as part of the isodendritic core

A very striking observation in the present study is that neurons giving rise to projections to the VTA are poorly localized in brain nuclei. They rather comprise an elongated formation of neurons stretching from prefrontal cortex to the medulla oblongata. Within this formation, no dominant input to the VTA can be readily discerned. The VTA, instead, appears to receive comparably strong innervations from many sources. This pattern of connections is very different, for instance, from the pattern in the striatopallidal or amygdalar system, in which nuclei receive strong inputs from a few clearly delineated sites of origin (e.g., Fig. 11, column D).

Rather, the underlying principle of the hodological and morphological organization of the VTA and most of its afferents is perhaps best reflected in the concept of the "isodendritic core of the brainstem" as articulated by Ramón-Moliner and Nauta (1966) and of the "reticular formation" as described by Leontovich and Zhukova (1963) and Scheibel and Scheibel (1958). According to these investigators, the "isodendritic core" (or "reticular formation") consists of a neuronal continuum with overlapping dendritic fields (Scheibel and Scheibel, 1958; Ramón-Moliner and Nauta, 1966) stretching from spinal cord to telencephalon (Leontovich and Zhukova, 1963). Isodendritic (or "reticular," "generalized") neurons are characterized by thick, long, poorly ramifying dendrites that are targeted by heterogeneous, diverse sets of afferents (Scheibel and Scheibel, 1958; Valverde, 1961; Ramon-Moliner, 1962; Leontovich and Zhukova, 1963; Ramón-Moliner and Nauta, 1966). The axons of isodendritic neurons are long, send out numerous collaterals (Scheibel and Scheibel, 1958; Jones and Yang, 1985), and terminate with little ramification (Leontovich and Zhukova, 1963). Thus, each isodendritic neuron can be targeted by fibers from a great number of various sites of origin, and the axon of such a neuron can conduct impulses to numerous distant neurons, altogether providing an optimal substratum for integrative function. The VTA and most of its afferents express all of the characteristics mentioned above. The sparsely branching dendrites of differently sized VTA neurons extend for long distances (Phillipson, 1979b), allowing contacts with many afferent fibers. A similar morphology is readily observed in most neurons that project to the VTA, not only in the "reticular" lateral hypothalamic and preoptic area (McMullen and Almli, 1981) but also in the ventral pallidum, medial hypothalamic nuclei (Leontovich and Zhukova, 1963; Millhouse, 1978), diagonal band of Broca (Arendt et al., 1986; Dinopoulos et al., 1988), lateral part of the lateral habenula (Leontovich and Zhukova, 1963; Iwahori, 1977), and brainstem nuclei (see, e.g., Leontovich and Zhukova, 1963; Ramón-Moliner and Nauta, 1966). Most structures that innervate the VTA directly also have projections of equivalent or greater density to one or more other brain structures that also project to the VTA (Fig. 15), suggesting an extensive collateralization of afferents of the VTA. In view of these characteristics, together with the observed affiliation within a continuous formation extending throughout the core of the brain, the VTA and most of its afferents can be regarded as bona fide components of the phylogenetically old isodendritic core and optimally suited for integrative functions.

The VTA, however, also receives afferents from brain nuclei that are not isodendritic in nature. The nucleus accumbens and lateral septum, for example, both contain medium-sized, densely spiny neurons with ramifying short dendrites and small dendritic fields (Alonso and Frotscher, 1989; Meredith et al., 1992, 1995). The medium spiny neurons are the recipients of a relatively homogeneous input and, thus, are well suited for discriminative functions. Relaying different cortical information, the lateral septum and accumbens access the VTA and its "isodendritic" afferent system in different ways. The accumbens sends a strong projection to the ventral pallidum and a moderate one to the lateral preoptic area, lateral hypothalamus, and VTA, whereas the lateral septum projects strongly to lateral preoptic area and lateral hypothalamus and sends presumably only a minor projection to the VTA. One might speculate that afferents that are part of the isodendritic core exert a certain tone on VTA neurons and that these afferents of the isodendritic core in turn are accessed by allo- and idiodendritic or "specialized" nuclei. This arrangement provides a substrate to convey cortically derived information to the VTA in some limited cases directly, but largely via a phylogenetically old multisynaptic system.

Fig. 14 (*Overleaf*). Overview and comparison of patterns of terminal arborization in the VTA after injections of the anterograde tracers PHA-L or BDA into multiple forebrain sites (see Fig. 13). The injection sites are shown as **insets** in the upper left and higher magnifications of anterogradely labeled fibers in the VTA as **insets** in the lower right of pictures showing the patterns of anterograde labeling in the VTA (**A–F**). Note that all anterogradely labeled fibers exhibit varicosities (insets at lower right), which are thought to reflect synaptic specialization. For comparison with the innervation of the VTA, another projection site of each case is shown to the right (**A′–F′**). Every structure injected in these experiments projects as least as strongly to another structure (that, in turn, also projects to the VTA; see Fig. 15). For example, the lateral hypothalamus (injection site: A inset) projects to the VTA (A) but also with similar robustness to the lateral habenula (LHb; A′). After BDA is injected into the ventral pallidum (F inset), it is transported anterogradely to the VTA (F) as well as retrogradely to the accumbens shell (F second inset), from which it is also anterogradely transported to the VTA, thus showing the pattern of the combined innervation of the nucleus accumbens shell and ventral pallidum in the VTA. ac, anterior commissure; Acb, accumbens; fr, fasciculus retroflexus; IP, interpeduncular nucleus; LH, lateral hypothalamic area; LHb, lateral habenula; LPO, lateral preoptic area; LS, lateral septum; MHb, medial habenula; PFC, prefrontal cortex; rpAcb, rostral pole of accumbens nucleus, VP, ventral pallidum. Scale bars = 100 μm in A (applies to A–F), A′ (applies to A′–F′), injection-site insets; 25 μm in high-magnification insets.

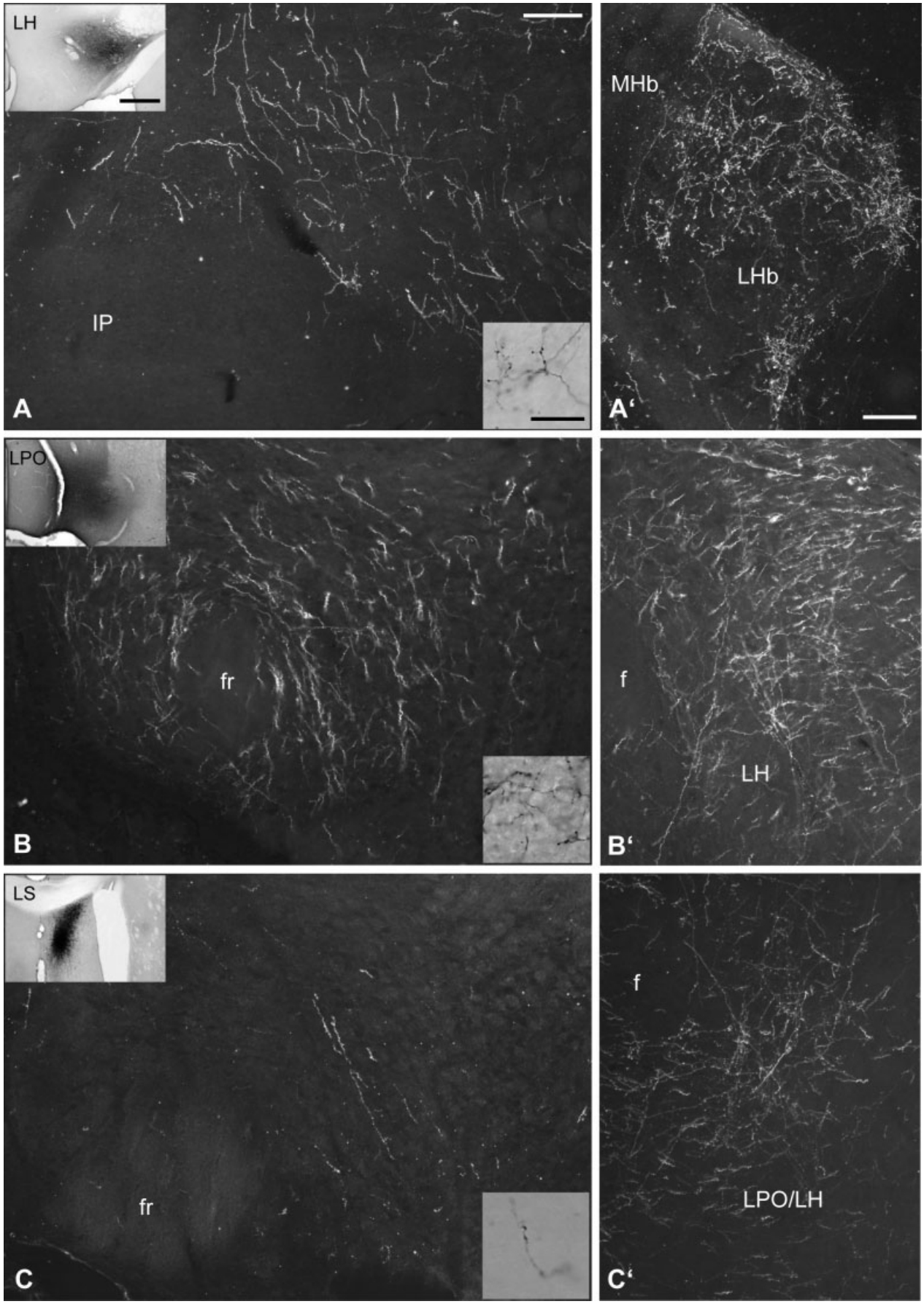

Figure 14

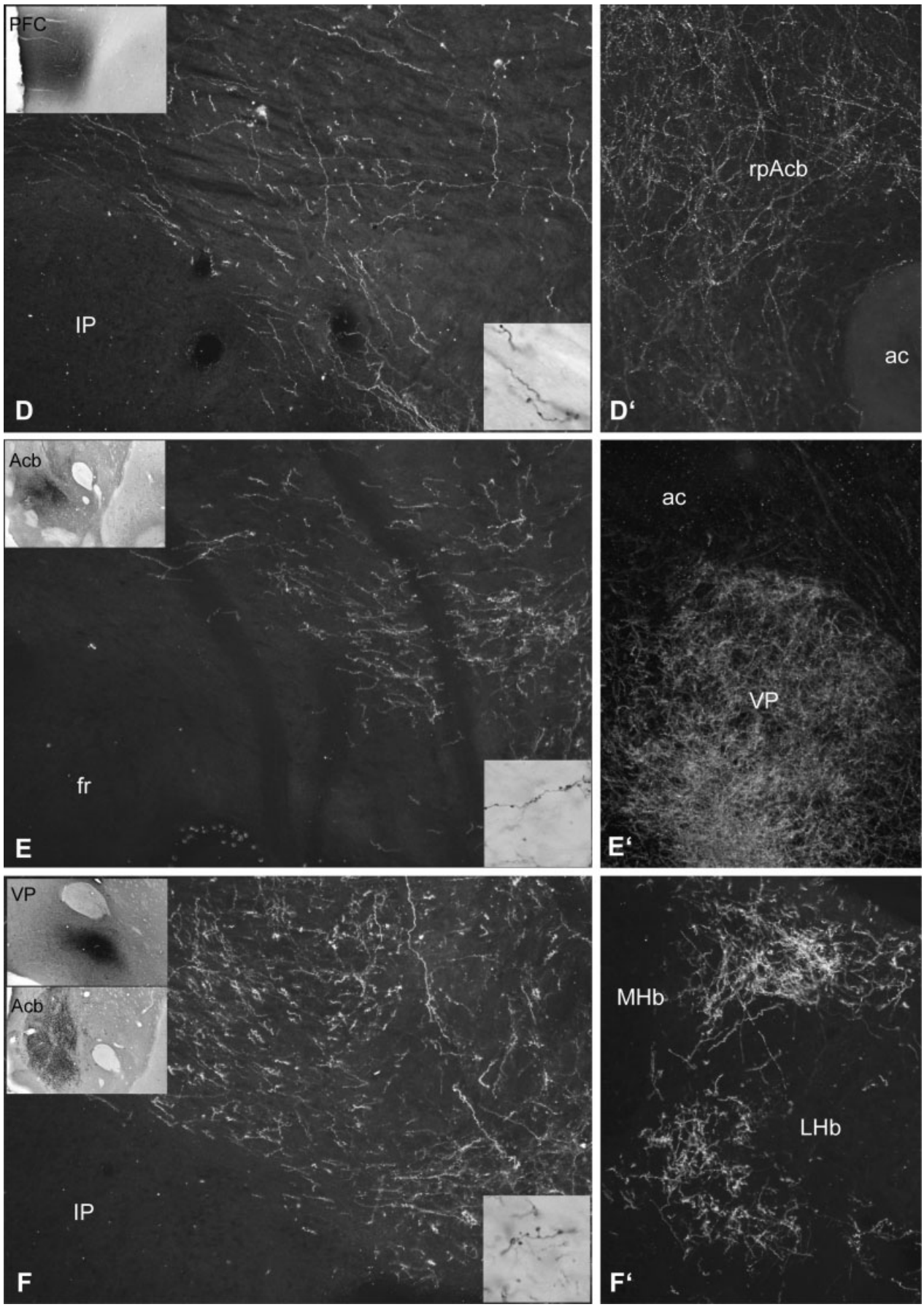

Figure 14 (Continued)

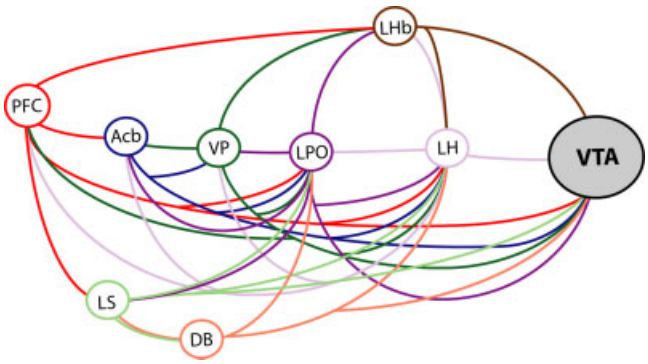

Fig. 15. Synopsis of the anterograde tracing experiments. All of the structures observed in this study to project to the VTA (see Fig. 13) also innervate with similar or greater robustness other brain structures (see Fig. 14) that, in turn, also provide strong inputs to the VTA, indicating an important characteristic of the innervation of the VTA, i.e., that constitute an anatomical network. Each line in this diagram is supported by one or more tracing cases.

In addition, the possibility arises that some structures from which the VTA receives an input represent transitional forms bridging isodendritic and specialized phenotypes. Neurons of the ventral pallidum, e.g., exhibit the morphological characteristics of isodendritic neurons, but hodological features of specialized structures. Their long, aspiny dendrites are targeted by a profuse, main (i.e., striatal) input. From these features alone, the ventral pallidum can be regarded as a transitional structure. In addition, ventral pallidal neurons intermingle with isodendritic neurons of the lateral preoptic area, substantiating the ventral pallidum as a transition between specialized and isodendritic structures.

Topography of VTA afferents

Although the principal morphology of VTA neurons is uniform (long dendrites, with no or only few spines), neurons differ in size, density, and orientation in different parts of the VTA. From these characteristics, the VTA has been divided into different subnuclei (Olszewski and Baxter, 1954; Phillipson 1979b; Halliday and Törk, 1984; Oades and Halliday, 1987). This and the reported functional differences between rostral and caudal VTA (see, e.g., Ikemoto and Wise, 2002; Bolaños et al., 2003; Rodd et al., 2004) could reflect a topographic organization of inputs to subnuclei or parts of the VTA.

The data from the present study, however, indicate that there is only a broad topography, with a great amount of overlap in the innervation of the VTA. Injection of FG into the lateral compared with the medial part of the VTA results only in a small lateral shift of the entire formation of retrogradely labeled neurons in the basal forebrain (Fig. 11), which is supported by our anterograde tracing data showing that the sites investigated in this study, in general, innervate the entire VTA (see Fig. 14). It did seem, however, that, after injections of anterograde tracer in some forebrain sites (e.g., lateral preoptic area, horizontal limb of diagonal band of Broca, and lateral septum), the resulting anterograde labeling in the VTA was somewhat more lateral than medial or more rostral than caudal. The number of cases per structure, however, was inadequate to formulate conclusions, necessitating separate studies to address this question specifically.

The nucleus accumbens is exceptional in this regard. A considerable amount of retrogradely labeled neurons in the rostral pole of the nucleus accumbens was observed only after an injection of FG into the lateral VTA. This observation is supported by PHA-L injections, which reveal a strong projection from the rostral pole only to the lateral VTA (Zahm and Heimer, 1993, their Figs. 5, 6). Furthermore, the present retrograde and anterograde tracing data suggest that only the medial VTA receives an input from the dorsomedial shell, whereas progressively more lateral parts of the VTA are targeted by progressively more ventral and lateral parts of the accumbens shell, suggesting a stricter topography in the connections between nucleus accumbens and VTA than the other afferents investigated in this study. However, it should be recalled that the accumbens also projects indirectly to the VTA via relays in the ventral pallidum and lateral preoptic and lateral hypothalamic area, which project to the VTA with a less refined topography. It might be anticipated that the overlap of the indirect inputs could serve to degrade the impact of the direct projections from the accumbens to the VTA.

Comparison to previous studies

To our best knowledge, the most recent study intending to show all of the afferents of the VTA is the benchmark description by Phillipson (1979a). Using horseradish peroxidase (HRP) as the retrograde tracer, Phillipson's very carefully conducted work continues to this day to serve neuroscientists working in many different areas as an important and comprehensive reference. Since 1979, however, tracers with increased sensitivity in terms of both uptake and visualization have been introduced (see the introductory paragraphs). Therefore, we undertook a reexamination of the afferent connections of the VTA, with FG as the retrograde tracer. While the uptake of HRP is coupled directly to the synaptic activity of terminals (Warr et al., 1981) and is reduced markedly when this activity is inhibited (Singer et al., 1977; Turner, 1977), FG is avidly incorporated by axonal terminals independently of neuronal activity. HRP is typically visualized by exploiting its enzymatic peroxidase activity with the aid of DAB or TBM as chromogens (Warr et al., 1981), whereas FG can be visualized with exquisite sensitivity by immunocytochemistry, which serves to increase signal greatly (Chang et al., 1990). Thus, not surprisingly, a major difference between Phillipson's and our study is the number of retrogradely labeled neurons visualized per structure. In the present data set, 20–50 times more retrogradely labeled neurons were observed in given structures than previously reported (Table 1). Probably also because of the heightened sensitivity of the methods, we observed retrogradely labeled neurons in many more structures than previously reported, e.g., in the claustrum/endopiriform nucleus complex; in several medial hypothalamic nuclei, such as paraventricular, ventromedial, and perifornical hypothalamic nucleus, tuber cinereum, and dorsal hypothalamic area; and in a number of brainstem nuclei, such as laterodorsal tegmental nucleus, pedunculopontine nucleus, paramedian raphe, and intermediate and gigantocellular reticular field (Table 1). The lateral septum had been regarded as one of only two structures (together with the hippocampus) receiving a dense dopaminergic innervation but not projecting to the VTA or another dopaminergic cell group in the ventral mesencephalon (Phillipson, 1979a; Oades

TABLE 1. Retrogradely Labeled Neurons in Various Brain Structures after Fluoro-Gold Injections into the VTA

		Present study	
	Phillipson (ipsilateral)	Ipsilateral	Contralateral
Forebrain			
Cortex			
Dorsal peduncular	−	+++++	++++
Infralimbic	++	++++	+++
Prelimbic	++	++++	+++
Cingulate	+	+++	+
Agranular insular	+	+++	+
Claustrum	−	+++++	++++
Endopiriform nucleus	−	++++	++
Olfactory tubercle	++	++	++
Nucleus accumbens			
Rostral pole	−	+++++	−
Shell	+++	+++++++	+
Core	−	++	−
Bed nucleus of stria terminalis	+++	++++++	++++
Amygdala	+		
Ant. amygdaloid area		++++	+
Medial nucleus		++++	+
Central nucleus		+	−
Substantia innominata	+++		
Ventral pallidum		+++++++	+++++
Sublenticular subst. innominata		++++	+++
Septum			
Lateral, dorsal part	−	+++	−
Lateral, intermediate part	−	+++++++	+++
Lateral, ventral part	−	++	−
Septofimbrial nucleus	−	+++++	++
Medial/Diagonal band of Broca	+++	++++++	++++
Hypothalamus			
Median preoptic area	−	+++	+++
Medial preoptic area	+	++++++	+++++
Lateral preoptic area	+++	+++++++	+++++
Magnocellular preoptic area	++	++	+
Anterior hypothalamic area	+	+++++	+++
Paraventricular nucleus	−	+++++	+++
Ventromedial hypothal. ncl	−	+	+
Tuber cinereum	−	+++++	++++
Perifornical nucleus	−	++++	+
Lateral hypothalamic area	+++	++++++	++++
Postdorsal hypothalamus	+		
Dorsal hypothalamic area		+++++	+++
Posterior hypothal. ncl		+++++	++++
Supramammillary nucleus	−	+	+
Zona incerta	+	+++++	++
Fields of Forel	+	−	−
Thalamus/epithalamus			
Parafascicular ncl	+	++	+
Paraventricular ncl	−	+++	++
Medial habenula	+++	++++++	++++++
Lateral habenula	++	+++++++	+++++++
Midbrain			
Superior colliculus	++	+++++	+++
Periaqueductal gray	+	++++++	+++++
Substantia nigra	++		
Pars compacta		++++	+++
Pars reticulata		+++	++
Deep mesencephalic field	−	+++++	+++++
Anterotegmental nucleus	−	++++	++++
Ventral tegmental nucleus	−	++++	−
Dorsal tegmental nucleus	−	++	++
Pons and medulla oblongata			
Oral field of pontine reticular formation	−	+++++	+++++
Dorsal raphe	+++	++++++	++++++
Median raphe	+++	+++++	+++++
Paramedian raphe	−	+++++	++++
Pontine raphe	+	+++	+++
Pedunculopontine nucleus	−	++	++
Laterodorsal tegmental ncl	−	++++	++++
Cuneiform nucleus	++	+++	++
Parabrachial nucleus	+++	++++	+++
Locus ceruleus	+	++++	++++
Principal nucleus nV	++	−	−
Caudal field of pontine reticular formation	+	++++	++++
Lateral reticular field	+	++	++
Intermediate reticular field	−	++	++
Gigantocellular reticular field	−	++	++
Cerebellum			
Dentate nucleus	+++	n.d.	n.d.

+, 1–10; ++, 10–20; +++, 20–50; ++++, 50–100; +++++, 100–500; ++++++, 500–1,000; +++++++, >1,000; n.d., not determined.

and Halliday, 1987). However, numerous retrogradely labeled neurons in the lateral septum were observed in the present study after FG deposits in the VTA. A connection from the lateral septum to the VTA is in accordance with published anterograde tracing data (Risold and Swanson, 1997) and from the work presented here showing antero-

gradely labeled fibers with varicosities (which are commonly thought to reflect functional contacts) in the VTA following injections of the anterogradely transported BDA into the lateral septum (Fig. 14C). Another novel finding of the present study with potentially substantial functional importance is that the input to the VTA, in general, is bilateral, comprising lesser descending and comparable ascending innervations from the contralateral side of the injection.

The tracer FG provides great morphological detail on retrogradely labeled neurons (see above). In the present study, it could be demonstrated for the first time that neurons projecting to the VTA express thick, long, sparsely branching dendrites, a morphological characteristic of reticular or "isodendritic" neurons. This is in accordance with the concept of Leontovich and Zhukova (1963) and others that reticular structures are not confined to the brainstem but extend to (and include parts of) the telencephalon. In subsequent studies, however, it was demonstrated that forebrain structures labeled as being "reticular" by Leontovich and Zhukova (1963) consist of different cell types with different cell morphologies (Iwahori, 1977; Millhouse, 1978; Dinopoulos et al., 1988). Based on the present study, though, it appears that expressing isodendritic (reticular) morphologies is a characteristic of neurons projecting to the VTA. Even in structures in which isodendritic neurons constitute only a minor fraction (e.g., in some nuclei of the medial hypothalamus), the retrogradely labeled neurons were of the isodendritic type.

Studies investigating the connection of a particular brain structure with the VTA describe the organization of terminations in the VTA as ". . . thin axons with varicosities" (Charara et al., 1996; Fadel and Deutch, 2002; Omelchenko and Sesack, 2005). Here we suggest, based on direct comparison of patterns of innervation from 14 different forebrain structures, that this is a common feature of afferents in the VTA. In addition, all structures investigated in the present study terminated with sparse arborization in the VTA. This seems to apply not only to the afferents derived from forebrain structures, insofar as the innervation of the VTA from the pedunculopontine (investigated in monkey) and laterodorsal tegmental (investigated in rat) nuclei shows the same features (Charara et al., 1996; Omelchenko and Sesack, 2005).

Functional considerations

The VTA is critically involved in reward-related behaviors and response to novelty (see the introductory paragraphs). Primary reward, stimuli that predict reward, and novel circumstances elicit a change in the firing frequency from tonic to phasic in 60–80% of dopaminergic neurons in the VTA and substantia nigra pars compacta (Schultz et al., 1998). A reward, however, is not always a reward, or, as Wolfram Schultz (1998) states, ". . . Rewards come in various physical forms . . . and depend on the particular environment of the subject." So, how does the VTA recognize a primary reward? The VTA receives a direct input neither from the outside environment, such as from visual, auditory, or somatosensory receptors, nor from the internal milieu, such as from osmo- and chemoreceptors. Explanations for this conundrum might be found in the special organization of VTA afferents and the morphology and location of the VTA itself. Neurons projecting to the VTA are very widespread in their distribution but are localized within an elongated formation stretching throughout the core of the brain. This organization not only features numerous neurons that project directly to the VTA but allows for rapid access to these VTA-projecting neurons by many brain areas. For instance, information from the internal milieu, conveyed via circumventricular organs and medial hypothalamus, can be easily transmitted via the lateral hypothalamus to the VTA. In the VTA itself, long dendrites enmeshed in major fiber bundles provide a morphological basis for a tremendous integration of different inputs.

A structure or system with a great integrative capacity, on the other hand, might be less well suited for discriminative function. VTA neurons do not differentiate between primary rewards vs. conditioned appetitive stimuli nor between different appetitive stimuli and sensory modalities (Schultz et al., 1998). The present study supports the view that the function of the VTA is to signal significance or expectation, in contrast, e.g., to neurons in the prefrontal cortex (PFC), which can discriminate between different rewards (Schultz et al., 1998).

This should not be interpreted as indicating that the VTA and its connections represent a diffuse system. To the contrary, a certain level of anatomical specificity, mainly in the efferents of the VTA, is implicit in the observation that essentially separate groups of VTA neurons project to different terminal fields (Swanson, 1982). These groups of neurons were not situated in different parts of the VTA but intermingled considerably. Furthermore, the VTA consists of γ-aminobutyric acid (GABA)-ergic and dopaminergic projection neurons (Swanson, 1982), some of which contain the peptidergic cotransmitters neurotensin and cholecystokinin (Seroogy et al., 1988). Using retrograde and anterograde tracing techniques in combination with electron microscopy, Carr and Sesack (2000) observed that, whereas the PFC projects both to dopaminergic and GABAergic neurons of the VTA, PFC-innervated GABAergic neurons project to the nucleus accumbens but not the PFC, in contrast to PFC-innervated dopaminergic neurons, which project to the PFC but not nucleus accumbens. Similarly, the laterodorsal tegmental nucleus is reported to innervate the entire VTA uniformly, inhibitory and excitatory, both dopaminergic and GABAergic neurons (Omelchenko and Sesack, 2005). Dopaminergic neurons, however, are targeted significantly more by axons from the laterodorsal tegmental nucleus that exhibit asymmetric synaptic contacts (which putatively are excitatory), whereas GABAergic VTA neurons are targeted more by axons with symmetric synaptic differentiation (putative inhibitory). In addition, whereas dopaminergic neurons receiving asymmetric synapses from the laterodorsal tegmental nucleus project to the PFC and nucleus accumbens, those targeted by axons with symmetrical synapses project to the PFC but not nucleus accumbens. One should keep in mind, though, that none of these structures projects exclusively or mainly to the VTA but that all project with similar intensity to other structures, which in turn also project to the VTA.

To summarize, this study reveals heterogeneous and widespread sources of input to the VTA, which, because of the distinctive overall organization, might provide a basis for exceptional integrative capacity. It seems probable that the VTA contributes in a variety of ways to the assembly of organismal responses to, e.g., natural and synthetic reinforcers. Thus, the VTA may be a structure in

which homeostatic signals, e.g., from the nucleus of the solitary tract relayed via the parabrachial nucleus (Saper, 2002) and from hypothalamic neurons expressing anorexigenic or orexigenic peptides, such as proopiomelanocortin, melanin-concentrating hormone, and orexin/hypocretin (Elias et al., 1999; Dallvechia-Adams et al., 2002; Korotkova et al., 2003), and hedonic signals, e.g., from PFC and nucleus accumbens, are integrated (Saper et al., 2002) and transmuted into a motivational drive. Dissecting out the interneuronal interactions among neurons in the VTA, the formation of isodendritic neurons with which it is associated, and the idiodendritic structures accessing the VTA and this formation remains a challenge for further studies.

ACKNOWLEDGMENTS

The authors acknowledge the superb technical assistance of Evelyn Williams and Randal Nonneman.

LITERATURE CITED

Alonso JR, Frotscher M. 1989. Organization of the septal region in the rat brain: a Golgi/EM study of lateral septal neurons. J Comp Neurol 286:472–487.

Andres KH, Düring M von, Veh RW. 1999. Subnuclear organization of the rat habenular complexes. J Comp Neurol 407:130–150.

Arendt T, Zveginstseva HG, Leontovich TA. 1986. Dendritic changes in the basal nucleus of Meynert and in the diagonal band nucleus in Alzheimer's disease—a quantitative Golgi investigation. Neuroscience 19: 1265–1268.

Bamford NS, Zhang H, Schmitz Y, Wu NP, Cepeda C, Levine MS, Schmauss C, Zakharenko SS, Zablow L, Sulzer D. 2004. Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals. Neuron 42:653–663.

Bolaños CA, Perrotti LI, Edwards S, Eisch AJ, Barrot M, Olson VG, Russell DS, Neve RL, Nestler EJ. 2003. Phospholipase Cγ in distinct regions of the ventral tegmental area differentially modulates mood-related behaviors. J Neurosci 2320:7569–7576.

Brandt HM, Apkarian AV. 1992. Biotin-dextran: a sensitive anterograde tracer for neuroanatomic studies in rat and monkey. J Neurosci Methods 45:35–40.

Carr DB, Sesack SR. 2000. Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. J Neurosci 20:3864–3873.

Chang HT, Kuo H, Whittaker JA, Cooper NGF. 1990. Light and electron microscopic analysis of projection neurons retrogradely labeled with Fluoro-Gold: notes on the application of antibodies to Fluoro-Gold. J Neurosci Methods 35:31–37.

Charara A, Smith Y, Parent A. 1996. Glutamatergic inputs from the pedunculopontine nucleus to midbrain dopaminergic neurons in primates: *Phaseolus vulgaris*-leucoagglutinin anterograde labeling combined with postembedding glutamate and GABA immunohistochemistry. J Comp Neurol 364:254–266.

Contestabile A, Flumerfelt BA. 1981. Afferent connections of the interpeduncular nucleus and the topographic organization of the habenulointerpeduncular pathway: an HRP study in the rat. J Comp Neurol 196:253–270.

Dado RJ, Burstein R, Cliffer KD, Giesler GJ Jr. 1990. Evidence that Fluoro-Gold can be transported avidly through fibers of passage. Brain Res 533:329–333.

Dallvechia-Adams S, Kuhar MJ, Smith Y. 2002. Cocaine- and amphetamine-regulated transcript peptide projections in the ventral midbrain: colocalization with gamma-aminobutyric acid, melanin-concentrating hormone, dynorphin, and synaptic interactions with dopamine neurons. J Comp Neurol 448:360–372.

Dinopoulos A, Parnavelas JG, Uylings HBM, van Eden CG. 1988. Morphology of neurons in the basal forebrain nuclei of the rat: a Golgi study. J Comp Neurol 272:461–474.

Elias CF, Aschkenasi C, Lee C, Kelly J, Ahima RS, Bjorbaek C, Flier JS, Saper CB, Elmquist JK. 1999. Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. Neuron 23:775–786.

Fadel J, Deutch AY. 2002. Anatomical substrates of orexin-dopamine interactions: laterial hypothalamic projections to the ventral tegmental area. Neuroscience 111:379–387.

Floresco SB, West AR, Ash B, Moore H, Grace AA. 2003. Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. Nat Neurosci 6:968–973.

Georges F, Aston-Jones G. 2002. Activation of ventral tegmental area cells by the bed nucleus of the stria terminalis: a novel excitatory amino acid input to midbrain dopamine neurons. J Neurosci 22:5173–5187.

Gerfen CR, Sawchenko PE. 1984. An anterograde neuroanatomical tracing method that shows the detailed morphology of neurons, their axons and terminals: immunohistochemical localization of an axonally transported plant lectin, *Phaseolus vulgaris*-leucoagglutinin (PHA-L). Brain Res 290:219–238.

Gerfen CR, Sawchenko PE. 1985. A method for anterograde axonal tracing of chemically specified circuits in the central nervous system: combined *Phaseolus vulgaris*-leucoagglutinin (PHA-L) tract tracing and immunohistochemistry. Brain Res 343:144–150.

Groenewegen HJ, Berendse HW, Haber SN. 1993. Organization of the output of the ventral striatopallidal system in the rat: ventral pallidal efferents. Neuroscience 57:113–142.

Groenewegen HJ, Berendse HW, Wouterlood FG. 1994. Organization of the projections from the ventral striato-pallidal system to ventral mesencephalic dopaminergic neurons in the rat. In: Percheron G, et al., editors. The basal ganglia. New York: Plenum Press. p 81–93.

Hallanger AE, Wainer BH. 1988. Ascending projections from the pedunculopontine tegmental nucleus and the adjacent mesopontine tegmentum in the rat. J Comp Neurol 274:483–515.

Halliday GM, Törk I. 1984. Electron microscopic analysis of the mesencephalic ventromedial tegmentum in the cat. J Comp Neurol 230:393–412.

Hamill GS, Jacobowitz DM. 1984. A study of afferent projections to the rat interpeduncular nucleus. Brain Res Bull 13:527–539.

Heimer L, Zahm DS, Churchill L, Kalivas PW, Wohltmann C. 1991. Specificity in the projection patterns of accumbal core and shell in the rat. Neuroscience 41:89–125.

Herkenham M, Nauta WJH. 1979. Efferent connections of the habenular nuclei in the rat. J Comp Neurol 187:19–48.

Ikemoto S, Wise RA. 2002. Rewarding effects of the cholinergic agents carbachol and neostigmine in the posterior ventral tegmental area. J Neurosci 22:9895–9904.

Iwahori N. 1977. A Golgi study on the habenular nucleus of the cat. J Comp Neurol 171:319–344.

Jones BE, Yang TZ. 1985. The efferent projections from the reticular formation and the locus coeruleus studied by anterograde and retrograde axonal transport in the rat. J Comp Neurol 242:56–92.

Jones S, Kornblum JL, Kauer JA. 2000. Amphetamine blocks long-term synaptic depression in the ventral tegmental area. J Neurosci 20:5575–5580.

Kelley AE. 2004. Memory and addiction: shared neural circuitry and molecular mechanisms. Neuron 44:161–179.

Korotkova TM, Sergeeva OA, Eriksson KS, Haas HL, Brown RE. 2003. Excitation of ventral tegmental area dopaminergic and nondopaminergic neurons by orexins/hypocretins. J Neurosci 23:7–11.

Leontovich TA, Zhukova GP. 1963. The specificity of the neuronal structure and topography of the reticular formation in the brain and spinal cord of carnivora. J Comp Neurol 121:347–379.

Lewis BL, O'Donnell P. 2000. Ventral tegmental area afferents to the prefrontal cortex maintain membrane potential "up" states in pyramidal neurons via D1 dopamine receptors. Cereb Cortex 10:1168–1175.

Luppi PH, Sakai K, Salvert D, Fort P, Jouvet M. 1987. Peptidergic hypothalamic afferents to the cat nucleus raphe pallidus as revealed by a double immunostaining technique using unconjugated cholera toxin as a retrograde tracer. Brain Res 402:339–345.

Luppi PH, Fort P, Jouvet M. 1990. Iontophoretic application of unconjugated cholera toxin B subunit (Ctb) combined with immunohistochemistry of neurochemical substances: a method for transmitter identification of retrogradely labeled neurons. Brain Res 534:209–224.

Marchand ER, Riley JN, Moore RY. 1980. Interpeduncular nucleus afferents in the rat. Brain Res 193:339–352.

McMullen NT, Almli CR. 1981. Cell types within the medial forebrain bundle: a Golgi study of preoptic and hypothalamic neurons in the rat. Am J Anat 161:323–340.

Melis M, Perra S, Muntoni AL, Pillolla G, Lutz B, Marsicano G, Di Marzo V, Gessa GL, Pistis M. 2004. Prefrontal cortex stimulation induces 2-arachidonoyl-glycerol-mediated suppression of excitation in dopamine neurons. J Neurosci 24:10707–10715.

Meredith GE, Agolia R, Arts MPM, Groenewegen HJ, Zahm DS. 1992. Morphological differences between projection neurons of the core and shell in the nucleus accumbens of the rat. Neuroscience 50:149–162.

Meredith GE, Ypma P, Zahm DS. 1995. Effects of dopamine depletion on the morphology of medium spiny neurons in the shell and core of the rat nucleus accumbens. J Neurosci 15:3808–3820.

Millhouse OE. 1978. Cytological observations on the ventromedial hypothalamic nucleus. Cell Tissue Res 191:473–491.

Neuhoff H, Neu A, Liss B, Roeper J. 2002. I_h channels contribute to the different functional properties of identified dopaminergic subpopulations in the midbrain. J Neurosci 22:1290–1302.

Oades RD, Halliday GM. 1987. Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. Brain Res Rev 12:117–165.

Olszewski J, Baxter D. 1954. Cytoarchitecture of the human brain stem. Philadelphia: J.B. Lippincott.

Omelchenko N, Sesack SR. 2005. Laterodorsal tegmental projections to identified cell populations in the rat ventral tegmental area. J Comp Neurol 483:217–235.

Overton PG, Clark D. 1997. Burst firing in midbrain dopaminergic neurons. Brain Res Rev 25:312–334.

Paladini CA, Williams JT. 2004. Noradrenergic inhibition of midbrain dopamine neurons. J Neurosci 24:4568–4575.

Paxinos G. 1995. The rat nervous system, 2nd ed. San Diego: Academic Press.

Paxinos G, Watson C. 1998. The rat brain in stereotaxic coordinates, 4th ed. San Diego: Academic Press.

Phillipson OT. 1979a. Afferent projections to the ventral tegmental area of Tsai and interfascicular nucleus: a horseradish peroxidase study in the rat. J Comp Neurol 187:85–98.

Phillipson OT. 1979b. A Golgi study of the ventral tegmental area of Tsai and interfascicular nucleus in the rat. J Comp Neurol 187:99–116.

Pieribone VA, Aston-Jones G. 1988. The iontophoretic application of Fluoro-Gold for the study of afferents to deep brain nuclei. Brain Res 475:259–271.

Ramón-Moliner E. 1962. An attempt at classifying nerve cells on the basis of their dendritic patterns. J Comp Neurol 119:211–227.

Ramón-Moliner E, Nauta WJH. 1966. The isodendritic core of the brainstem. J Comp Neurol 126:311–366.

Rebec GV, Grabner CP, Johnson M, Pierce RC, Bardo MT. 1997a. Transient increases in catecholaminergic activity in medial prefrontal cortex and nucleus accumbens shell during novelty. Neuroscience 76:707–714.

Rebec GV, Christensen JCR, Guerra C, Bardo MT. 1997b. Regional and temporal differences in real-time dopamine efflux in the nucleus accumbens during free-choice novelty. Brain Res 776:61–67.

Risold PY, Swanson LW. 1997. Connections of the rat lateral septal complex. Brain Res Rev 24:115–195.

Risold PY, Canteras NS, Swanson LW. 1994. Organization of projections from the anterior hypothalamic nucleus: a *Phaseolus vulgaris*-leucoagglutinin study in the rat. J Comp Neurol 348:1–40.

Rodd ZA, Melendez RI, Bell RL, Kuc KA, Zhang Y, Murphy JM, McBride WJ. 2004. Intracranial sef-administration of ethanol within the ventral tegmental area of male wistar rats: evidence for involvement of dopamine neurons. J Neurosci 24:1050–1057.

Saal D, Dong Y, Bonci A, Malenka R. 2003. Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. Neuron 37:577–582.

Saper CB. 2002. The central autonomic nervous system: conscious visceral perception and autonomic pattern generation. Annu Rev Neurosci 25: 433–469.

Saper CB, Swanson LW, Cowan WM. 1979. An autoradiographic study of the efferent connections of the lateral hypothalamic area in the rat. J Comp Neurol 183:689–706.

Saper CB, Chou TC, Elmquist JK. 2002. The need to feed: homeostatic and hedonic control of eating. Neuron 36:199–211.

Satoh K, Fibiger HC. 1986. Cholinergic neurons of the laterodorsal tegmental nucleus: efferent and afferent connections. J Comp Neurol 253:277–302.

Scheibel ME, Scheibel AB. 1958. Structural substrates for integrative patterns in the brainstem reticular core. In: Jasper HH, Proctor LD, Knighton RS, Noshay WC, Costello RT, editors. Reticular formation of the brain. Boston: Little Brown. p 31–55.

Schmued LC, Fallon JH. 1986. Fluoro-Gold: a new fluorescent retrograde axonal tracer with numerous unique properties. Brain Res 377:147–154.

Schmued LC, Heimer L. 1990. Iontophoretic injection of Fluoro-Gold and other fluorescent tracers. J Histochem Cytochem 38:721–723.

Schultz W. 1998. Predictive reward signal of dopamine neurons. J Neurophysiol 80:1–27.

Schultz W, Dayan P, Montague PR. 1997. A neural substrate of prediction and reward. Science 275:1593–1599.

Schultz W, Tremblay L, Hollerman JR. 1998. Reward prediction in primate basal ganglia and frontal cortex. Neuropharmacology 37:421–429.

Seroogy KB, Mehta A, Fallon JH. 1988. Neurotensin and cholecystokinin coexistence within neurons of the ventral mesencephalon: projections to forebrain. Exp Brain Res 68:277–289.

Sesack SR, Deutch AY, Roth RH, Bunney BS. 1989. Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract tracing study with *Phaseolus vulgaris* leucoagglutinin. J Comp Neurol 290:213–242.

Simon H, Lee Moal M, Calas A. 1979. Efferents and afferents of the ventral tegmental A10 region studied after local injection of [^{3}H]leucine and horseradish peroxidase. Brain Res 178:17–40.

Singer W, Holländer H, Vanegas H. 1977. Decreased peroxidase labeling of lateral geniculate neurons following deafferentation. Brain Res 120: 133–137.

Spanagel R, Weiss F. 1999. The dopamine hypothesis of reward: past and current status. TINS 22:521–527.

Swanson LW. 1976. An autoradiographic study of the efferent connections of the preoptic region in the rat. J Comp Neurol 167:227–256.

Swanson LW. 1982. The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunoflourescence study in the rat. Brain Res Bull 9:321–353.

Thomas MJ, Malenka RC, Bonci A. 2000. Modulation of long-term depression by dopamine in the mesolimbic system. J Neurosci 20:5581–5586.

Turner PT. 1977. Effect of pentobarbital on uptake of horseradish peroxidase by rabbit cortical synapses. Exp Neurol 54:24–32.

Ungless MA, Whistler J, Malenka RC, Bonci A. 2001. Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. Nature 411:583–586.

Valverde F. 1961. Reticular formation of the pons and medulla oblongata. A Golgi study. J Comp Neurol 116:71–99.

Veenman CL, Reiner A, Honig MG. 1992. Biotinylated dextran amine as an anterograde tracer for single- and double-labeling studies. J Neurosci Methods 41:239–254.

Vertes RP. 2004. Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse 51:32–58.

Vertes RP, Fortin WJ, Crane AM. 1999. Projections of the median raphe nucleus in the rat. J Comp Neurol 407:555–582.

Warr WB, de Olmos JS, Heimer L. 1981. Horseradish peroxidase—the basic procedure. In: Heimer L, RoBards MJ, editors. Neuroanatomical tract-tracing methods. New York: Plenum Press. p 207–262.

White FJ. 1996. Synaptic regulation of mesocorticolimbic dopamine neurons. Annu Rev Neurosci 19:405–436.

White FJ, Wang RY. 1983. Differential effects of classical and atypical antipsychotic drugs on A9 and A10 dopaminergic neurons. Science 22:1054–1057.

Woulfe J, Beaudet A. 1992. Neurotensin terminals form synapses primarily with neurons lacking detectable tyrosine hydroxylase immunoreactivity in the rat substantia nigra and ventral tegmental area. J Comp Neurol 321:163–176.

Zahm DS, Heimer L. 1993. Specificity in the efferent projections of the nucleus accumbens in the rat: comparison of the rostral pole projection patterns with those of the core and shell. J Comp Neurol 327:220–232.

Zahm DS, Williams E, Wohltmann C. 1996. Ventral striatopallidothalamic projection: IV. Relative involvements of neurochemically distinct subterritories in the ventral pallidum and adjacent parts of the rostroventral forebrain. J Comp Neurol 364:340–362.

Zahm DS, Jensen SL, Williams ES, Martin JR III. 1999. Direct comparison of projections from the central amygdaloid region and nucleus accumbens shell. Eur J Neurosci 11:1119–1126.