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A brain atlas of a wild-type inbred strain of the medaka, *Oryzias latipes*

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Abstract In response to the growing interest in the medaka (*Oryzias latipes*) as a model vertebrate for studies on neurobiology, we provide here an atlas of the adult brain of a wild-type inbred strain of the medaka. We used the HNI inbred strain, which has been used as a standard wild-type medaka in many laboratories. The adult brains were fixed and frontally cut into complete serial sections. The sections were stained with Nissl and Bodian methods. In the present atlas, external features of the brain, photographs of representative levels of the Nissl and Bodian sections, and drawings of these sections are presented. The brain structures were identified based on the recent comparative and hodological studies in various teleost species. In order to demarcate brain structures, hodological experiments using a carbocyanine dye (DiI) as a neuronal tracer have also been carried out. The atlas provides the basis for further developmental, neuroanatomical, neurophysiological, and behavioral investigations using the medaka.

Introduction

In this report, we provide a standard brain atlas of a wild-type inbred strain of the medaka (*Oryzias latipes*).

The knowledge of the organizations of teleost brains is indispensable for developmental, neuroanatomical, neurophysiological, and behavioral investigations in comparative neurology of vertebrates. Teleosts are the most numerous group of vertebrates, encompassing about 25000 species (Nelson, 1994). According to Lauder and Liem (1983), the living teleosts contain four major radiations, the Osteoglossomorpha, Elopomorpha, Clupeomorpha, and Euteleostei (the most diverse group of teleosts).

Small teleost fishes, such as the medaka and the zebrafish (*Danio rerio*), are increasingly being used as vertebrate model systems to study neurobiology (Yamamoto, 1975; Egami *et al.*, 1990; Kimmel *et al.*, 1991; Iwamatsu, 1993; Kimmel, 1993; Westerfield, 1993; Haffter *et al.*, 1996; Driever *et al.*, 1996; Ishikawa, 1997). Both medaka and zebrafish belong to the Euteleostei (Lauder and Liem, 1983; Nelson, 1994). The medaka (an order of the Beloniformes) belongs to the superorder Acanthopterygii, the most numerous group in the Euteleostei (Nelson, 1994; Naruse, 1996). On the other hand, the zebrafish (belonging to the order Cypriniformes) belongs to a superorder of the Ostariophysi, another highly specialized group within the Euteleostei (Nelson, 1994).

The ostariophysan teleosts including zebrafish have had a unique evolutionary history among the Euteleostei. The comparative studies on visual pathways in the teleost brains suggest that early ostariophysans may have had reduced vision and that the elaborate visual systems of cyprinids reevolved later (Northcutt and Wullimann, 1988; Striedter and Northcutt, 1989). Cyprinids, such as *Carassius auratus* and *Cyprinus caprio*, have tetraploidic origins (Ohno, 1970; Ojima, 1983). It is reported that the zebrafish has seven *Hox* gene clusters, probably as a result of entire genome duplication (Amores *et al.*, 1998). The entire length of the zebrafish genome is about twice that of the medaka genome (Ojima, 1983). Indeed, the brain structures of the cyprinids including zebrafish are strikingly different in several important features from those of acanthopterygian teleost fishes including the medaka (Rupp *et al.*, 1996; Wulliman *et al.*, 1996; Ishikawa, 1997; Anken and Bourrat, 1998).

It is well known that there exists enormous interspecific diversity in brain morphology in teleosts (Lissner, 1923; Herrick, 1924; Evans, 1940; Uchihashi, 1953; Ito, 1978; Meek and Nieuwenhuys, 1997). The brain structures of teleost fishes may be different even among various strains of the same species. Our results using five inbred strains of the medaka revealed that the strains with

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different genotypes possess large variation in gross brain morphology (Ishikawa *et al.*, 1996; 1999). The results indicate that there exists large intraspecific variation in gross brain morphology in the medaka.

In order to minimize the variation in a brain atlas of the medaka, we must therefore use a genetically homogenous strain. Recently, Anken and Bourrat (1998) published a brain atlas of a body-colour mutant strain of the medaka. However, they did not state whether the used strain was an inbred or not. In the present study, we used one of the inbred strains of the medaka, HNI strain, which has been used as a standard wild-type medaka in many laboratories.

In our brain atlas, the brain structures were identified mainly based on the recent comparative and hodological information on various acanthopterygian fishes (Ito, 1978; Ito and Vanegas, 1983; Murakami *et al.*, 1983; Ito and Yoshimoto, 1991; Yamane *et al.*, 1996; Yoshimoto *et al.*, 1998). In order to help the identification of brain structures, hodological experiments using a carbocyanine dye (DiI) as a neuronal tracer have also been carried out. Our results were often inconsistent with those of Anken and Bourrat (1998) in delineation and nomenclature of various nuclei and fiber systems.

Materials and Methods

Medaka inbred strain

A medaka inbred strain, HNI-II (Hyodo-Taguchi, 1980, 1990; Hyodo-Taguchi and Sakaizumi, 1993) was used in the present study. The wild populations of the medaka in Japan can be divided into two major genetic groups, the Northern and Southern populations (Sakaizumi *et al.*, 1983). Genetic analysis using DNA sequence data of mitochondrial cytochrome b and 12s ribosomal RNA genes suggests that these two populations diversified more than one million years ago (for a review, see Naruse, 1996). The HNI-II strain is derived from a pair of parent fish belonging to the wild Northern population. The strain has been maintained by full sibling mating for 32 generations, and the probability of homozygosity within the strain is more than 99% (Hyodo-Taguchi, 1980, 1990).

Fish were bred and raised under a common standard set of conditions: about 10 fish were kept in 3 liters of still water in a plastic vessel, and maintained under constant water temperature (26–29°C) and photoperiod (14-h light/10-h dark cycle). The fish were given powdered fish food (Tetra-min, Tetra Werke Co., Mells, Germany) once a day. Under these conditions, the fish mature sexually in 3–6 months after hatching and start to perform mating behaviors at the beginning of the light period. In the medaka, as in other teleost fishes, individuals of the same age and from the same genetic brood vary in size. Sexual maturation is associated with the attainment of a minimal body size rather than age (Sohn and Crews, 1977; Campton and Gall, 1988). Thus, we used sexually matured male fish of a similar body size (about 3 cm total length) of the ages of 3–6 months old.

External features of brains

The fish were anaesthetized with MS222 (0.03%) and perfused through the conus arteriosus with a mixed solution of 2% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) using a small glass pipette. The brains were removed from the skull, post-fixed in the same fresh fixative by immersion for 1.5 h, rinsed in the phosphate buffer, and examined under a dissection microscope (Plates 1 and 2).

Nissl Preparation

The fixed brains were immersed in 20% sucrose in 0.1M phosphate buffer (pH 7.4) for 2 h, embedded in 5% agarose and 20% sucrose in water, and frozen in *n*-hexane (–50°C). Completely serial sections of 40 μm thick were frontally cut on a cryostat. Sections were Nissl stained with cresyl violet (Panels A in Plates 3–20). The drawings were made with the use of a camera lucida at 100×.

Bodian Preparation

Brains were fixed by immersion in the Bodian II solution, embedded in paraffin, cut frontally into a complete series of serial sections (15 μm), and stained according to the Bodian-Otsuka method (Otsuka *et al.*, 1960) to visualize fiber systems (Panels B in Plates 3–20). The drawings were made with the use of a camera lucida at 100×.

DiI experiments

Brains were fixed by immersion or perfusion with the 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). The fixed brains were postfixed at 4°C for at least 2 days. To apply DiI, the fixed brain was washed with the 0.1M phosphate buffer (pH 7.4), blotted dry, and a small hole was made

with a sharp needle at a region of the brain. A small crystal of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI, Molecular Probes, Junction City, Oregon) was then pushed into the hole, and the application site was covered with a solution of 6% gelatin (Type A, 300 Bloom, from porcine skin, Sigma, St. Louis, Missouri). The application sites were various regions of the telencephalon, optic tectum, and cerebellum. The brains were put in the 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) and stored in the dark at 37°C for 6 to 9 days. At the end of the diffusion time, the brains were blotted dry and embedded in 7% gelatin. The embedded specimens were stored in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) overnight in order to harden the gelatin and then serially sectioned (80 μm) frontally on a microslicer (DTK-3000, D.S.K., Osaka). The sections were mounted using 50% sucrose and observed under an Olympus epifluorescence microscope (Vanox, AX80) equipped with a rhodamine type filter set.

Nomenclature

We follow the neuroanatomical terms of Nieuwenhuys (1963) and Murakami *et al.* (1983) for telencephalic structures, those of Peter *et al.*, (1975), Sakamoto and Ito (1982) and Ito *et al.*, (1986) for diencephalic structures, and those of Uchiyama *et al.*, (1988) for pretectal structures. Other references were listed in the Index of Abbreviations.

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INDEX OF ABBREVIATIONS

ABBREVIATIONS	*STRUCTURES*	*ABBREVIATIONS*	*STRUCTURES*
a	Mauthner axon	G	granule population (McCormick and Hernandez, 1996)
AON	anterior octavus nucleus (McCormick, 1983)	GA	corpus glomerulosum pars anterior
AP	area postrema	GR	corpus glomerulosum pars rotunda
APT	area pretectalis	H	hypophysis
BO	bulbus olfactorius	HB	habenula
ca	commissura anterior	IM	nucleus intermedius
CAN	caudal nucleus (McCormick, 1983)	LC	locus coeruleus
cans	commissura ansulata	lfb	lateral forebrain bundle (fasciculus lateralis telencephali)
cc	canalis centralis	LI	lobus inferior
cce	commissura cerebelli	ll	lemniscus lateralis
CD	cornu dorsale	M	cellula Mauthneri (Mauthner cell)
CE	corpus cerebelli	MCN	magnocellular octavus nucleus (Meredith and Butler, 1983)
cgs	commissure of nucleus gustatorius secundarius	MED	medulla oblongata
ch	commissura horizontalis	mfb	medial forebrain bundle (fasciculus medialis telencephali)
cho	chiasma opticum	MN	nucleus medialis (McCormick, 1983)
CM	corpus mamillare	nALL	nervus lineae lateralis anterior
cmi	commissura minor	NAT	nucleus anterior tuberis (Sheldon, 1912)
cp	commissura posterior	NC	nucleus corticalis
CR	crista cerebellaris	NCC	nucleus commissuralis Cajal
ct	commissura transversa	NCLI	nucleus centralis of inferior lobe (Braford and Northcutt, 1983)
CV	cornu ventrale	NDLI	nucleus diffusus lobi inferioris
Dc	area dorsalis telencephali pars centralis	NDTL	nucleus diffusus tori lateralis
Dd	area dorsalis telencephali pars dorsalis	NE	nucleus entopeduncularis
dDl	dorsal region of Dl	NF	nucleus funiculi (funicular nucleus, Finger, 1983)
dDm	dorsal region of Dm	NFl	nucleus funiculi lateralis (lateral funicular nucleus, Finger, 1983)
Dl	area dorsalis telencephali pars lateralis	NFLM	nucleus of fasciculus longitudinalis medialis
DLT	nucleus dorsolateralis thalami (Sheldon, 1912; Peter *et al.*, 1975)	NFm	nucleus funiculi medialis (medial funicular nucleus, Finger, 1983)
Dm	area dorsalis telencephali pars medialis	NGS	nucleus gustatorius secundarius
DM	nucleus dorsomedialis thalami	NI	nucleus isthmi
DO	descending octavus nucleus (Meredith and Butler, 1983)	NIP	nucleus interpeduncularis
Dp	area dorsalis telencephali pars posterior	NLT	nucleus lateral tuberis (Sheldon, 1912)
E	epiphysis (pineal organ)	NLV	nucleus lateralis valvulae
EC	efferent cells of octavus nerve (Meredith and Butler, 1983)	NP	nucleus pretectalis
EG	eminentia granularis	NPAC	nucleus paracommissuralis
EW	nucleus Edinger-Westphal	NPC	nucleus of posterior commissure
fan	fibrae ansulatae (Sheldon, 1912)	nPLL	nervus lineae lateralis posterior
fe	fissura endorhinalis	NPPv	nucleus posterioris periventricularis (Peter *et al.*, 1975)
fd	funiculus dorsalis		
fl	funiculus lateralis		
flm	fasciculus longitudinalis medialis		
fr	fasciculus retroflexus		
fv	funiculus ventralis		

ABBREVIATIONS	*STRUCTURES*	*ABBREVIATIONS*	*STRUCTURES*
NPT	nucleus posterior thalami	slt	sulcus limitans telencephali (Nieuwenhuys, 1963)
NR	nucleus ruber (Goldstein, 1905)	SO	secondary octaval population (McCormick and Hernandez, 1996) = medial auditory nucleus of medulla (Finger and Tong, 1984)
NRL	nucleus recessus lateralis (Peter *et al.*, 1975)	sy	sulcus ypsiloniformis
NRP	nucleus recessus posterioris (Peter *et al.*, 1975)	TE	telencephalon
NRPH	nucleus raphes	tela ep	tela ependymalis
NTA	nucleus tangentialis (Meredith and Butler, 1983)	tgs	tractus gustatorius secundarius
NTMT	nucleus tractus mesencephalicus nervi trigemini	tgt	tractus gustatorius tertius
NVT	nucleus ventralis tuberis (Sheldon, 1912)	TL	torus longitudinalis
nI	nervus olfactorius	tmc	tractus mesencephalocerebellaris
nII	nervus opticus	TO	tectum opticum
nIII	nervus oculomotorius	tol	tractus olfactorius lateralis
NIII	nucleus nervi oculomotorii	tom	tractus olfactorius medialis
nIV	nervus trochlearis	tro	tractus opticus
NIV	nucleus nervi trochlearis	trod	tractus opticus dorsomedialis
nV	nervus trigeminus	trot	tractus rotundus
NVm	nucleus motorius nervi trigemini	trov	tractus opticus ventrolateralis
nVI	nervus abducens	TS	torus semicircularis
nVII	nervus facialis	ttb	tractus tectobulbaris
nVIII	nervus octavus	ttbc	tractus tectobulbaris cruciatus
nIX	nervus glossopharyngeus	ttbr	tractus tectobulbaris rectus
NIXm	nucleus motorius nervi glossopharyngei	tvs	tractus vestibulospinalis
nX	nervus vagus	tV	radix descendens nervi trigemini
NXm	nucleus motorius nervi vagi	VC	valvula cerebelli
OI	oliva inferior	Vd	area ventralis telencephali pars dorsalis
PGc	nucleus preglomerulosus pars medialis commissuralis (Peter *et al.*, 1975)	vDl	ventral region of Dl
PGm	nucleus preglomerulosus pars medialis	vDm	ventral region of Dm
PO	nucleus preopticus (Peter *et al.*, 1975)	vec	ventriculus communis
POm	nucleus preopticus pars magnocellularis (Peter *et al.*, 1975)	ved	ventriculus diencephali
PON	posterior octavus nucleus (McCormick, 1983)	vem	ventriculus mesencephali
POp	nucleus preopticus pars parvocellularis (Peter *et al.*, 1975)	ver	ventriculus rhombencephali
PS	nucleus pretectalis superficialis	Vi	area ventralis telencephali pars intermedia
pTGN	preglomerular tertiary gustatory nucleus	Vl	area ventralis telencephali pars lateralis
RF	reticular formation	VM	nucleus ventromedialis thalami
RFm	medial reticular zone	Vp	area ventralis telencephali pars posterior
rl	recessus lateralis	Vs	area ventralis telencephali pars supracommissuralis
rv	radix ventralis	Vv	area ventralis telencephali pars ventralis
SC	spinal cord	VIIL	lobus facialis
		XL	lobus vagi

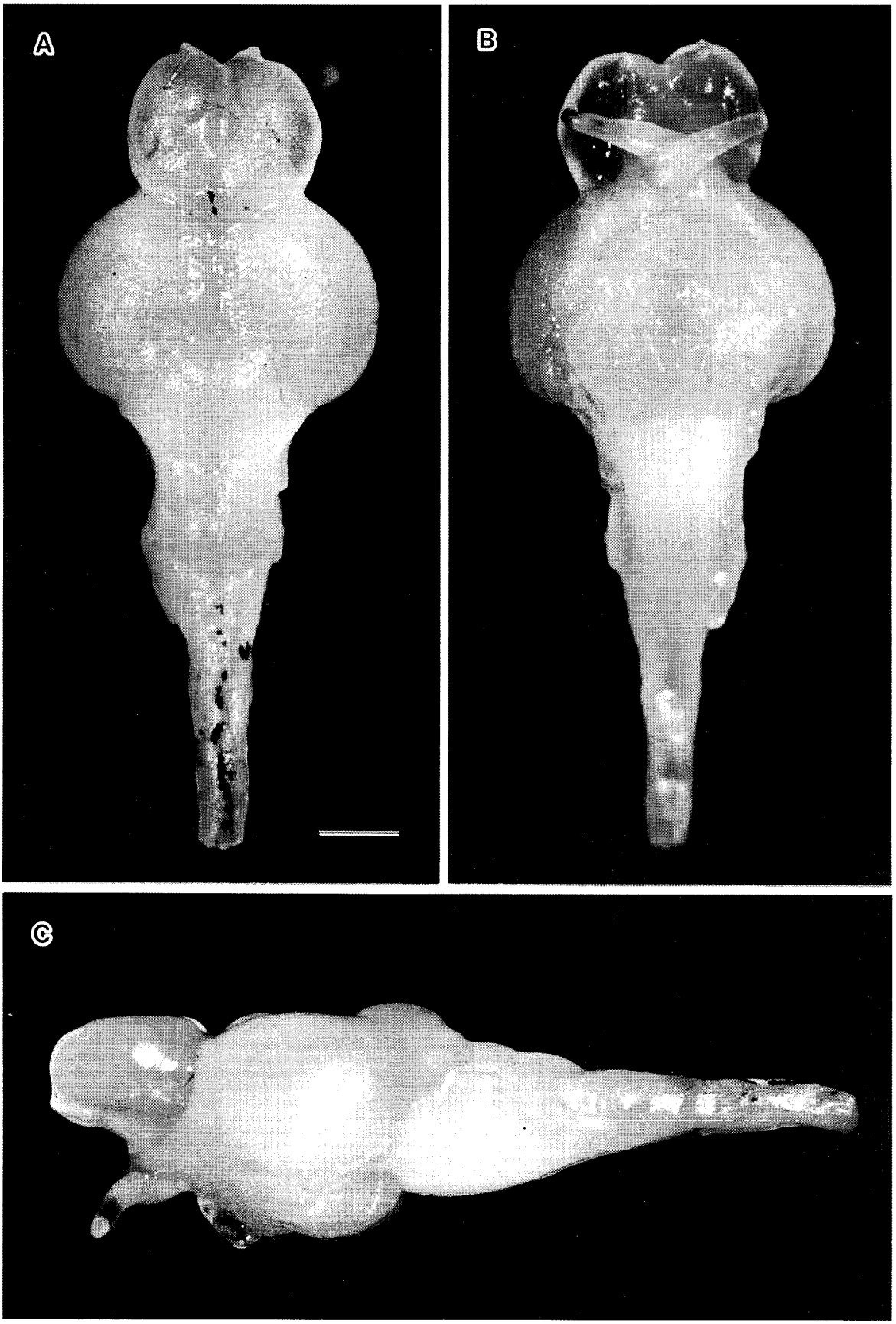

Plate 1. Dorsal (A), ventral (B) and left lateral (C) views of a brain of the HNI strain of the medaka. Scale bar = 0.5 mm.

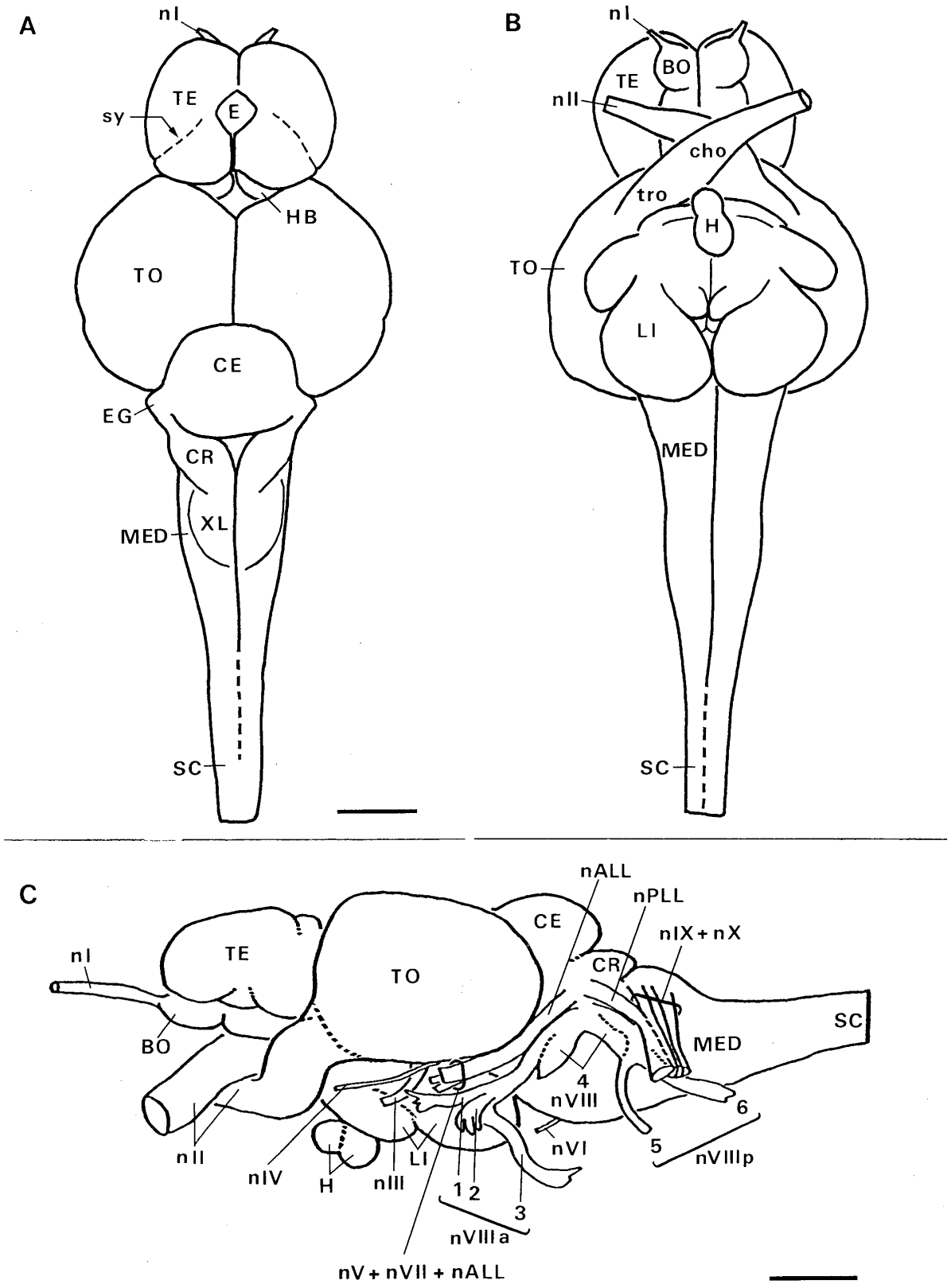

Plate 2. Line drawings of dorsal (A), ventral (B) and left lateral (C) views of medaka brains. The cranial nerves are shown in panel C. The octaval nerve (nVIII) divides into an anterior (nVIIIa), an intermediate (4), and a posterior (nVIIIp) component. The anterior component divides into 3 smaller nerve branches (1–3). The branch 1 supplies the crista ampullaris of anterior semicircular canal, the branch 2 distributes the macula of utriculus, and the branch 3 ends the crista ampullaris of horizontal semicircular canal. The branch 4 distributes the macula of succulus. The posterior component has 2 smaller branches (5 and 6). The branch 5 distributes the macula of lagena, and the branch 6 supplies the crista ampullaris of the posterior semicircular canal. For other abbreviations, see the list. Scale bars = 0.5 mm.

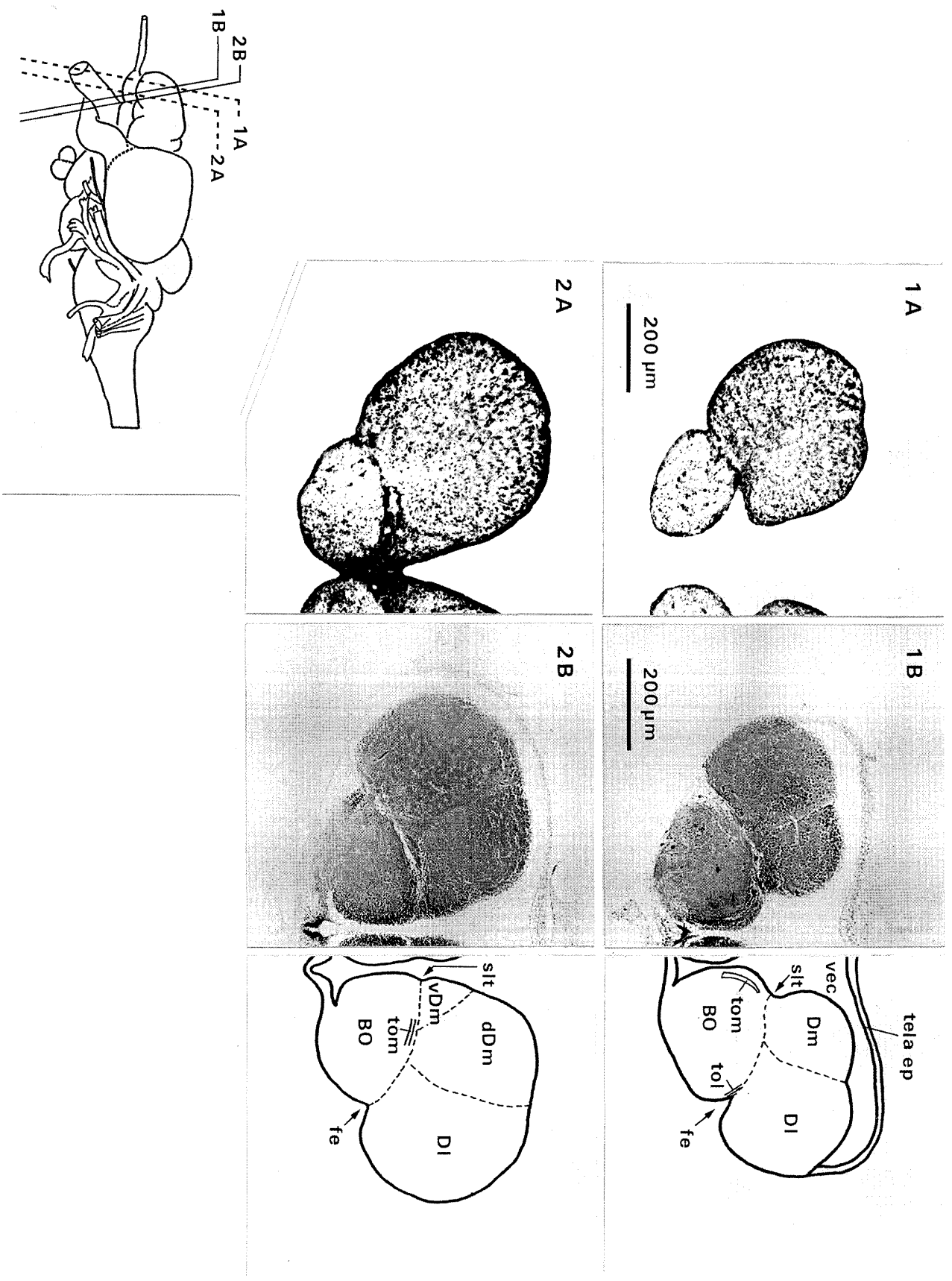
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PLATE 3.

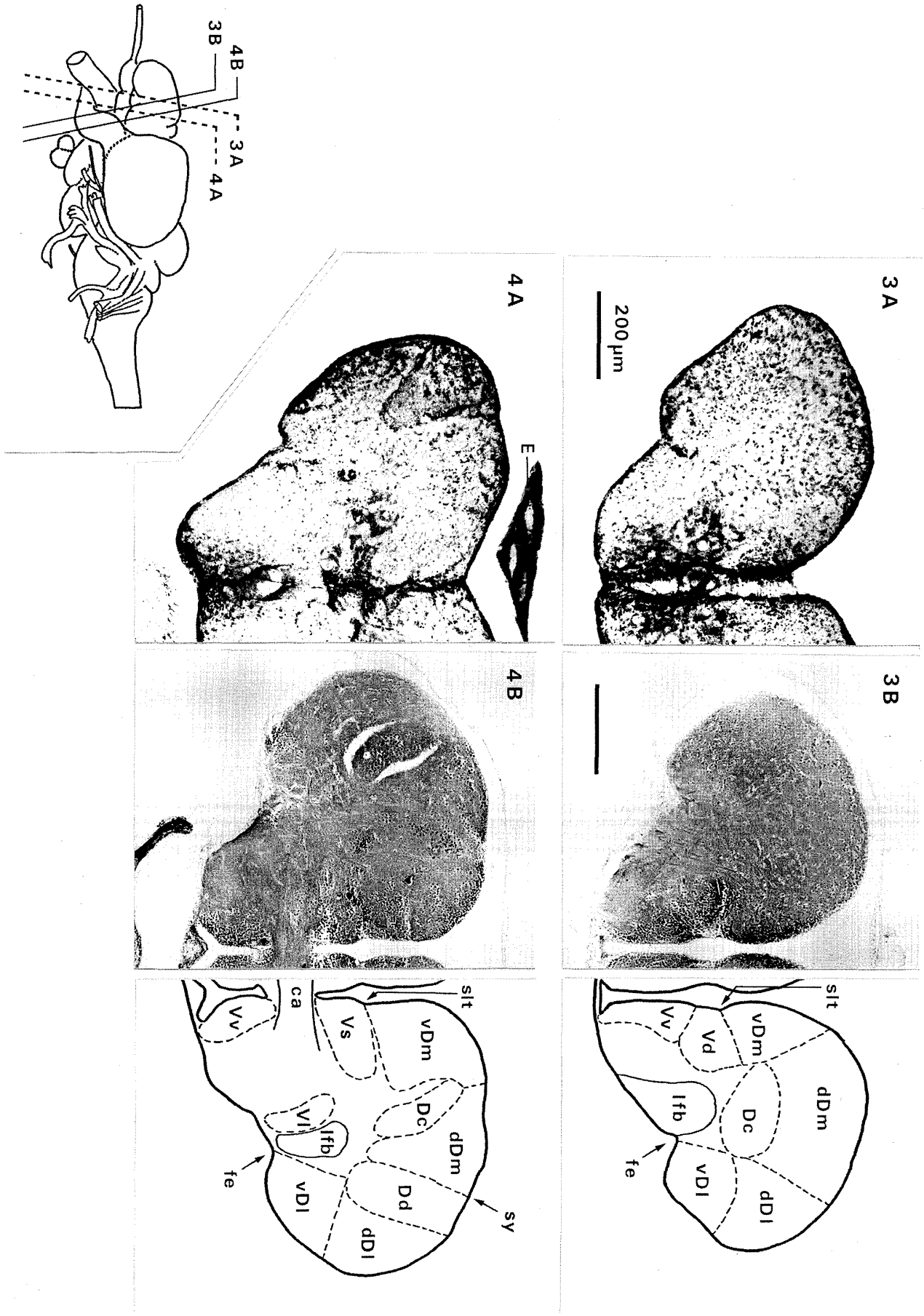
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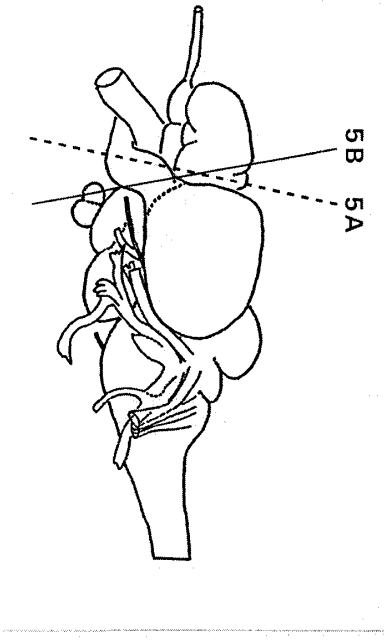
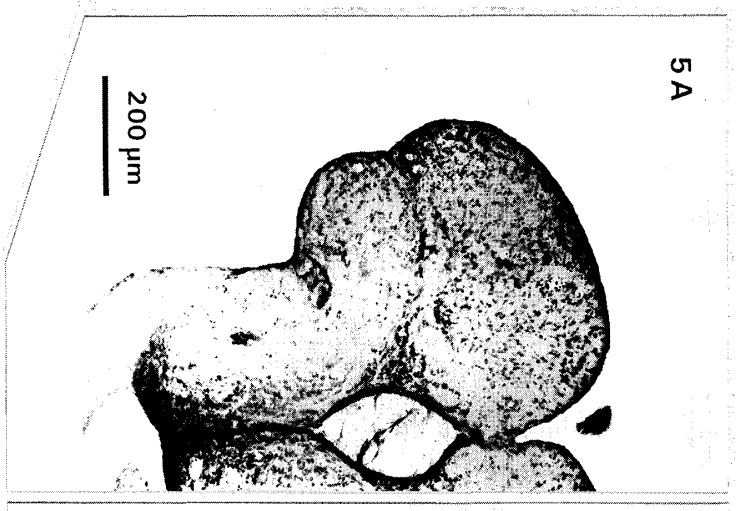
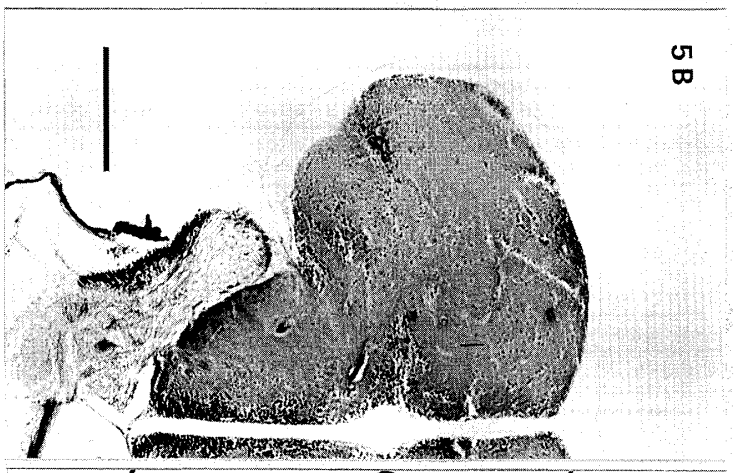
PLATE 4.


5B
5A


5A
200 µm


5B

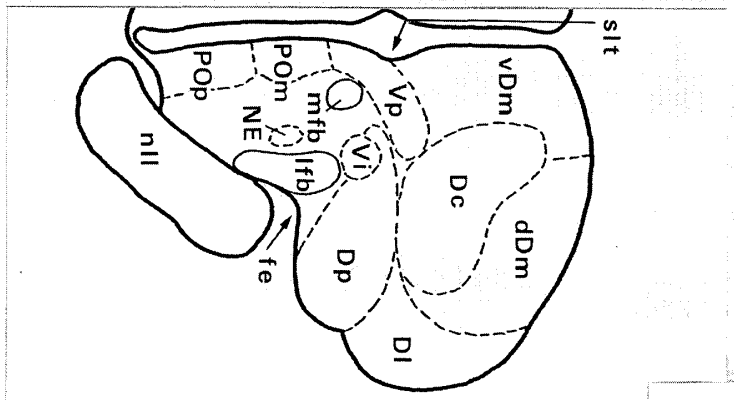
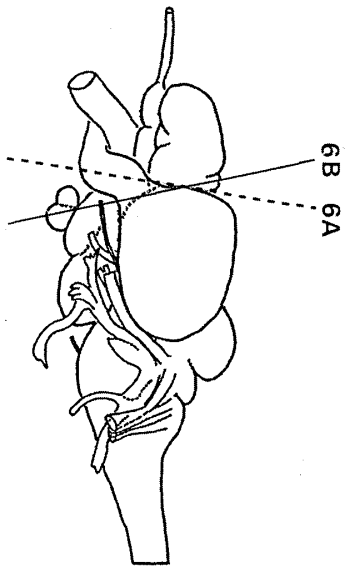

slt
vDm
POm
POp
Vp
mfb
NE
Vi
ffb
nll
Dc
dDm
Dp
fe
Dl

PLATE 5.


6B
6A


6A
200 µm

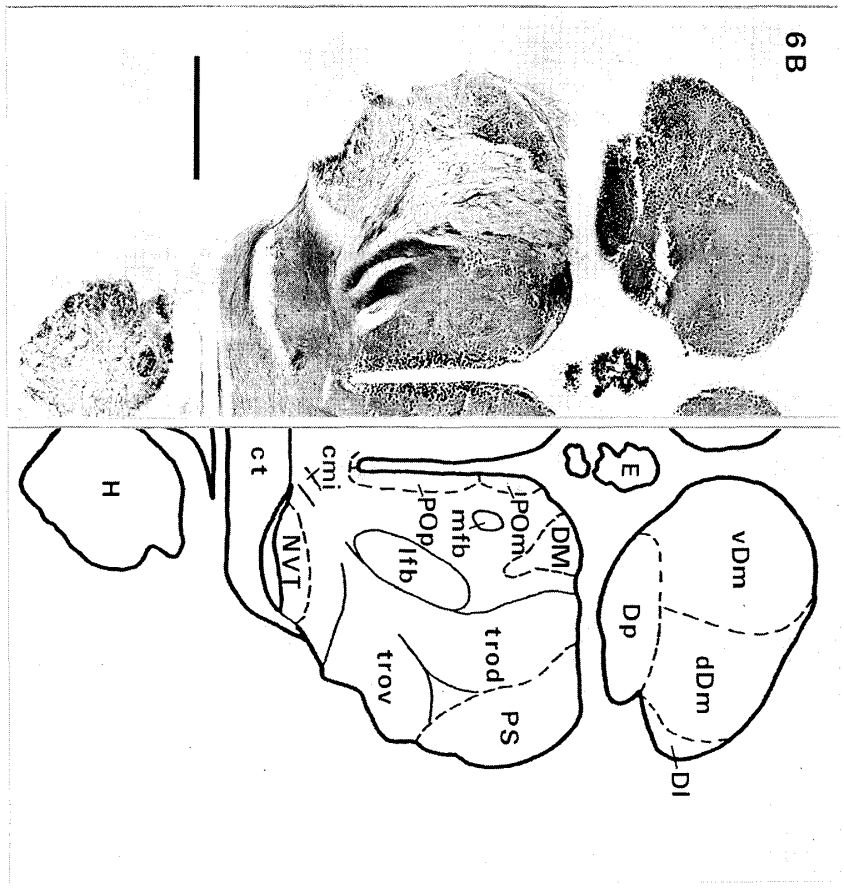

6B
H
ct
cmi
Xt
NVT
E
POm
DM
Q
mfb
POp
ltb
trod
trov
PS
vDm
Dp
dDm
Dl

PLATE 6.

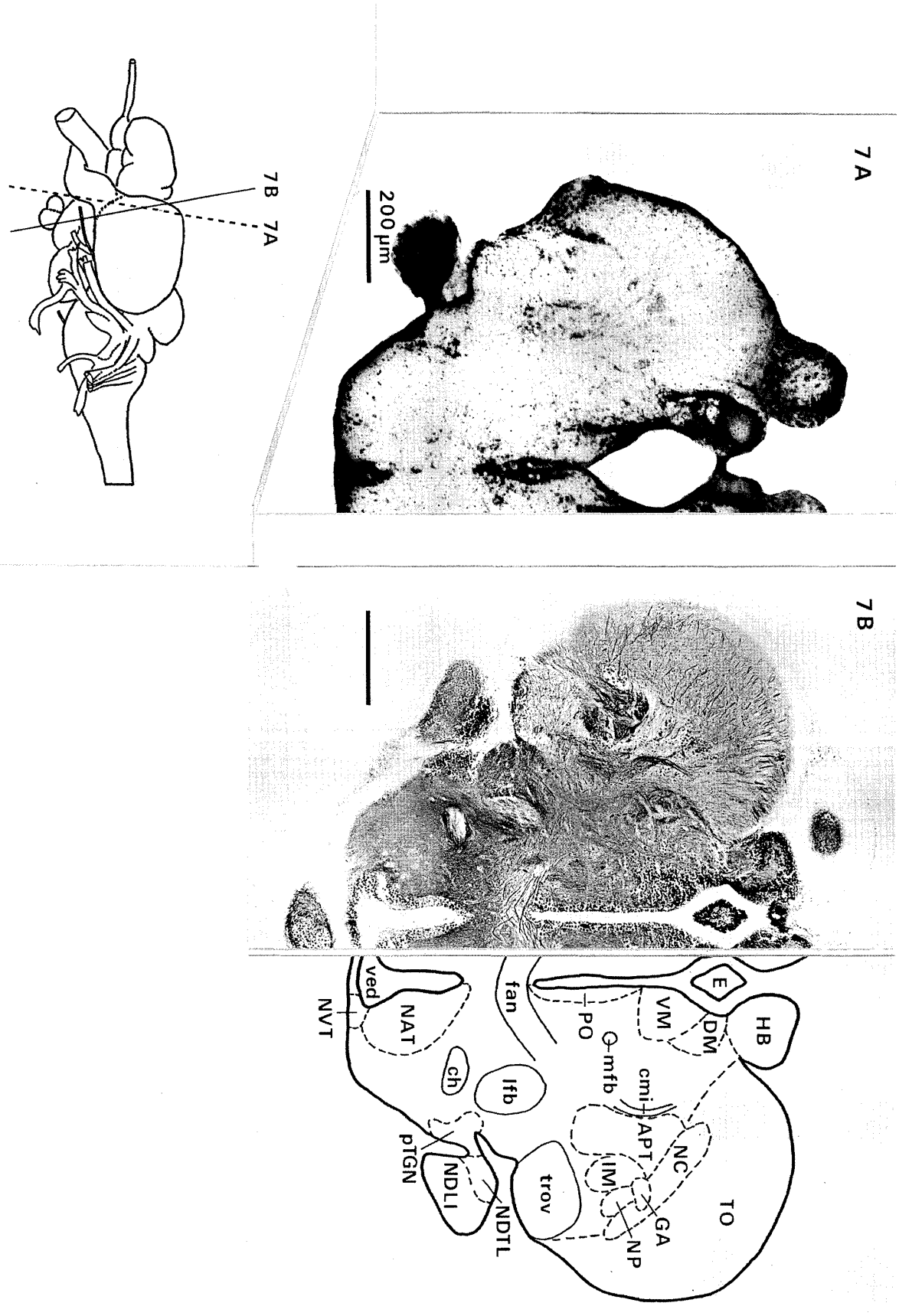

7B
7A
7A
200 µm
7B
ved
NVT
NAT
ch
fan
lfb
PO
mfb
cmi
VM
DM
E
HB
APT
IM
NC
GA
NP
TO
trov
pTGN
NDLI
NDTL

PLATE 7.

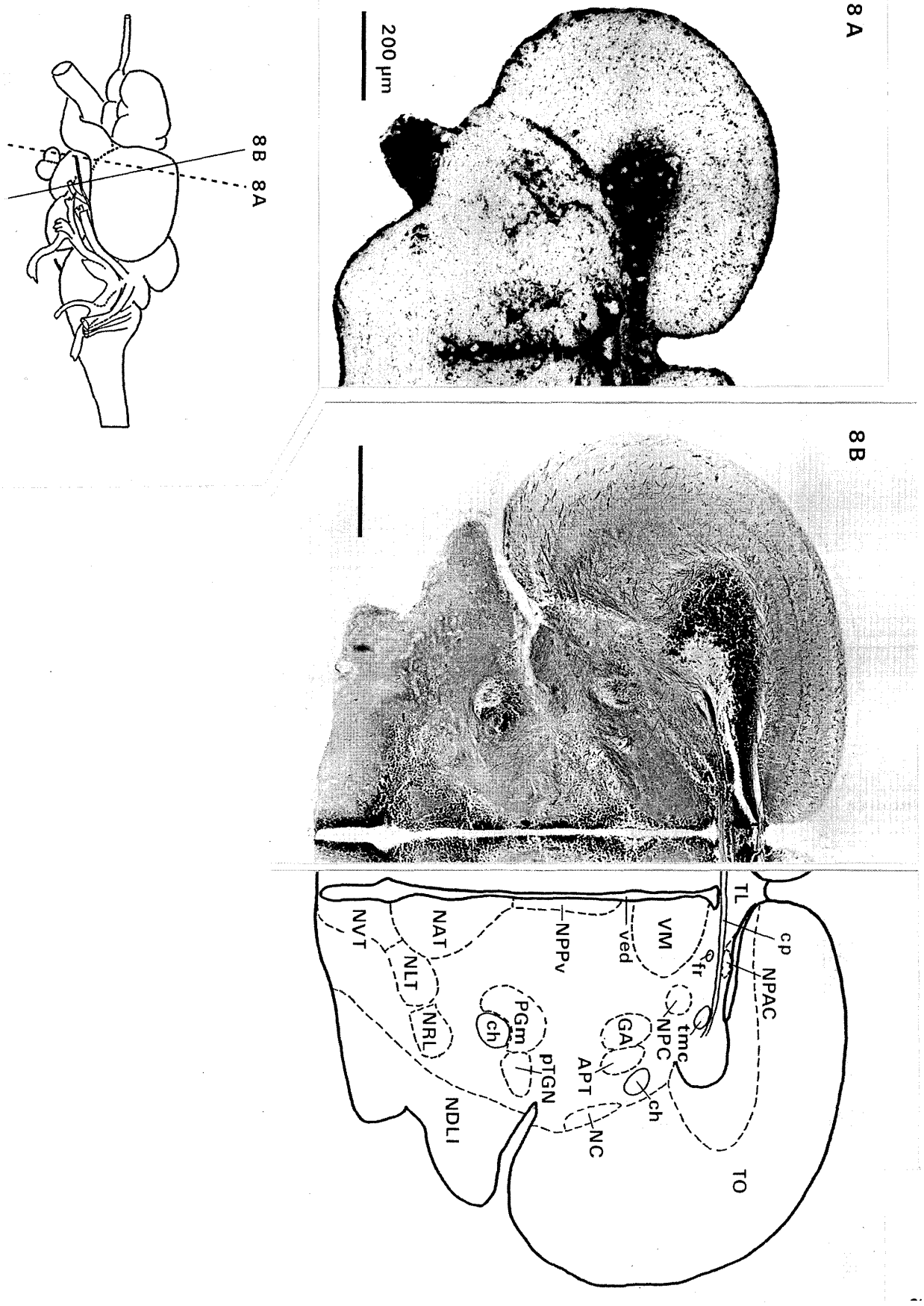

8B 8A
200 µm
8A
8B
TL
cp
NPAC
VM
ved
fr
NPPv
NAT
NVT
NLT
NRL
PGm
ch
tmc
NPC
GA
APT
ch
pTGN
NC
NDLI
TO

PLATE 8.

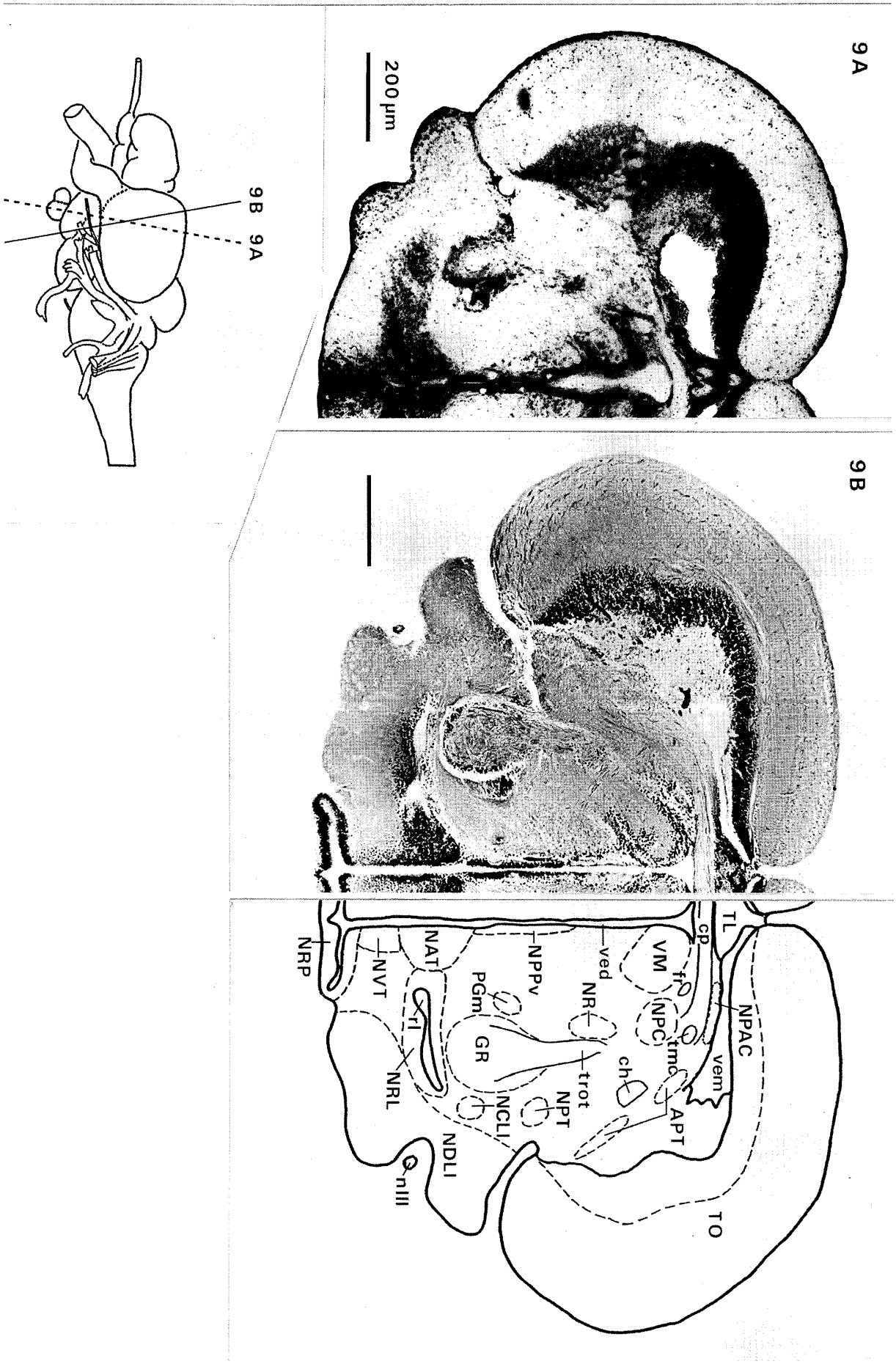

9B 9A
200 µm
9A
9B
NRP
NAT
NVT
NRL
NDLI
nIII
NPPv
PGm
GR
rl
NCLI
ved
NR
trot
NPT
VM
fr
NPC
tmc
ch
APT
cp
TL
NPAC
vem
TO

PLATE 9.

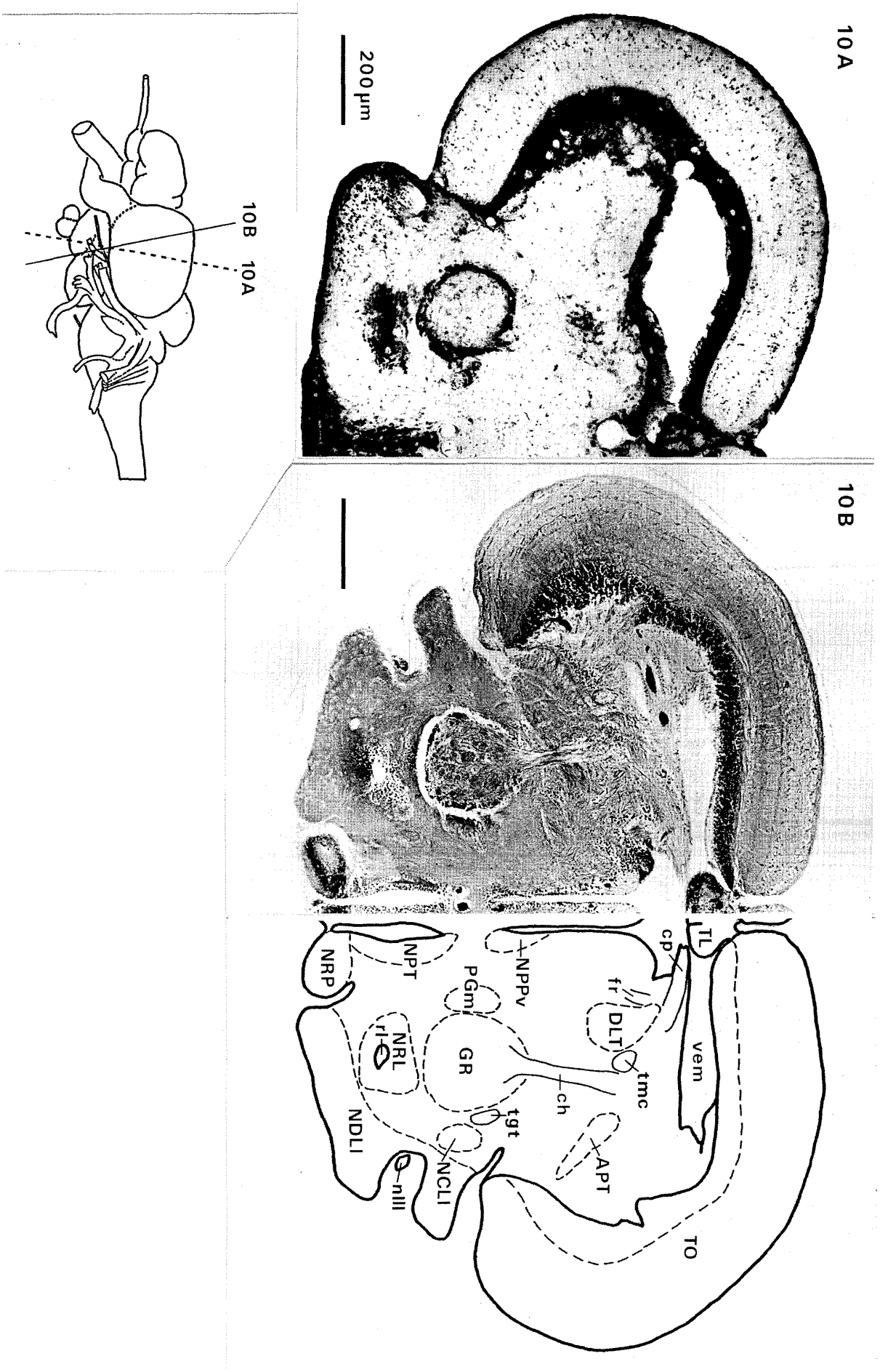

10B
10A
200 μm
10A
10B
NPT
NRP
NPPv
PGm
fr
DLT
cp
TL
vem
tmc
GR
NRL
rl
ch
tgt
APT
NDLI
NCLI
nIII
TO

PLATE 10.

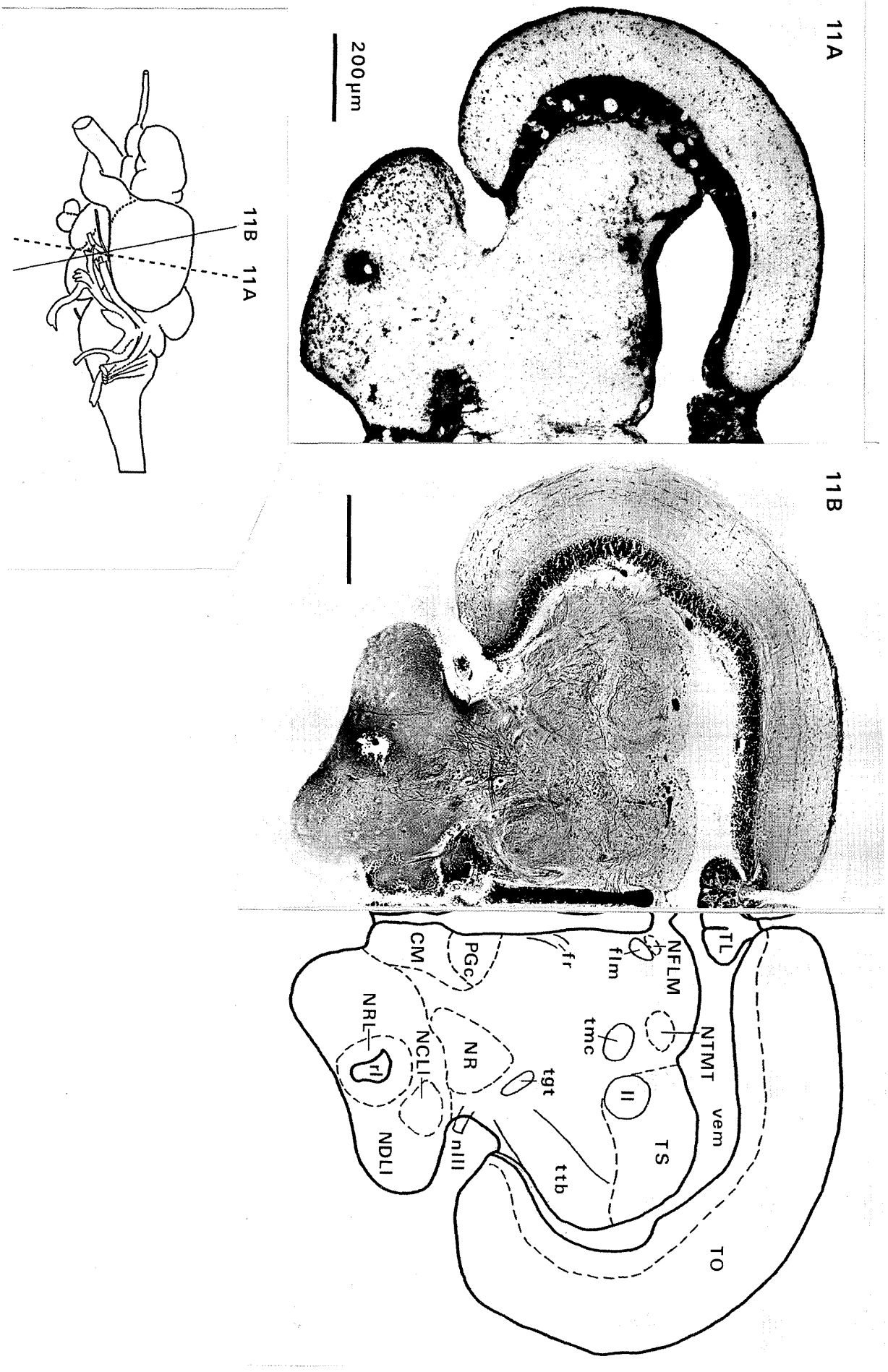

11A
200 µm
11B
11A
11B
CM
PGc
fr
flm
NFLM
TL
NRL
NCLI
NR
tmc
NTMT
vem
rl
tgt
II
TS
NDLI
nIII
ttb
TO

PLATE 11.

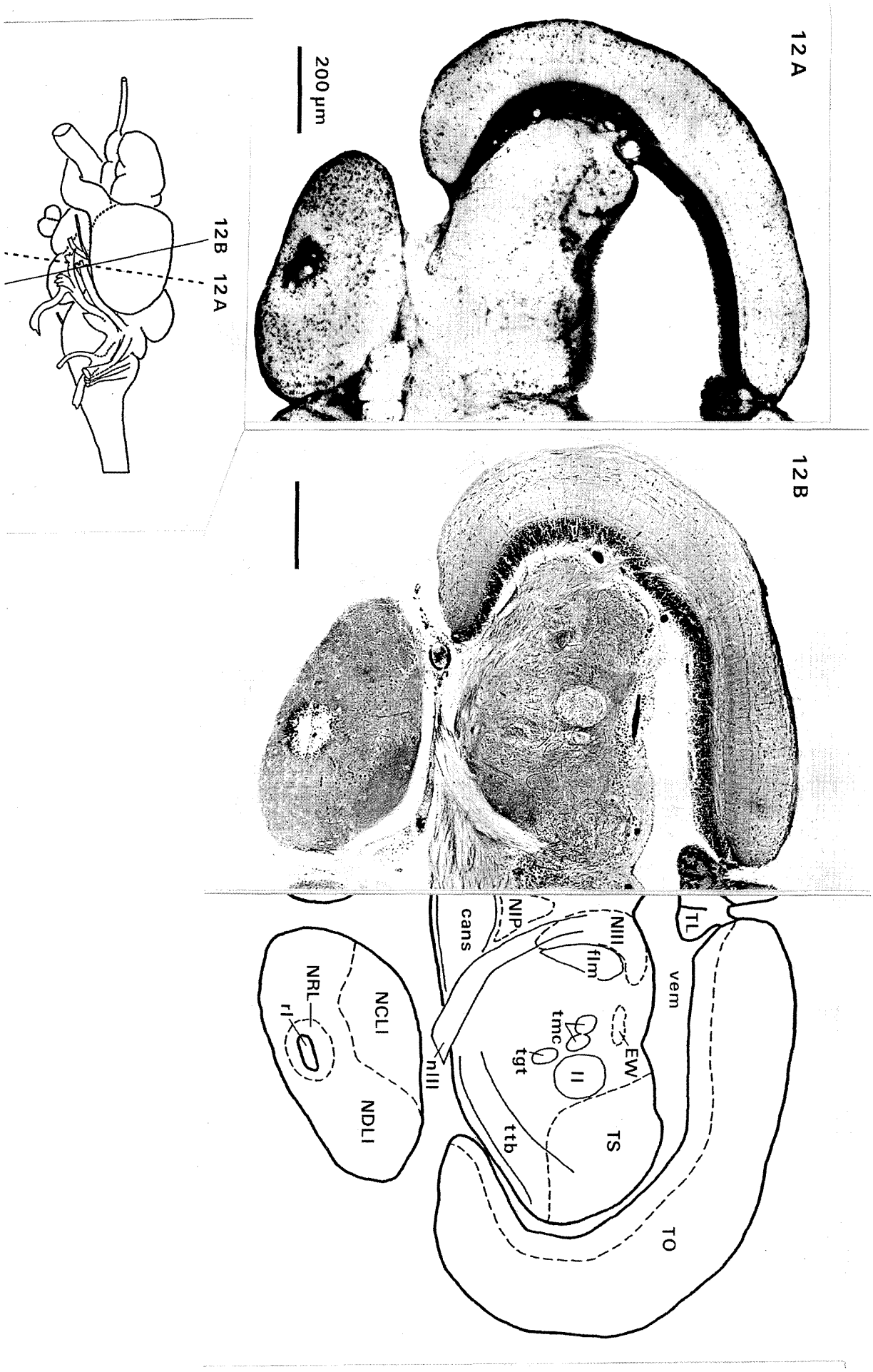

12A
12B
12A
200 µm
12B
NIP
cans
NIII
flm
TL
vem
tmc
EW
tgt
II
nIII
TS
ttb
TO
NRL
rl
NCLI
NDLI

PLATE 12.

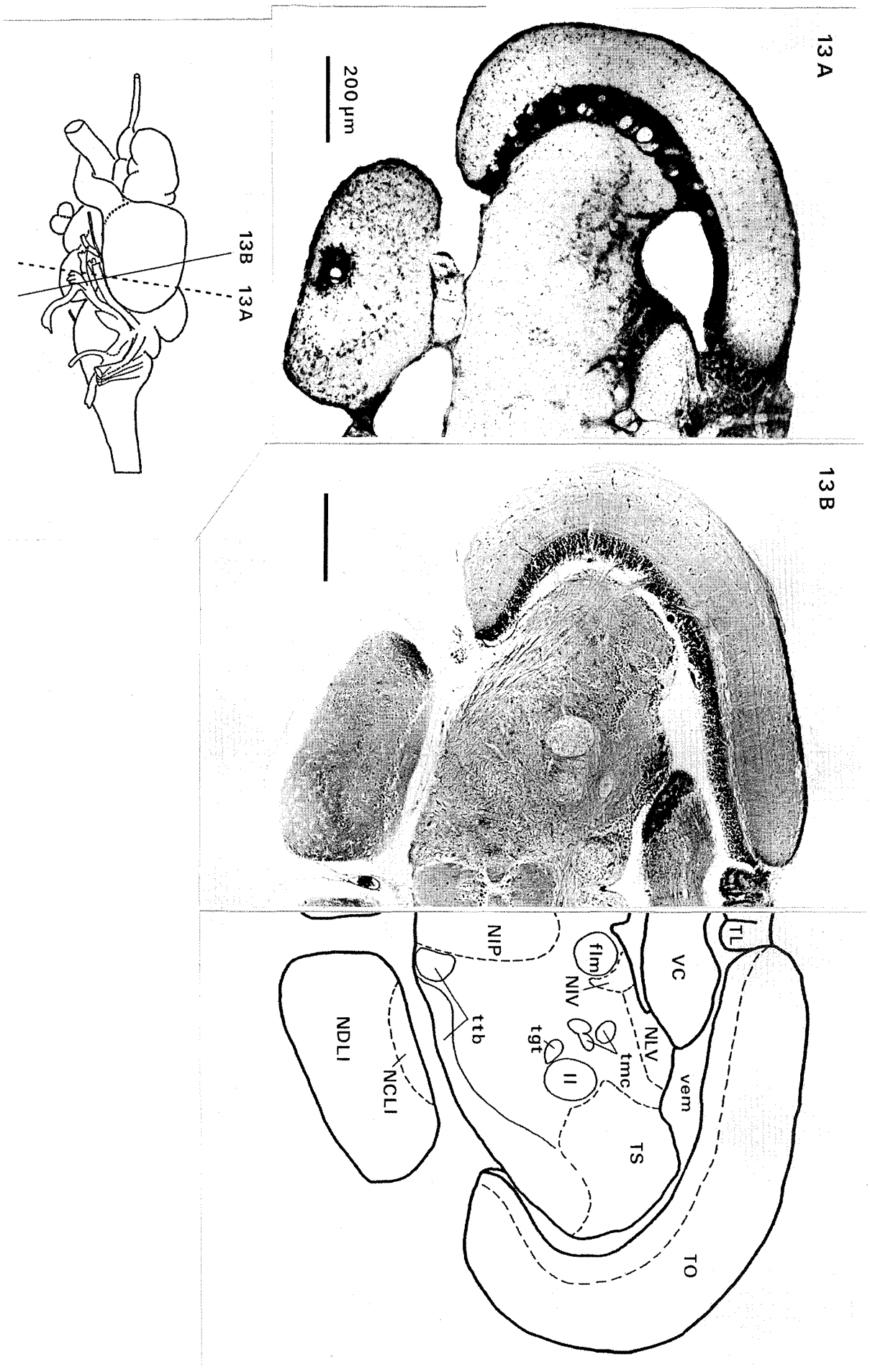

13A
13B
13A
200 µm
13B
NIP
flmlV
NIV
ttb
tgt
II
tmc
NLV
VC
TL
vem
NDLI
NCLI
TS
TO

PLATE 13.

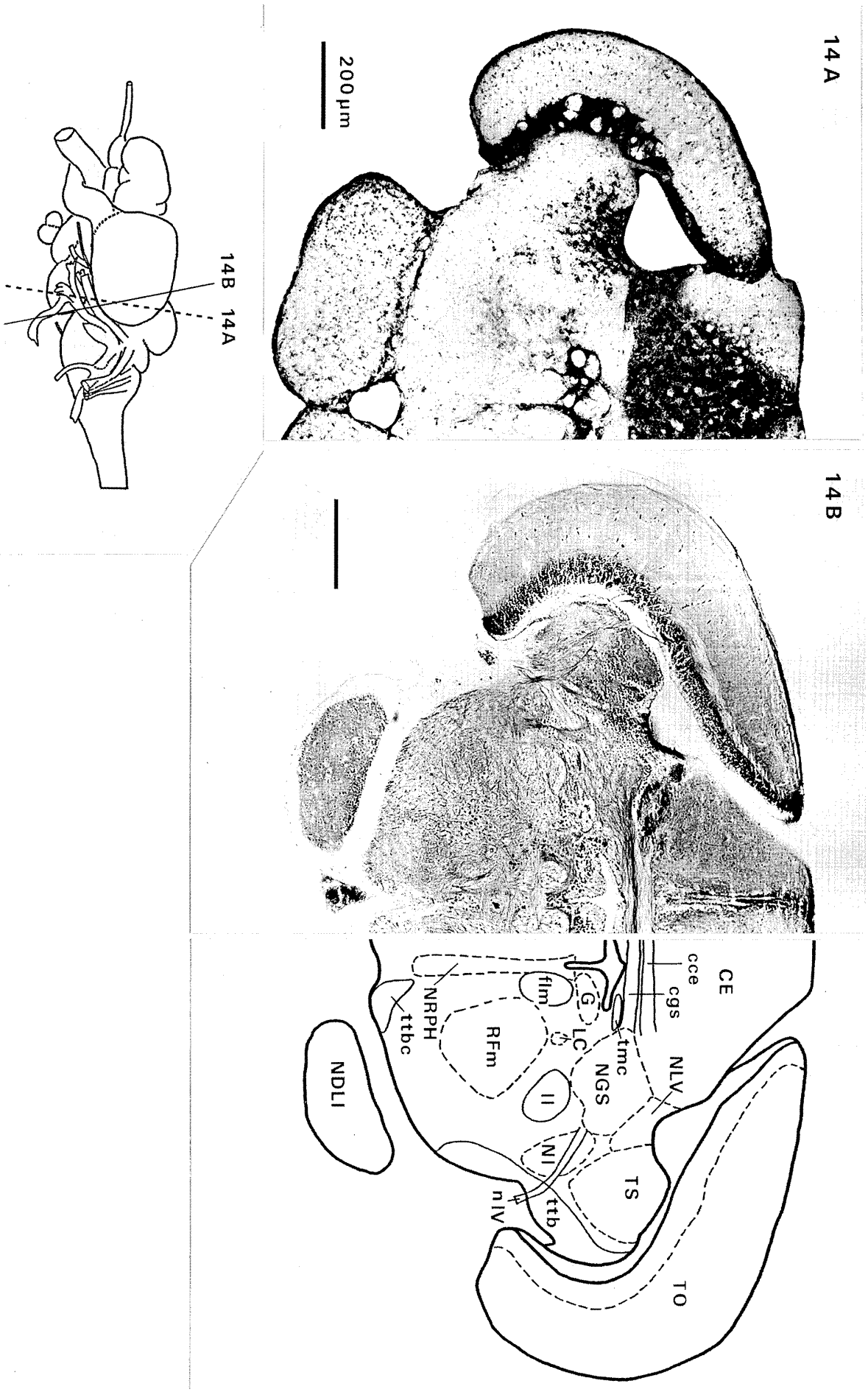

14B
14A
200 µm
14 A
14 B
CE
cce
cgs
flm
G
LC
NRPH
ttbc
RFm
tmc
NGS
NLV
II
NDLI
NI
TS
nIV
ttb
TO

PLATE 14.

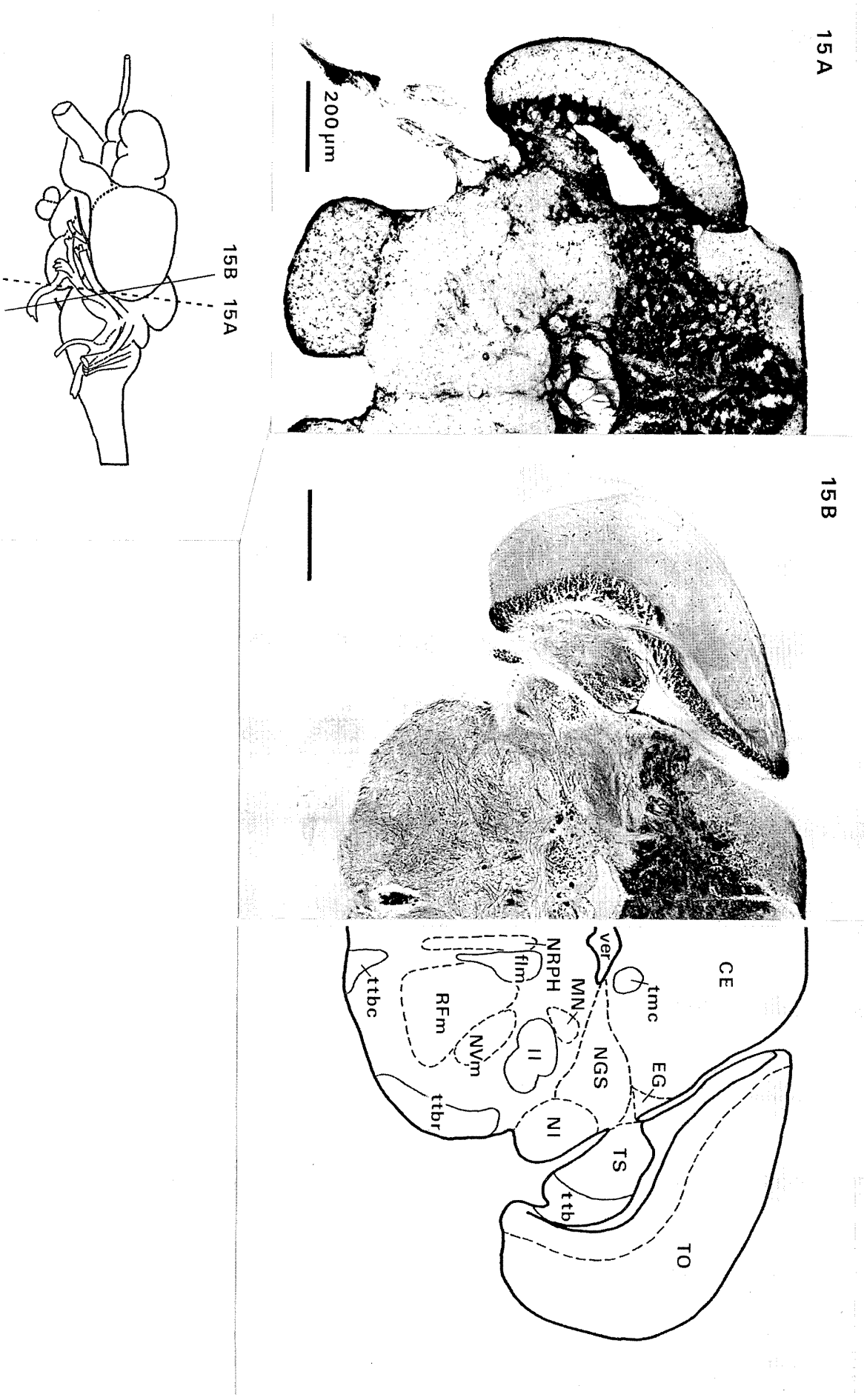
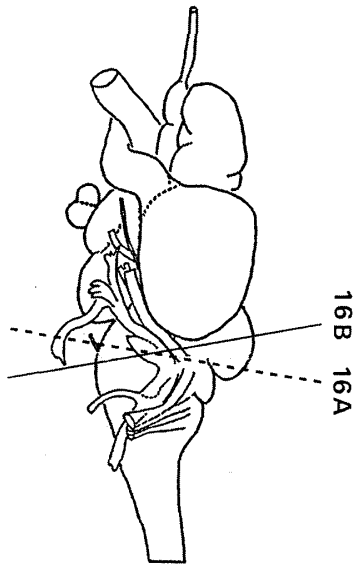

15A
15B
15A
200 µm
15B
CE
ver
tmc
MN
NRPH
flm
ttbc
RFm
NVm
II
NGS
EG
NI
ttbr
TS
ttb
TO

PLATE 15.


16B
16A


16A
200 µm

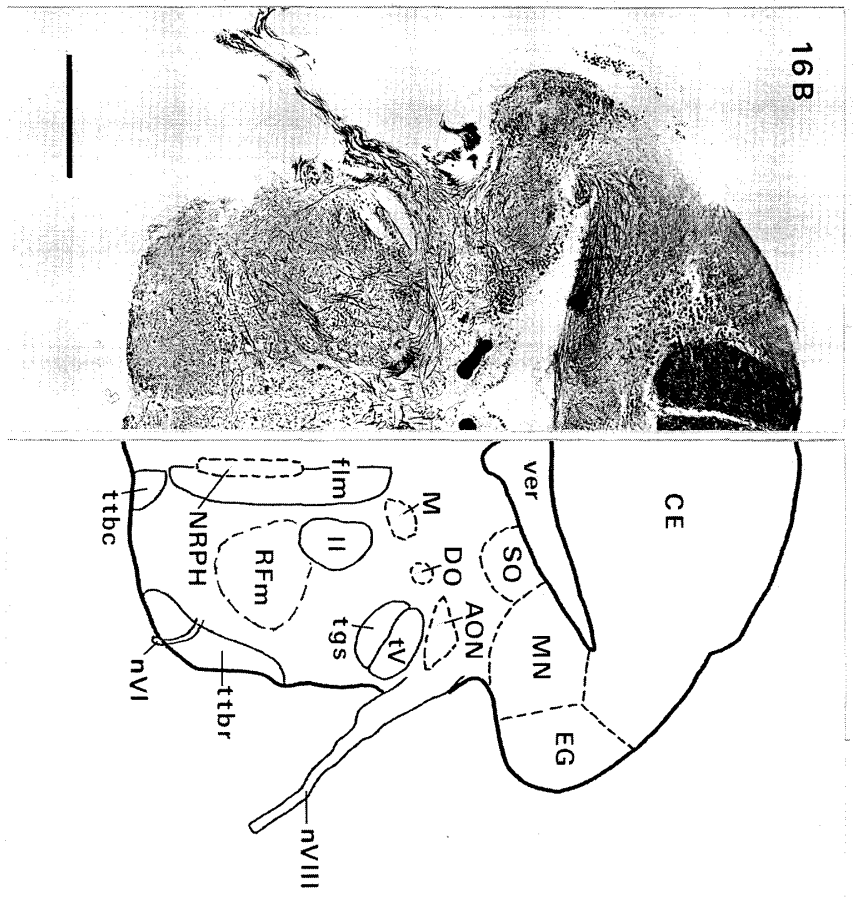
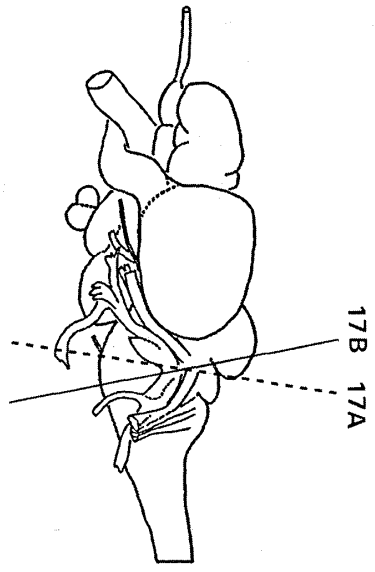
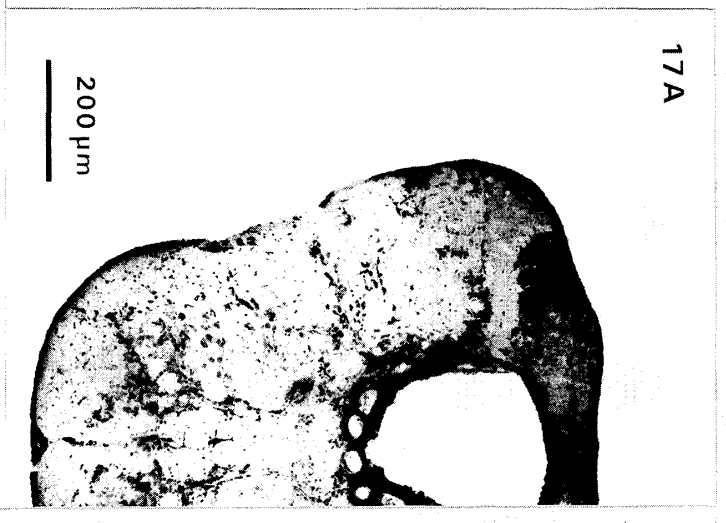

16B
flm
ttbc
NRPH
RFm
II
M
DO
AON
SO
ver
CE
tgs
tV
MN
EG
nVI
ttbr
nVIII

PLATE 16.


17B
17A


17A
200 µm

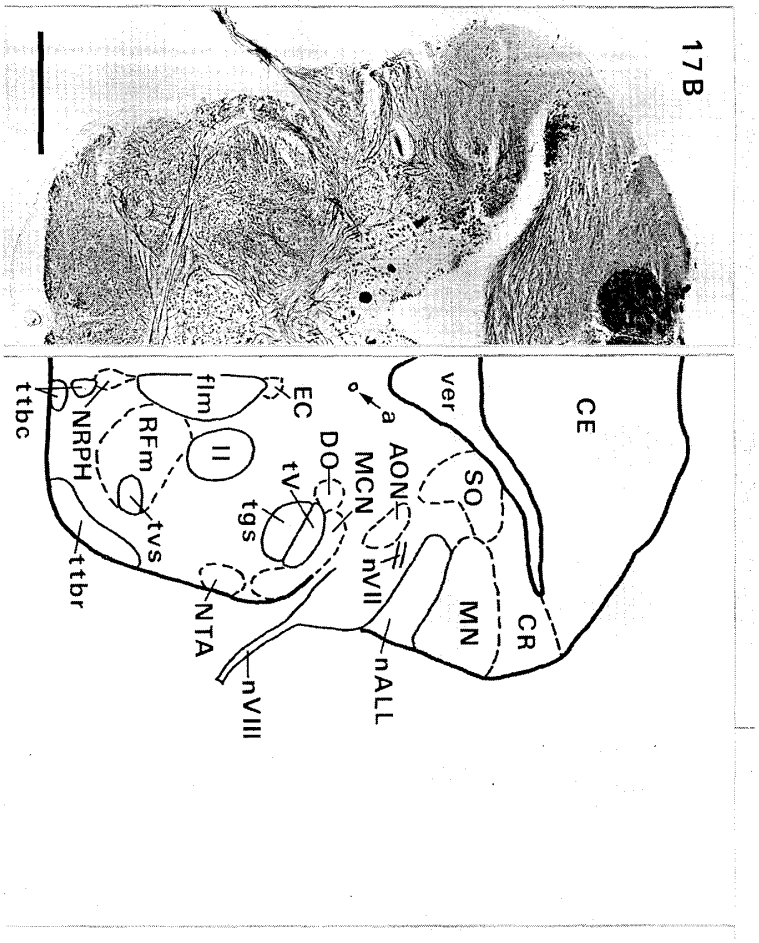

17B
CE
ver
a
AON
MCN
DO
SO
nVII
MIN
CR
nALL
nVIII
NTA
ttbr
tvs
tgs
tV
RFm
II
flm
EC
NRPH
ttbc

PLATE 17.

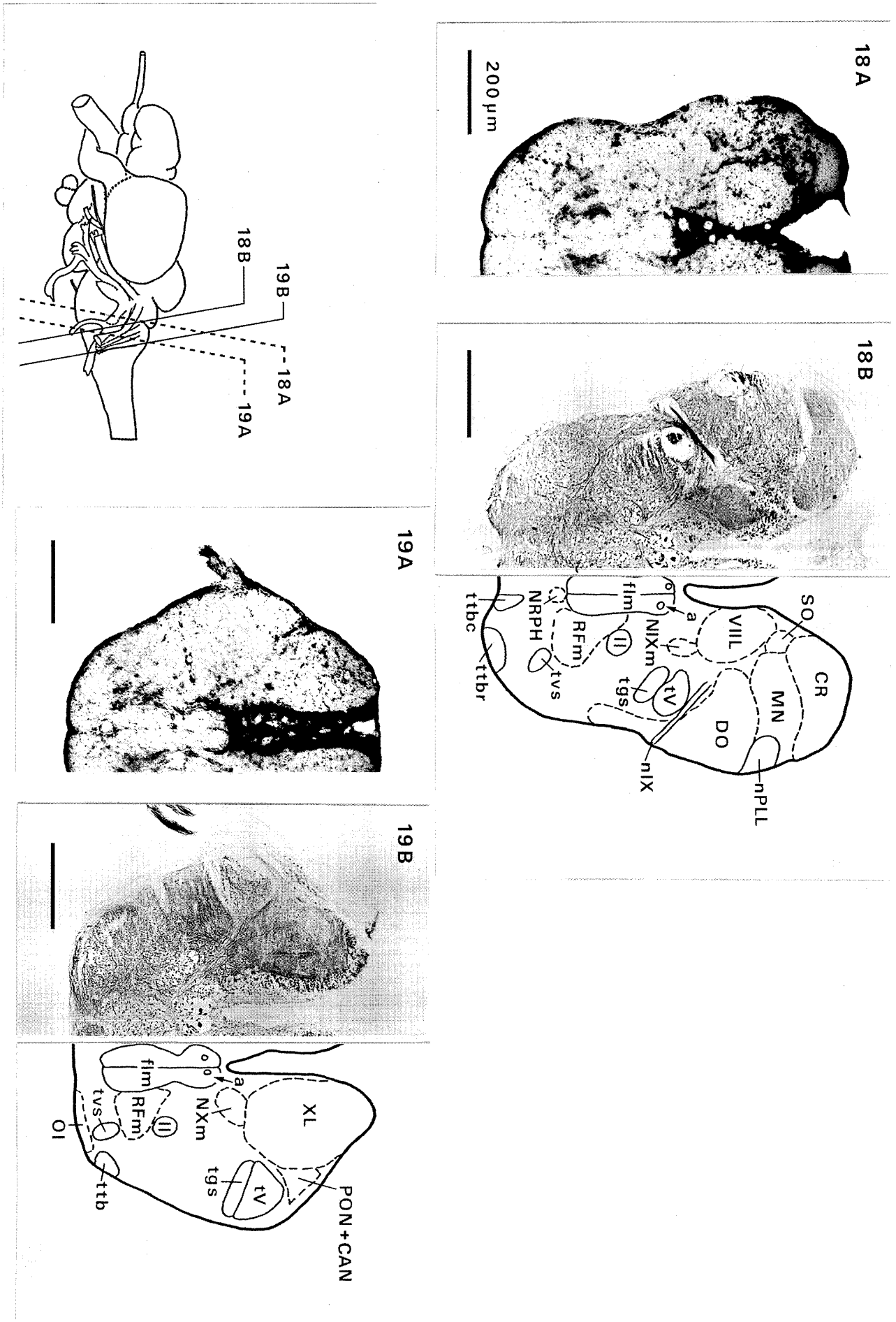

18A
200 µm
18B
SO
VIIL
CR
MN
DO
nPLL
nIX
NIXm
tV
tgs
tvs
NRPH
RFm
flm
II
a
ttbc
ttbr
19A
19B
18B
19B
18A
19A
XL
NXm
PON+CAN
OI
ttb

PLATE 18.

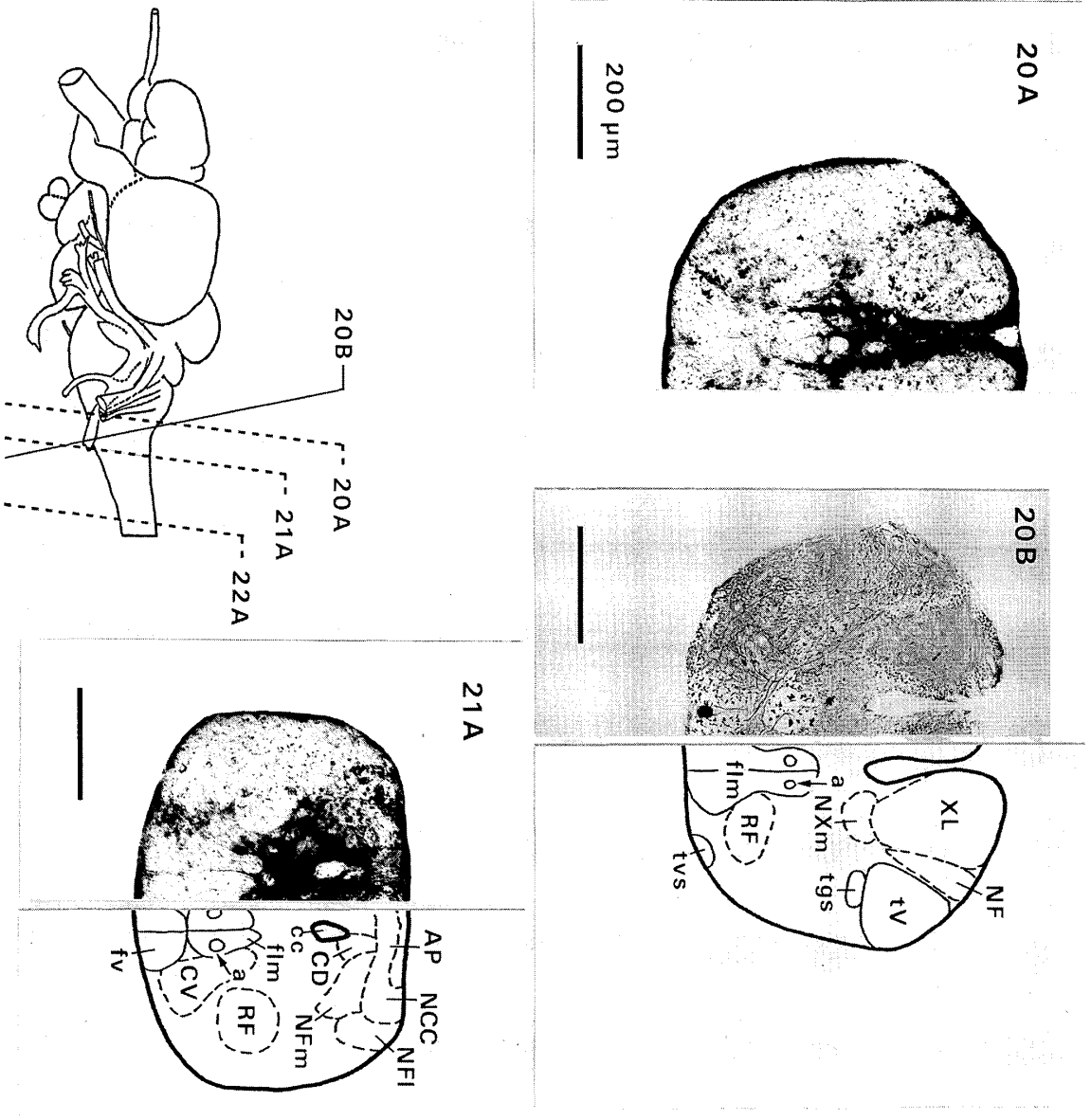

20B
20A
21A
22A
200 µm
20A
XL
NF
tV
tgs
NXm
RF
flm
tvs
21A
AP
NCC
NFl
CD
cc
NFm
flm
RF
CV
fv
22A
CD
cc
fd
fl
flm
fv
CV
rv

PLATE 19.

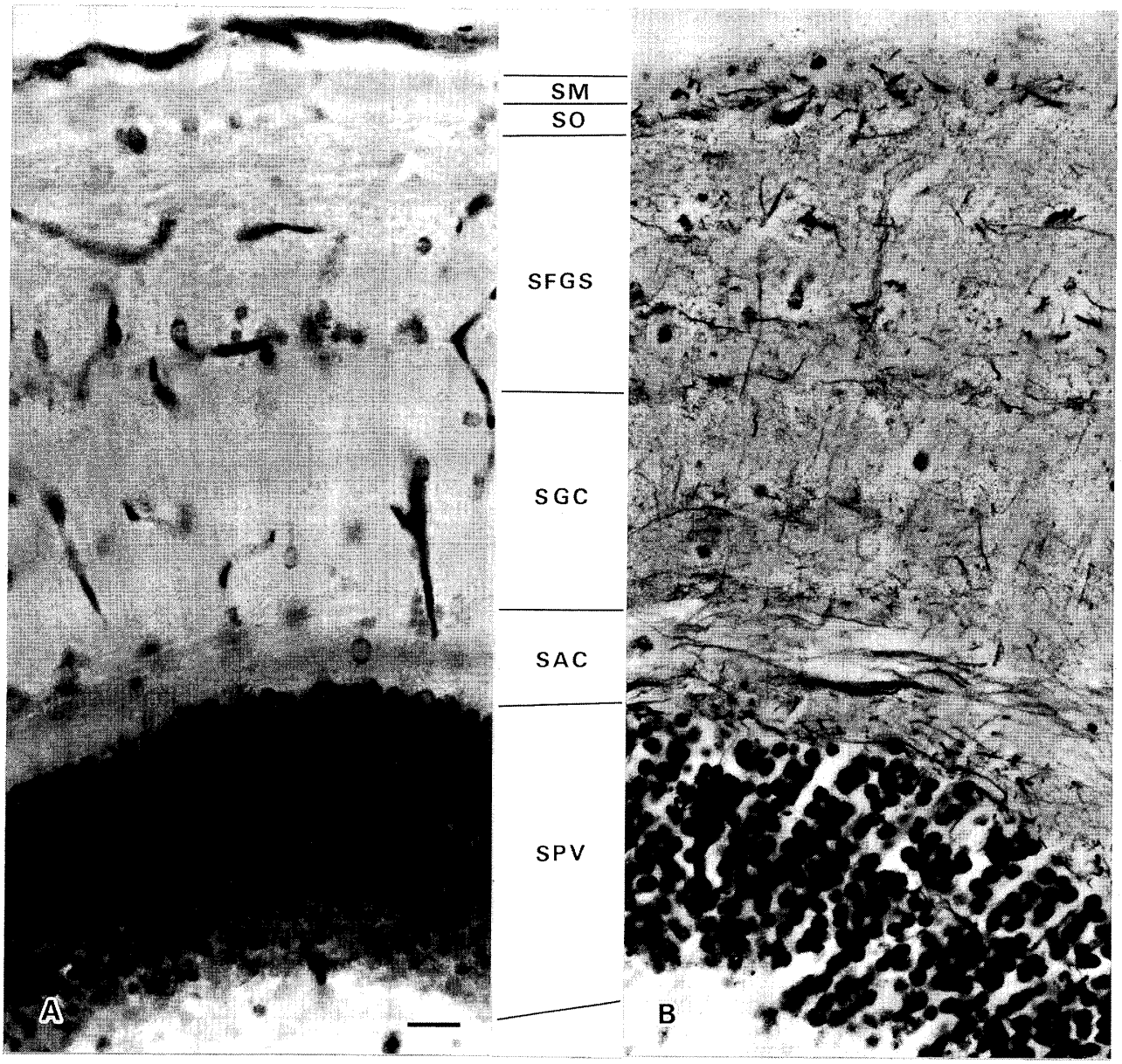


Plate 20. Frontal Nissl (A) and Bodian-stained (B) sections through the central part of the optic tectum. SM, stratum marginale; SO, stratum opticum; SFGS, stratum fibrosum et griseum superficiale; SGC, stratum griseum centrale; SAC, stratum album centrale; SPV, stratum periventriculare. Scale bar = 10 μm.