A stereotaxic atlas and implantation technique for nuclei of the diencephalon of Atlantic salmon (Salmo salar) parr

Re Peter, Lw Crim, Roland Billard

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Original article

A stereotaxic atlas and implantation technique for nuclei of the diencephalon of Atlantic salmon *(Salmo salar)* parr

RE Peter [1]*, LW Crim [2], R Billard [3]

[1] *University of Alberta, Department of Zoology, Edmonton, Alta T6G 2E9;*
[2] *Memorial University of Newfoundland, Marine Sciences Research Laboratory, St John's, Nfld A1C 5S7, Canada;*
[3] *Museum National d'Histoire Naturelle, Laboratoire d'Ichtyologie Générale et Appliquée, 43, rue Cuvier, 75231 Paris Cedex 05, France*

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Summary — A stereotaxic apparatus and technique for its inplantation in diencephalic nuclei of Atlantic salmon parr of 20 to 30 g body weight is described. An atlas of nuclei in the diencephalon is also presented.

diencephalic nuclei / stereotaxic apparatus / *Salmo*

Résumé — Atlas stéréotaxique et techniques d'implantation dans le diencéphale de saumon atlantique. *Un appareil stéréotaxique et les techniques d'implantation dans les noyaux diencéphaliques de saumon atlantique (*Salmo salar*) parr de 20 à 30 g sont décrits. Un atlas des noyaux dans le diencéphale est aussi présenté.*

noyaux de diencéphale / stéréotaxie / Salmonidés

INTRODUCTION

A stereotaxic atlas and technique for diencephalic nuclei is available for a limited number of teleost species, including goldfish, *Carassius auratus* (Peter and Gill, 1975), killifish, *Fundulus heteroclitus* (Peter *et al,* 1975), and rainbow trout, *Oncorhynchus mykiss* (= *Salmo gairdneri*) (Billard and Peter, 1982). In the stereotaxic techniques used for goldfish and killifish, the anterior mid margin of the posterior commissure (PC) is used as the zero point, and coordinates are based on measurements from the zero point and brain surfaces. In the technique used for rainbow trout, the zero point is based on skull coordinates and position of the fish in the head holder. When using PC as the zero point, the skull has to be widely opened and the brain exposed. This is relatively easy for fish of a small size. However, this approach may damage the brain and the accessory structures such as the pineal, saccus dorsalis and choroid membrane; the advantage is the generally high precision of sterotaxic placement. Using

* Correspondence and reprints

external coordinates trauma is minimized; however, the placement is generally less precise and fish of a limited size range from a standardized strain reared under homogeneous conditions are required.

In the present work, an atlas of diencephalic nuclei and a stereotaxic technique, using the PC as zero point, is described for Atlantic salmon *(Salmo salar)* parr ranging from 20 to 30 g in body weight.

MATERIALS, METHODS AND RESULTS

The stereotaxic apparatus

The apparatus is similar to that used for killifish (Peter *et al,* 1975). A 3-point system of attachment of the head is used, with a bar against the upper roof of the mouth, and a V-shaped bar on the upper rim of each bony orbital. The dimensions and distances of the orbital bars to the mouth bar, and the base plate, upon which the belly of the fish rests, are shown in figure 1. A micromanipulator for holding an electrode, or tubing for implant or infusion is attached to the base plate.

The fish and surgical procedures

Wild male and female Atlantic salmon parr, 2 or 3 years of age, were netted in June 1976 and July 1983 from Fitzgerald, near Placentia Bay, Newfoundland, Canada. Males had reached the age of onset of precocious sexual development (gonadosomatic index : 3–4%) and females were still sexually immature.

The surgical procedure used for the Atlantic salmon parr was derived from that described for goldfish (Peter and Gill, 1975) and killifish (Peter *et al,* 1975). Briefly, fish were anesthetized in phenoxy ethanol (0.5 ml/l) and wrapped in a damp tissue paper. Using a circular saw blade, a 3 sided flap was cut in the frontal bone; the most anterior cut was aligned with the posterior margin of the iris of the eyes and the second cut, parallel to the first, was made about 4 mm further posterior. If this second cut is made more posterior than indicated, damage to the optic tectum is likely occur.

Table I. Nomenclature and list of abbreviations.

AC, anterior commissure
AP, area pretectalis
CM, corpus mamillare
MT, midbrain tegmentum
NAPv, nucleus anteriosis periventricularis
NAT, nucleus anterior tuberis
NDL, nucleus dorsolateralis thalami
NDLl, nucleus diffusus lobi inferioris
NDM, nucleus dorsomedialis thalami
NDTL, nucleus diffusus tori lateralis
NE, nucleus entopeduncularis
NG, nucleus glomerulosus
NH, nucleus habenularis
NLT, nucleus lateralis tuberis
NP, nucleus pretectalis
NPA, nucleus preglomerulosus anterior
NPC, nucleus pretectalis centralis
NPGl, nucleus preglomerulosus lateralis
NPGm, nucleus preglomerulosus medialis
NPO, nucleus preopticus
NPP, nucleus preopticus periventricularis
NPPv, nucleus posteriosis periventricularis
NPS, nucleus pretectalis superficialis
NPR, nucleus pretectalis rotundus
NPT, nucleus posterior tuberis
NRL, nucleus recessus lateralis
NRP, nucleus recessus posterioris
NS, nucleus suprachiasmaticus
NSG, nucleus subglomerulosus
NSV, nucleus saccus vasculosus
NVM, nucleus ventromedialis thalami
OC, optic tectum
P, pituitary
PC, posterior commissure
SV, saccus vasculosus
Tel, telencephalon

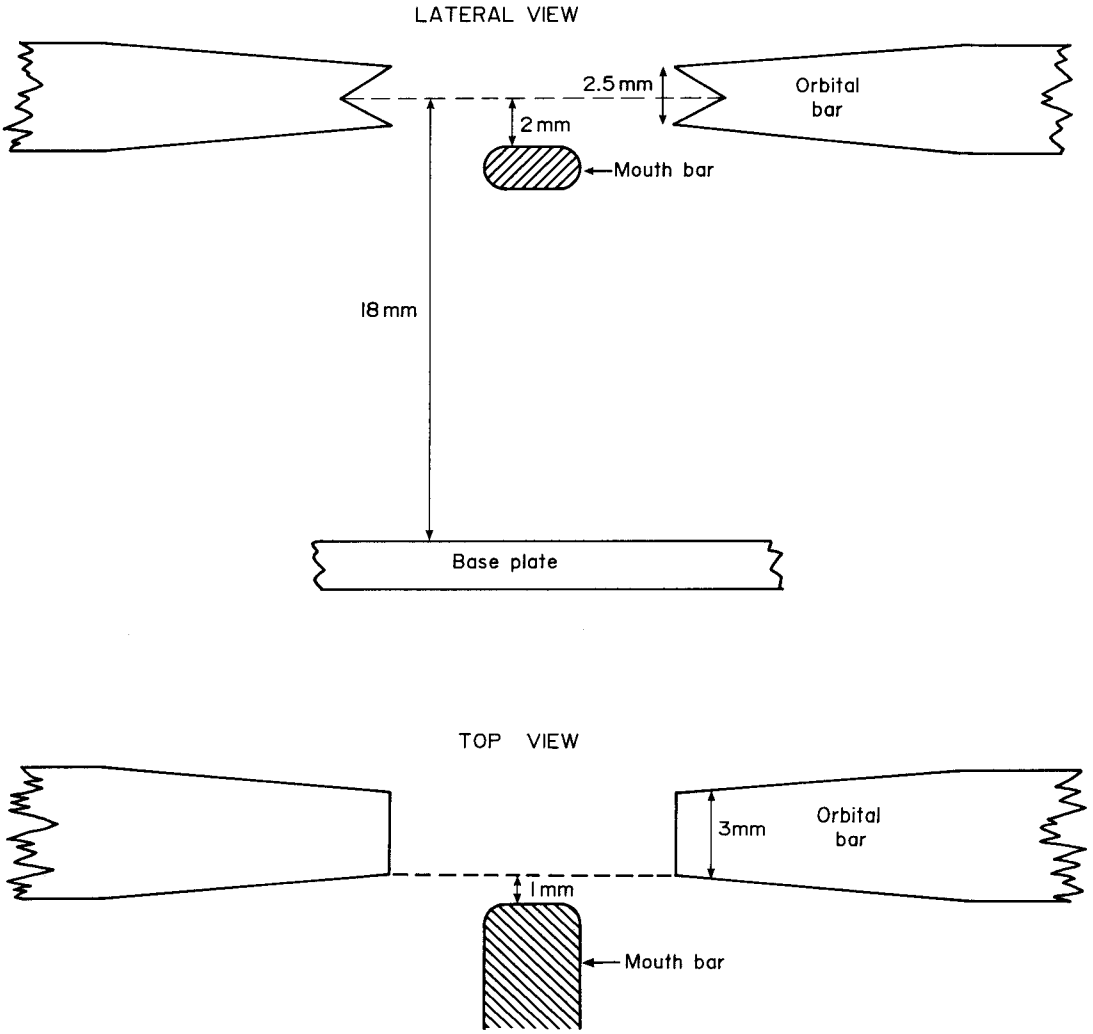


Fig 1. a Laterial view, to scale in mm, of the orbital bars and end views of the mouth bar and body support for the fish. **b** Top view, to scale in mm, of the orbital bars and mouth bar.

The third cut, parallel to the longitudinal axis of the fish, connects the first two cuts and was made in the frontal bone on the left side, about 2 mm above the edge of the orbital bones. Using a scalpel blade, the flap was folded to the right side of the fish along the uncut margin of the flap, and the fish was then clamped in the stereotaxic apparatus. While viewing through a dissecting microscope, and by using fine forceps and small twisted paper swabs to blot up fluids, the saccus dorsalis was pushed gently forward or laterally to the left side and the PC exposed. Throughout the operation, which lasted 5–10 min, the fish were kept cool by placing plastic bags with crushed ice around the body; the gills were not perfused with water during the operation.

Following the brain intervention, the top of the cranial cavity was filled with fish physiological saline (Burnstock, 1958). The frontal bone flap was then replaced and held with surgical silk thread looped across the top of the bone flap and stitched under the skin below the orbital bones. The fish were then returned to aerated and cooled water for recovery from the anesthetic. Survival was generally higher than 90%.

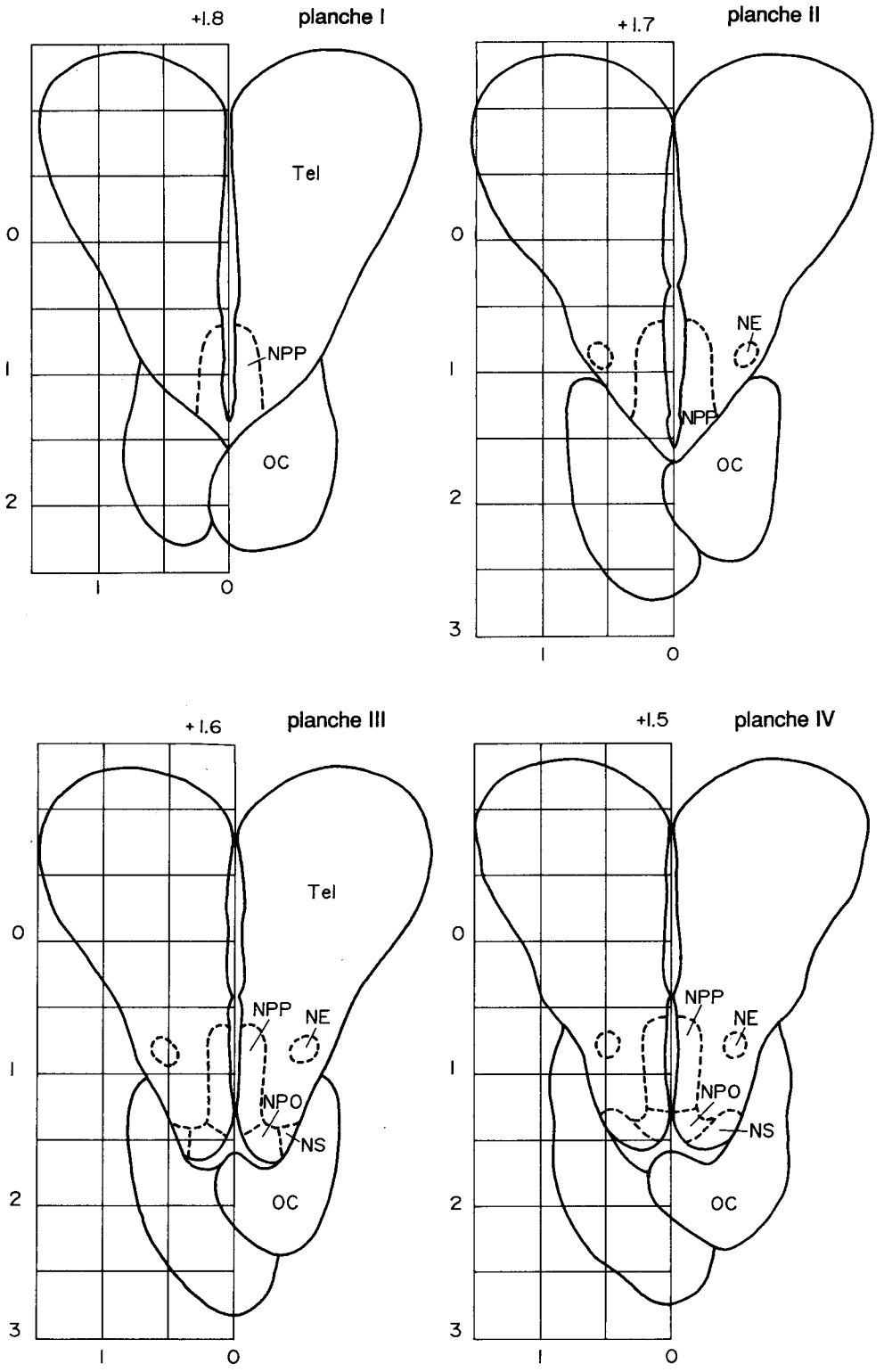
Preparation of the atlas

The atlas was prepared according to the procedures used for goldfish (Peter and Gill, 1975). Briefly, 5 male and 5 female Atlantic salmon parr weighing between 20 to 30 g were anesthetized and placed in the stereotaxic apparatus and surgery performed as described above. An insect pin was implanted in the floor of the cranium in the cerebellar region using the electrode holder. The whole head was fixed in Bouin's fixative for 4 d. The implanted electrode was used as a guide for the plane of cross-sectioning. All other histological procedures were carried out according to Peter and Gill (1975). A correction for shrinkage of 3% (Billard and Peter, 1982) was considered when tracing the grid placed on the left hand side of the drawings.

The atlas is shown in plates I-XXXI. There were no marked differences in the size and shape of the brain between individuals of different sex and size for the body weight range of 20–30 g. The brain that had been serially sectioned the most symmetrically was selected for photography and mapping of diencephalic nuclei. Sections 0.1 mm apart were photographed and drawn to scale. Each diencephalic nucleus was outlined on the photographs and drawings, and a grid with lines 0.5 mm apart placed on the left hand side of each drawing. The drawing labelled 0.0 (plate) shows the zero point in cross section (corresponding to the first section through PC). All atlas drawings anterior or posterior to 0.0 are indicated as + or –, respectively, with the distance given in mm from 0.0. The horizontal zero point from the anterior mid margin of PC was projected on all drawings and served as the reference for vertical positioning of an electrode or other apparatus during the stereotaxic procedure. It was preferable to use the anterior mid margin of PC as horizontal zero rather than the dorsal surface of the brain, as in goldfish (Peter and Gill, 1975) or killifish (Peter *et al*, 1975), because the highest surface was difficult to evaluate and was not stable in some areas (*eg* the optic tectum may collapse due to fluid loss following puncture).

Use of the atlas

After exposing the brain and taking note of the zero coordinates of the anterior mid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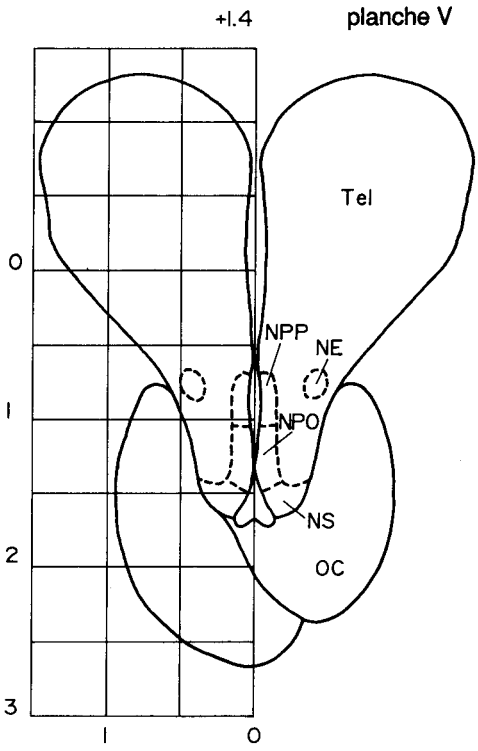
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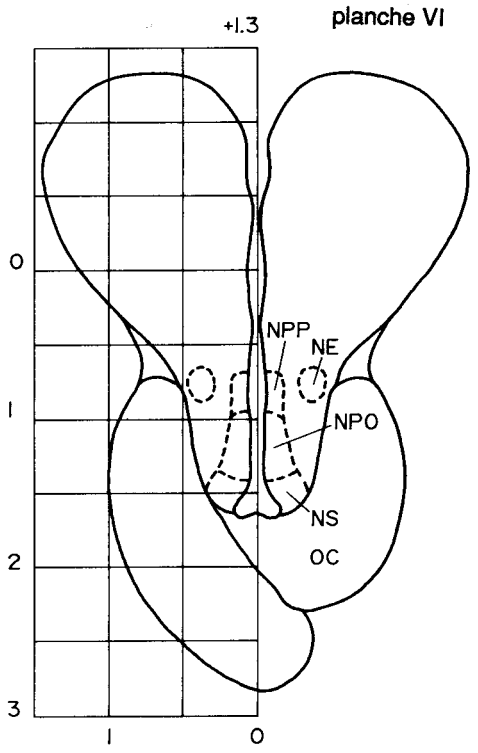
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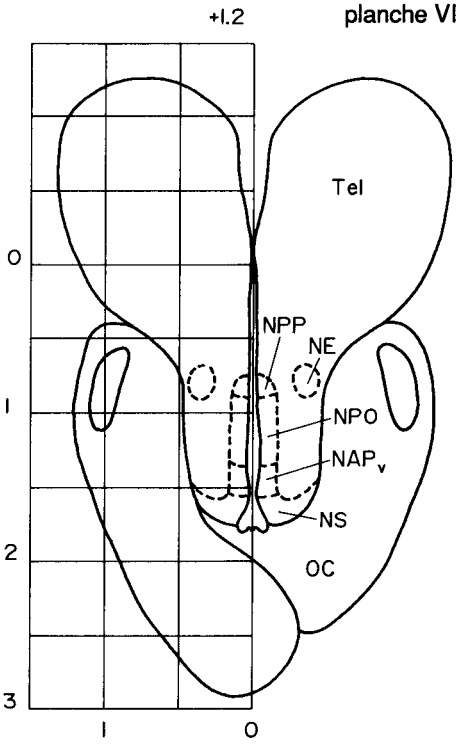
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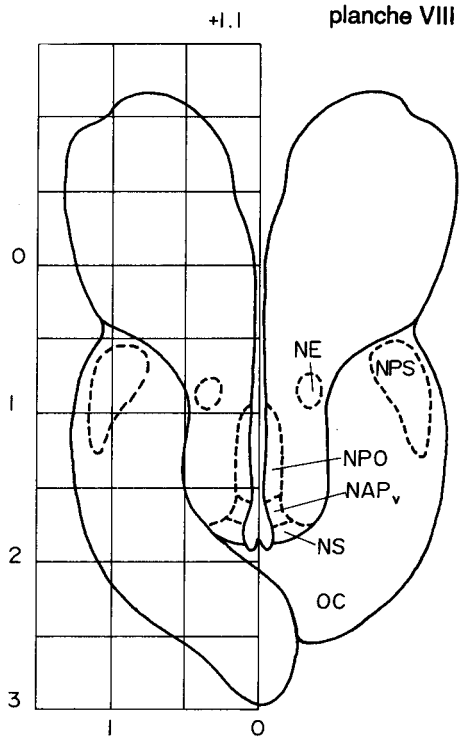
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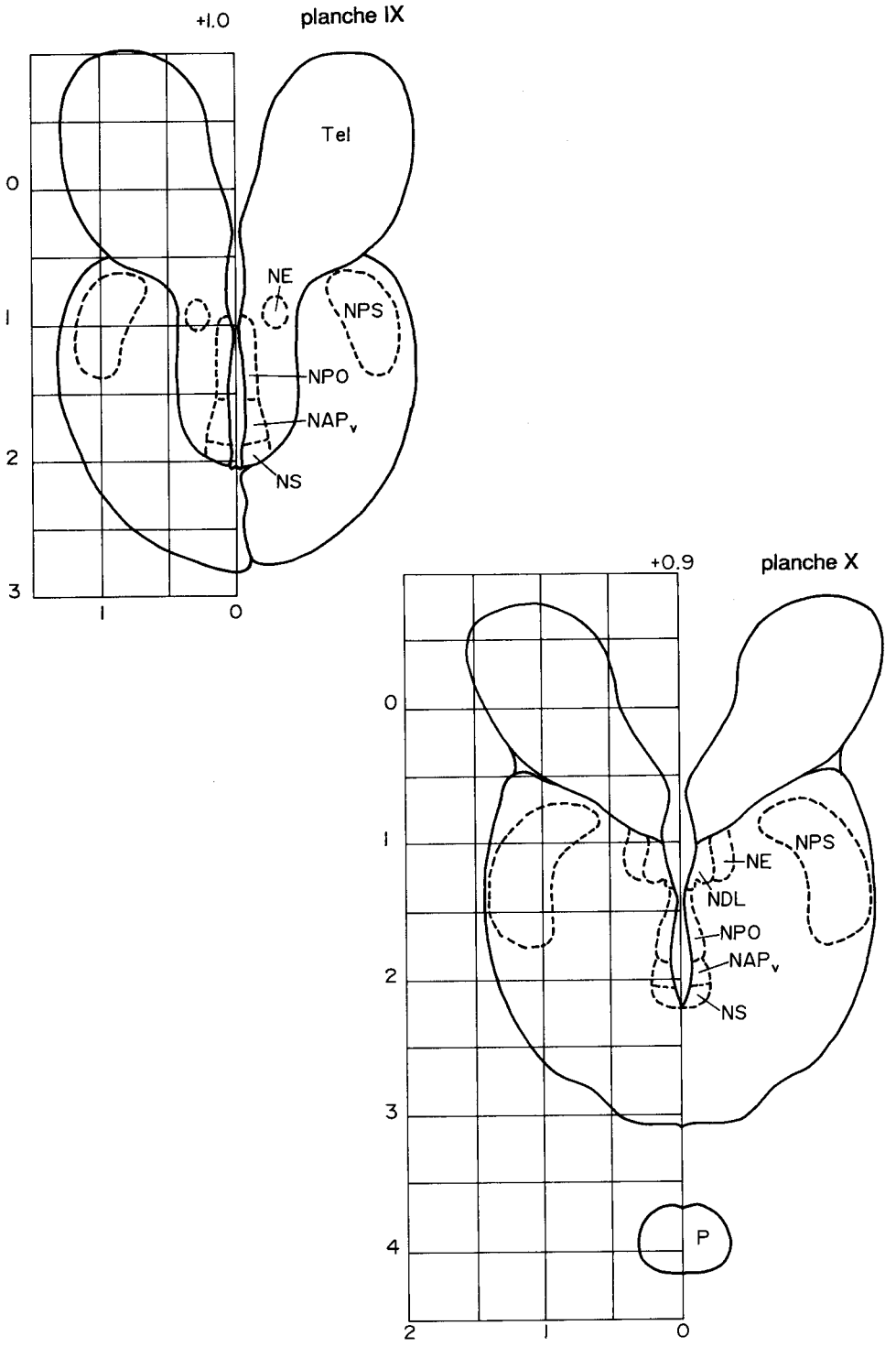
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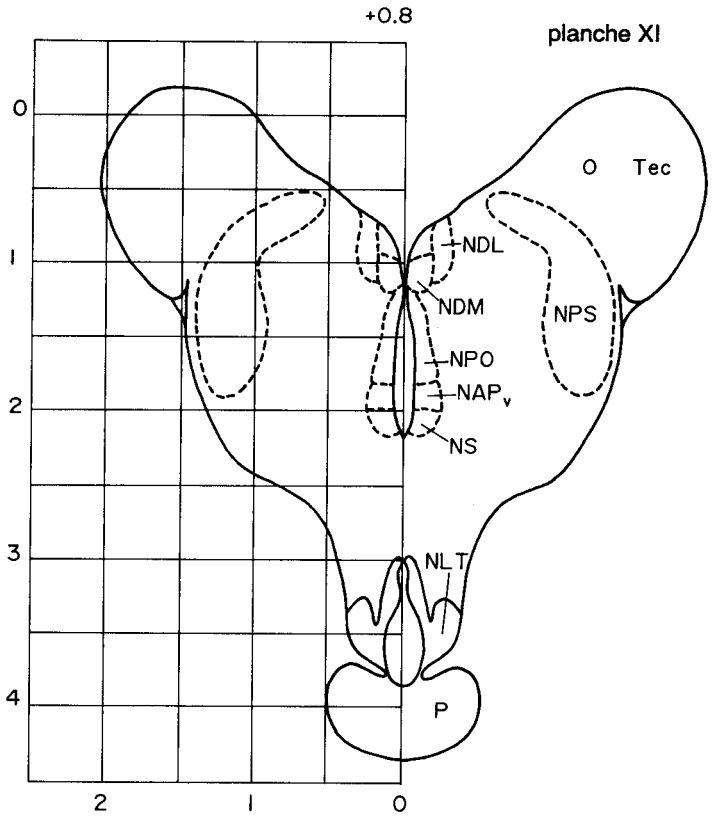
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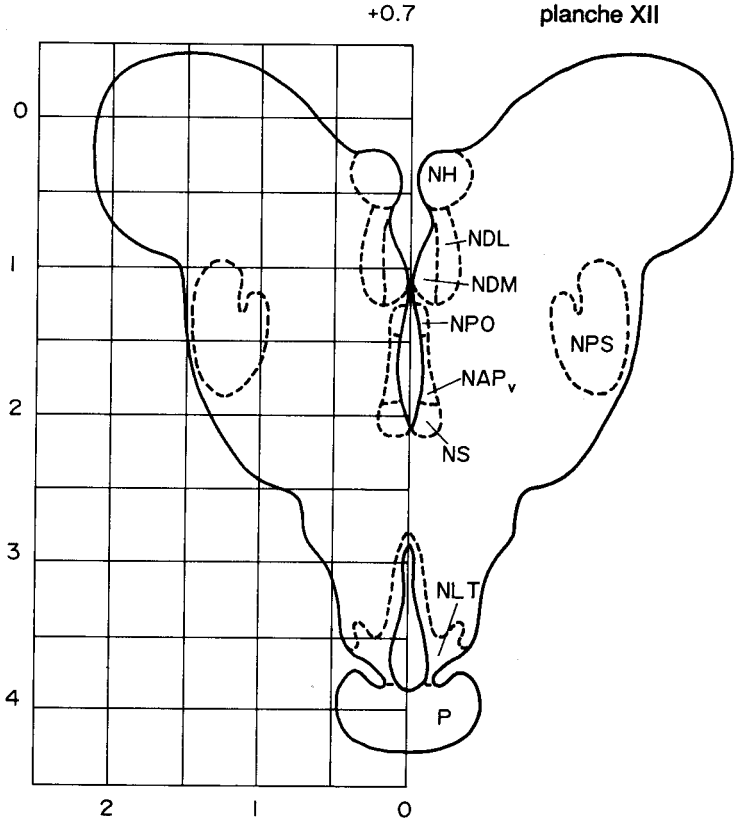
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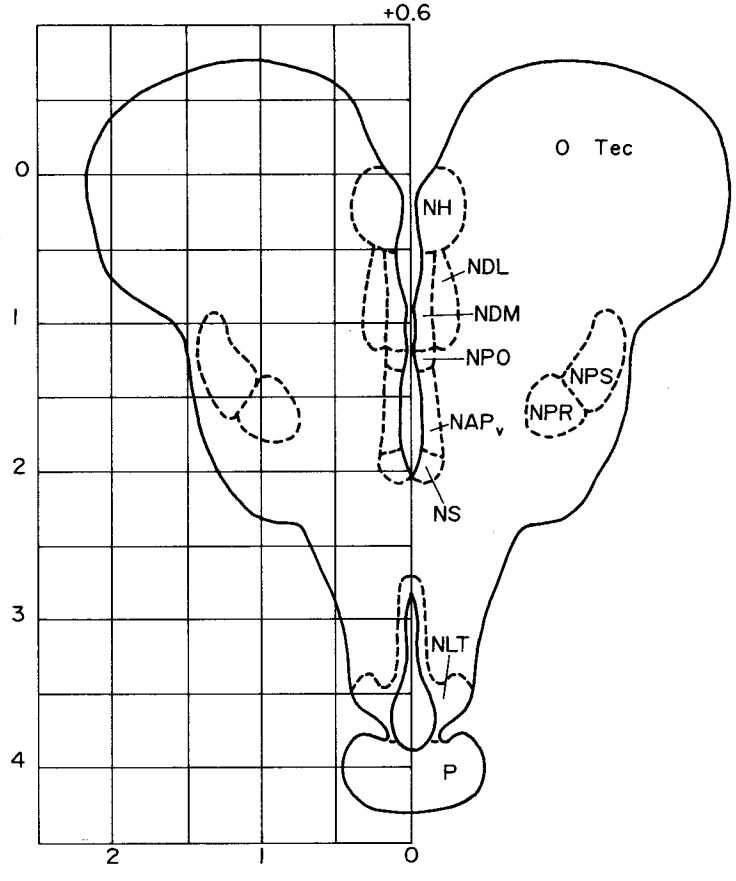

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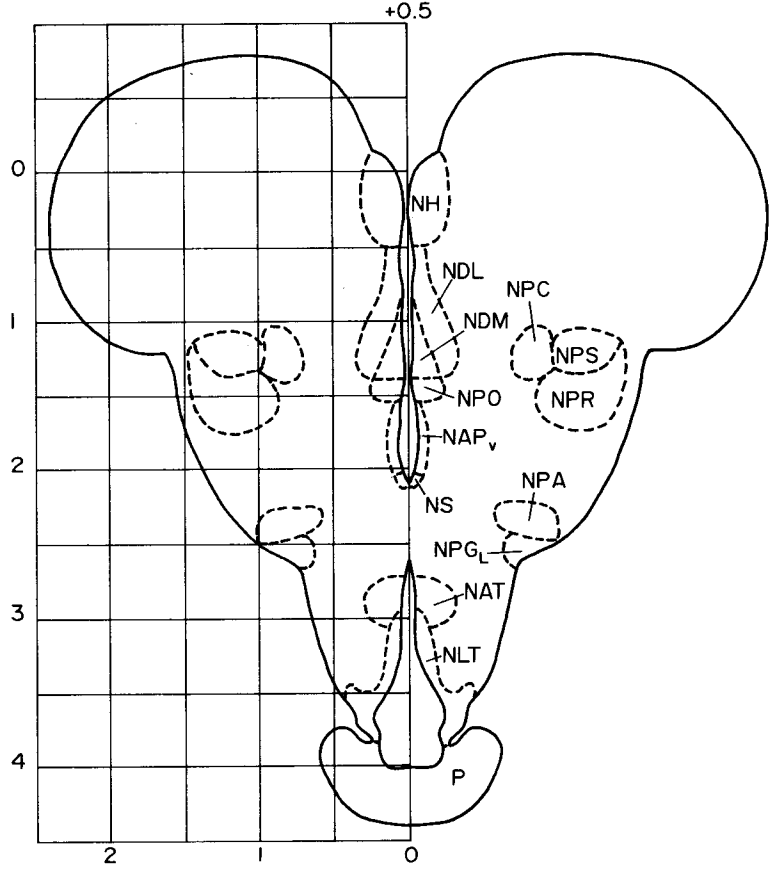

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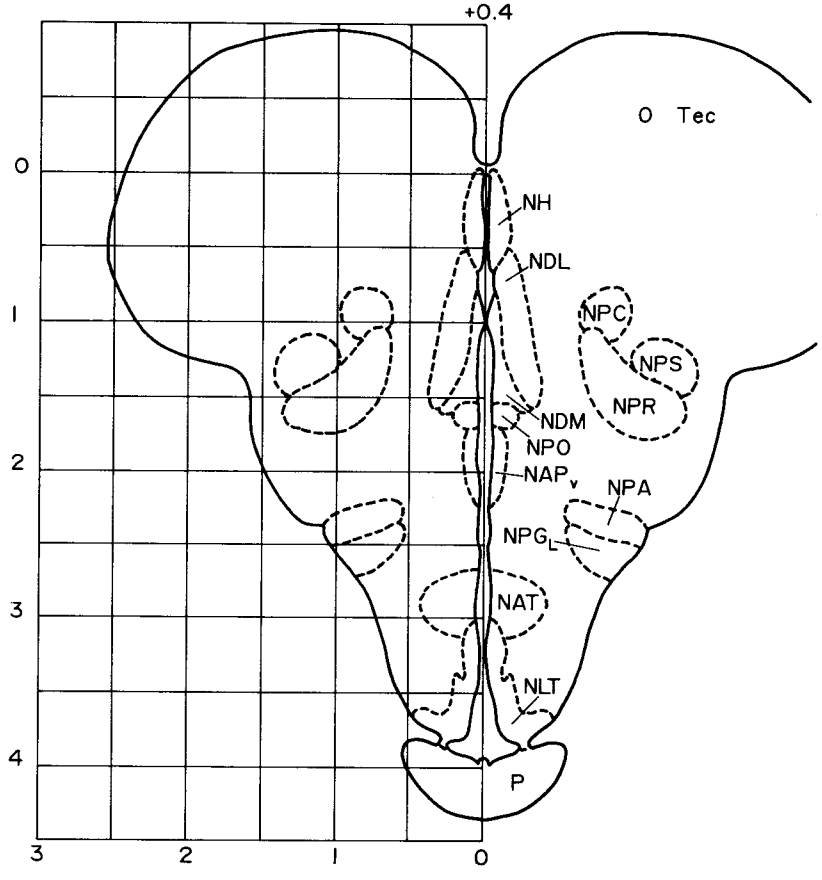

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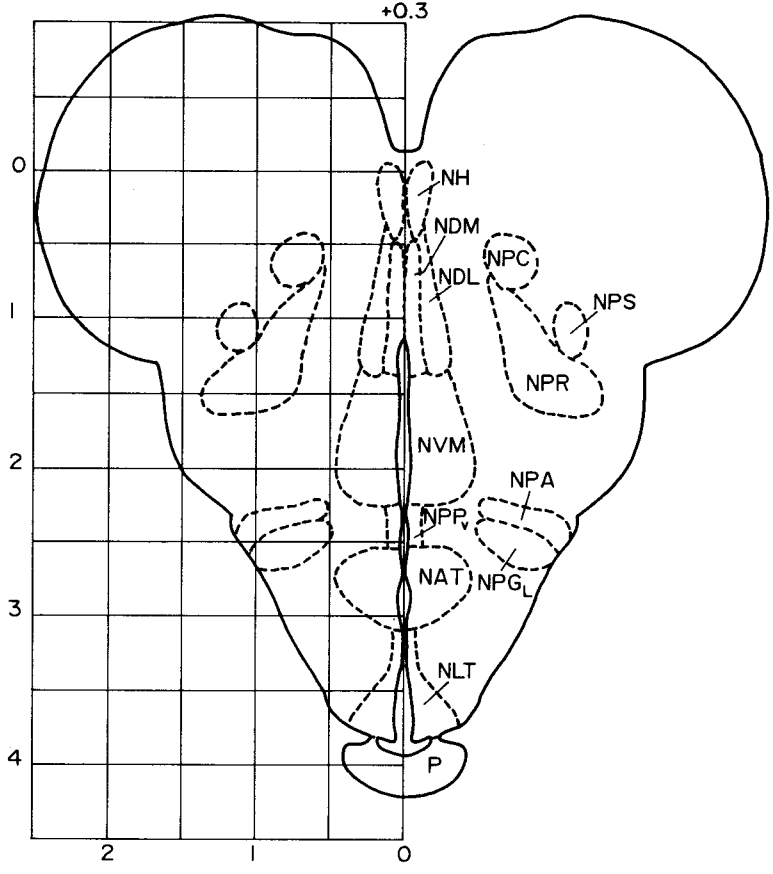

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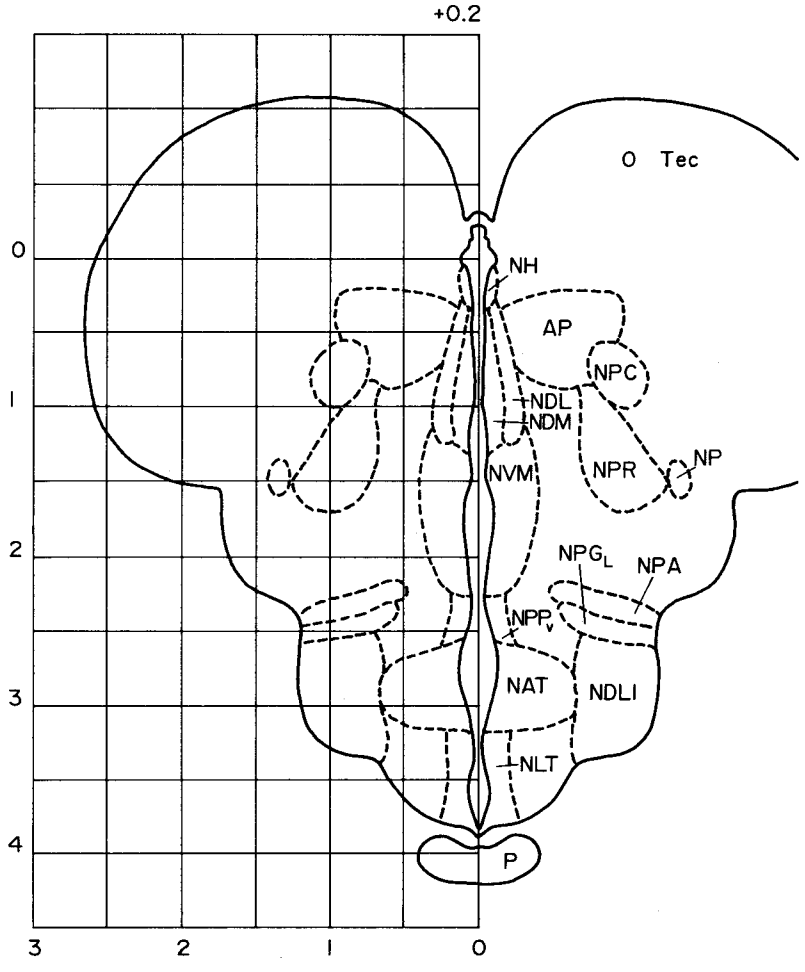

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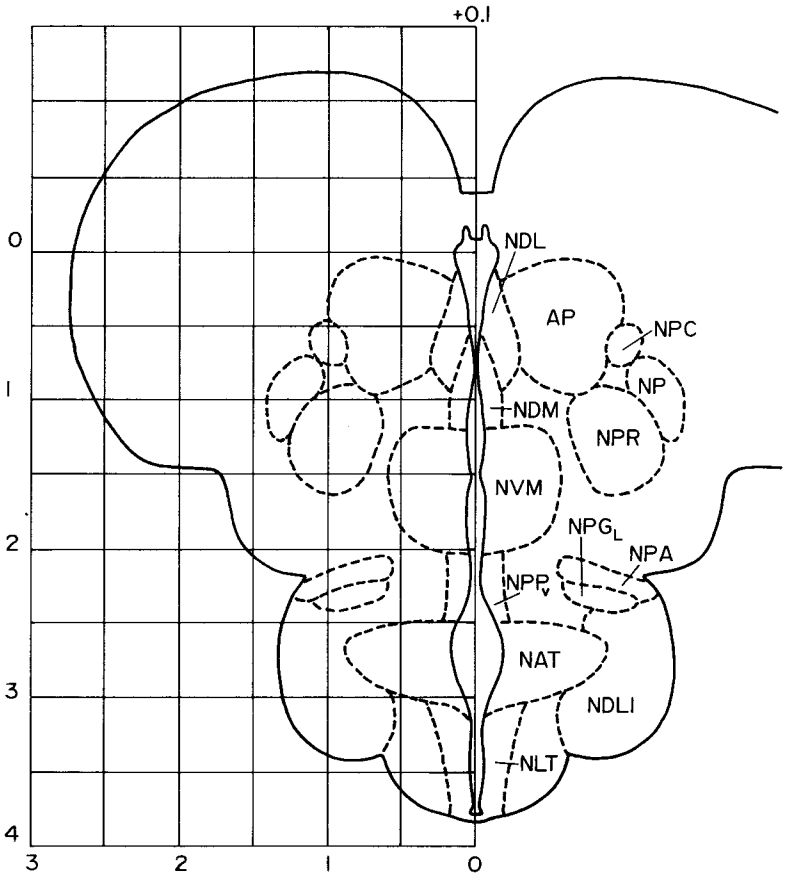

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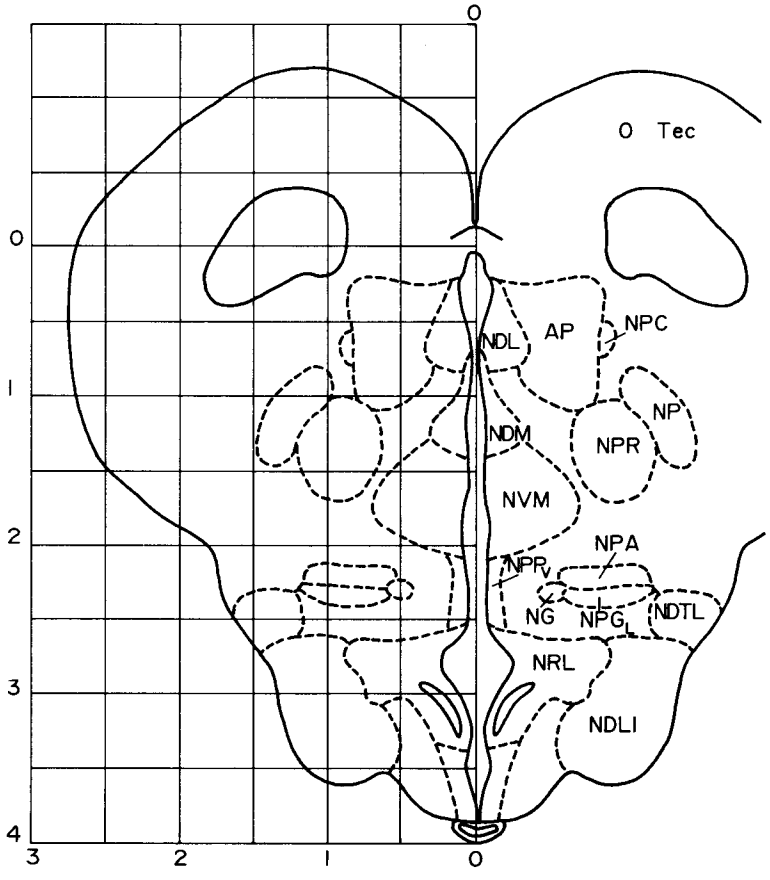

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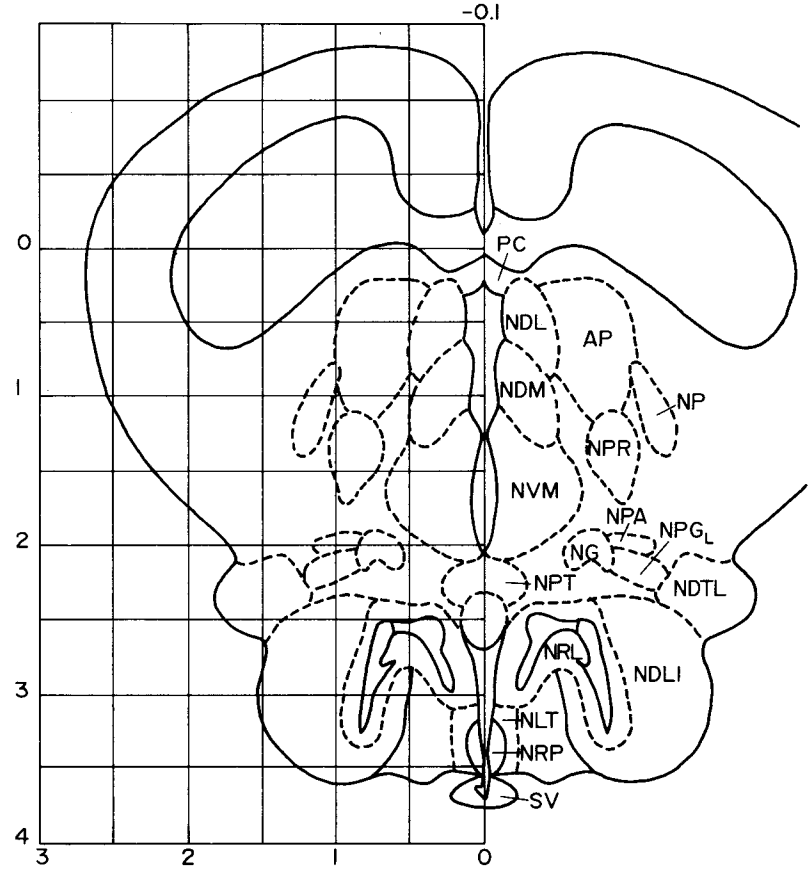

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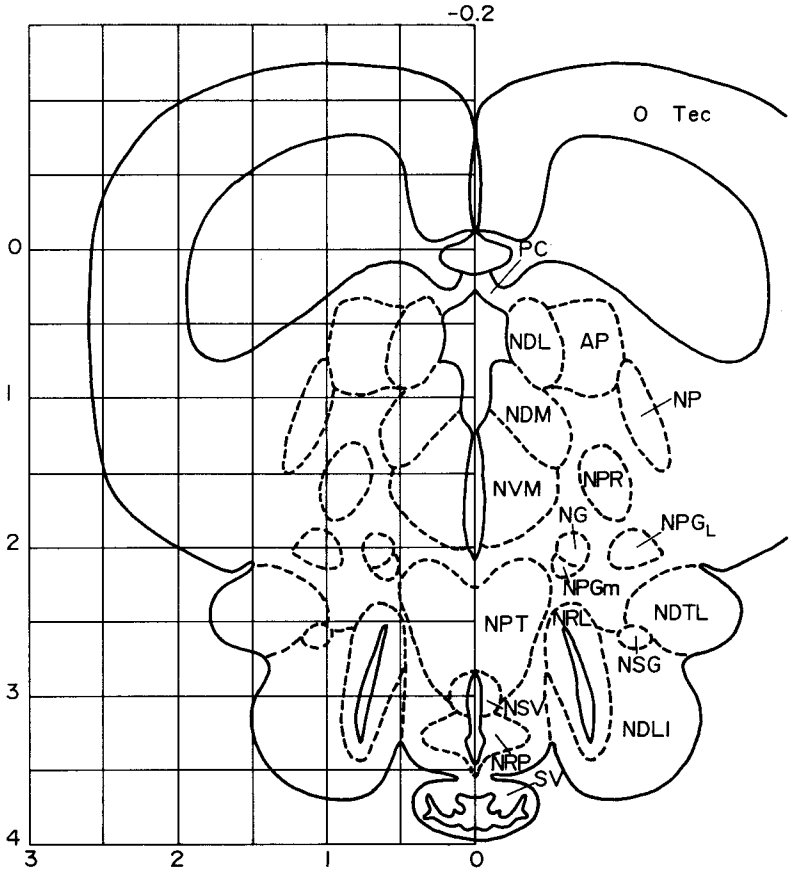

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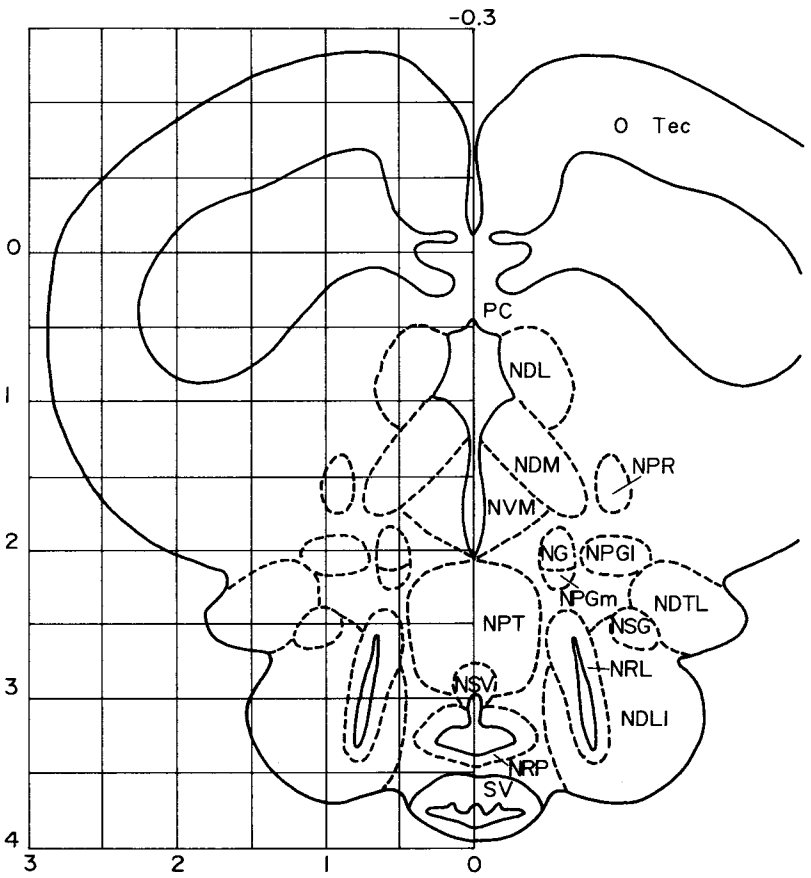

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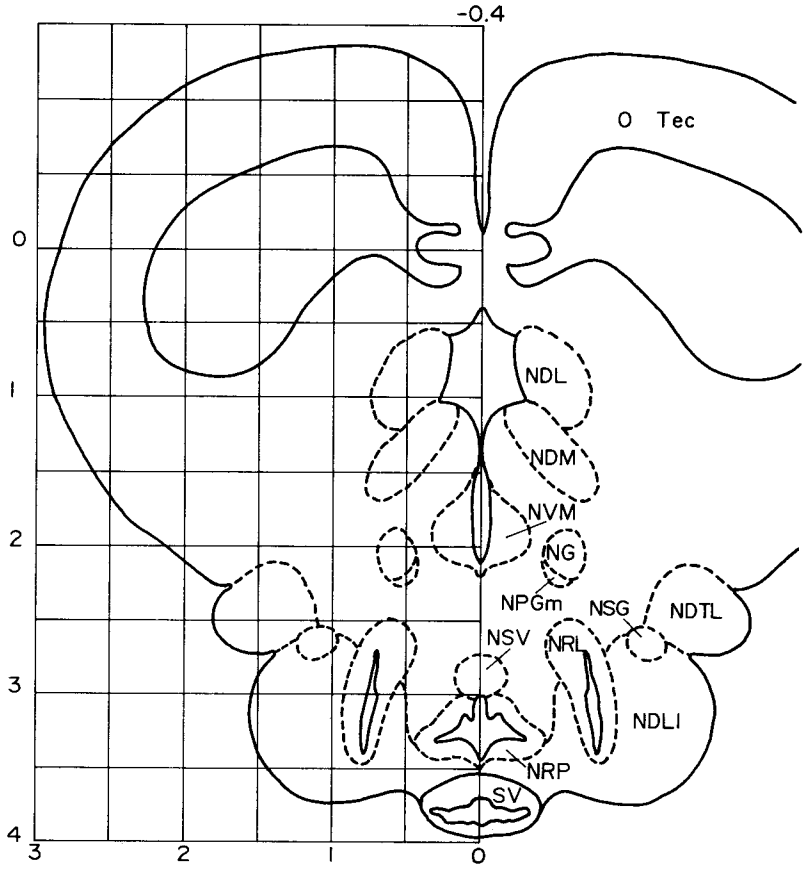

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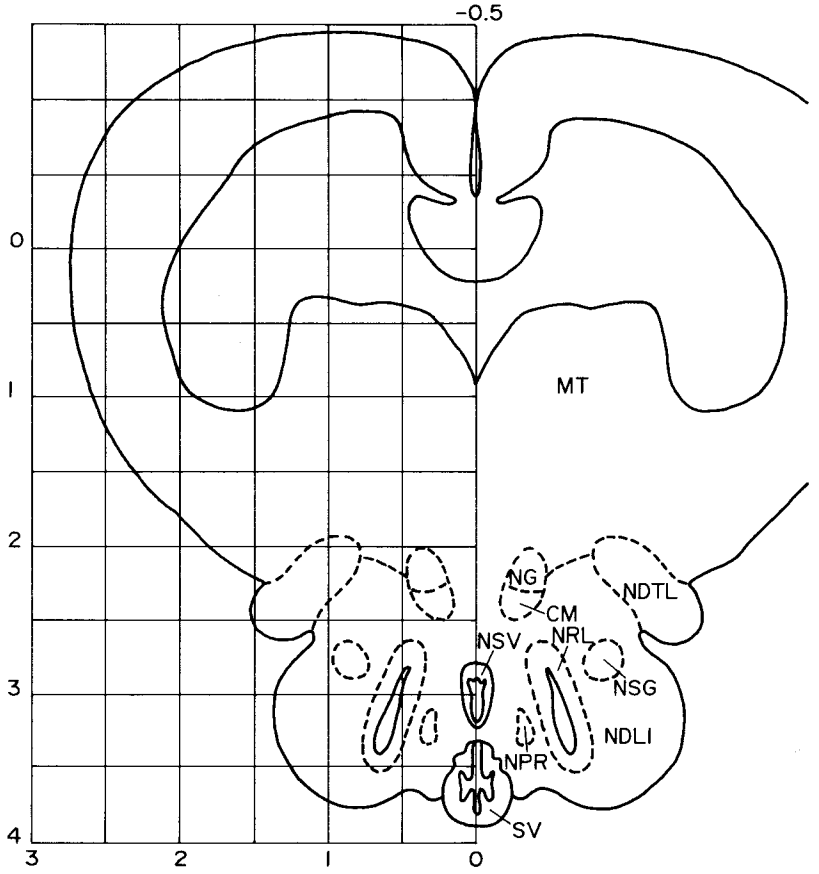

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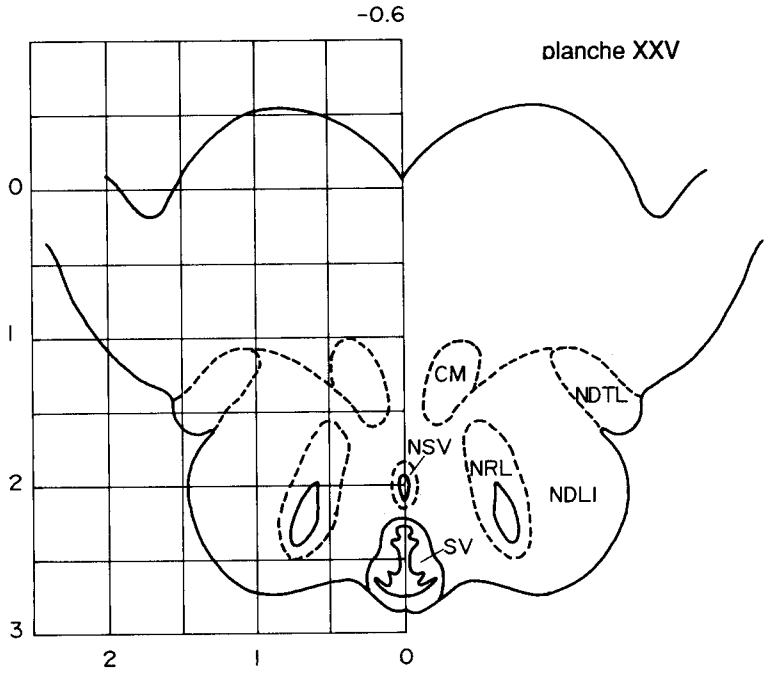
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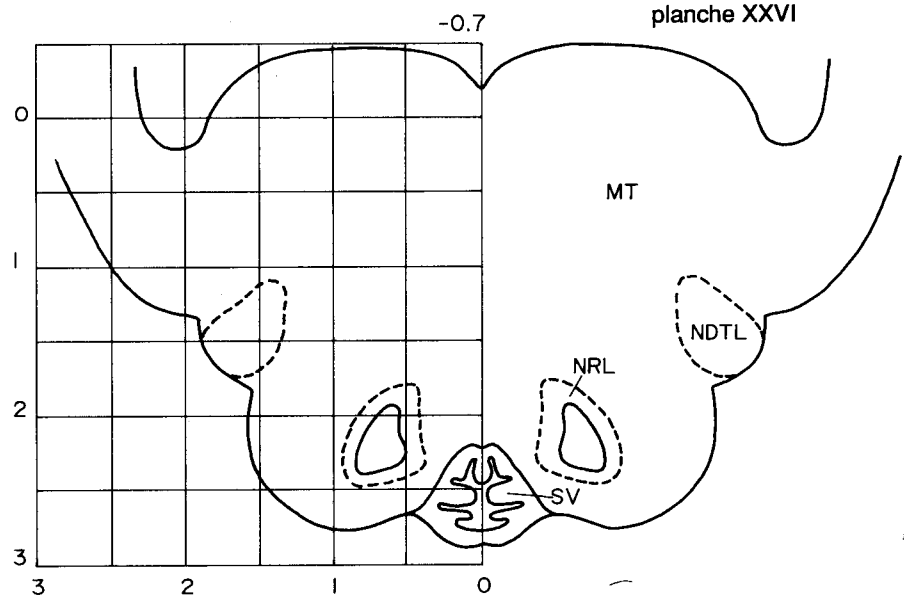
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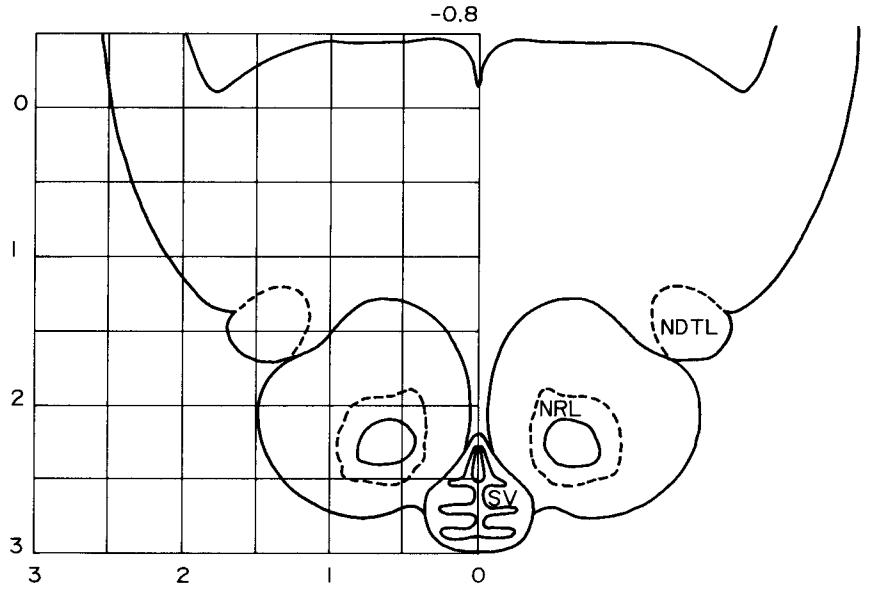

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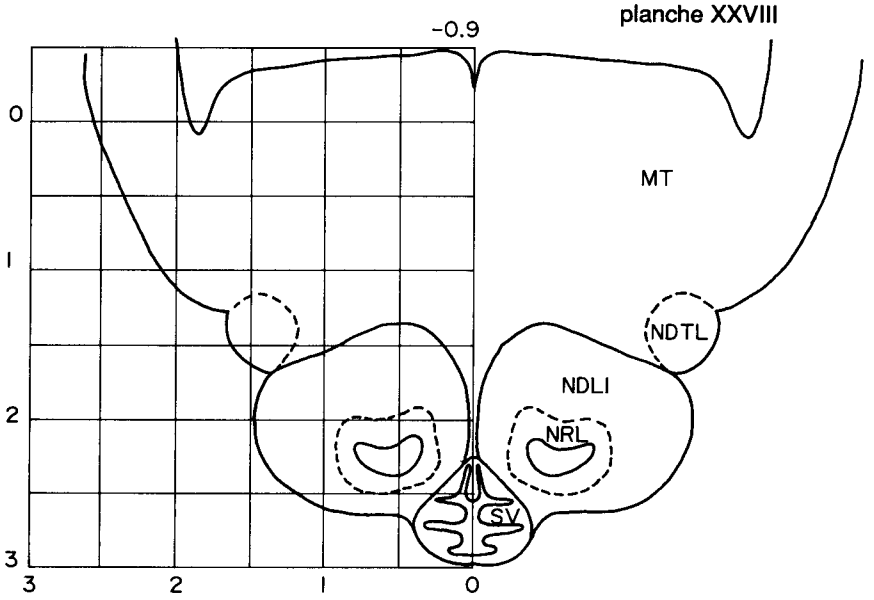

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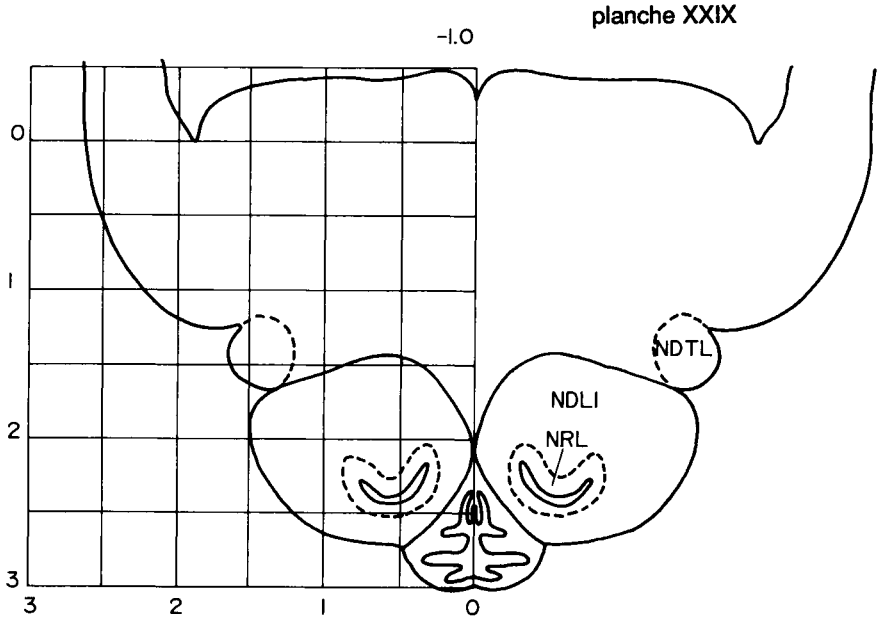

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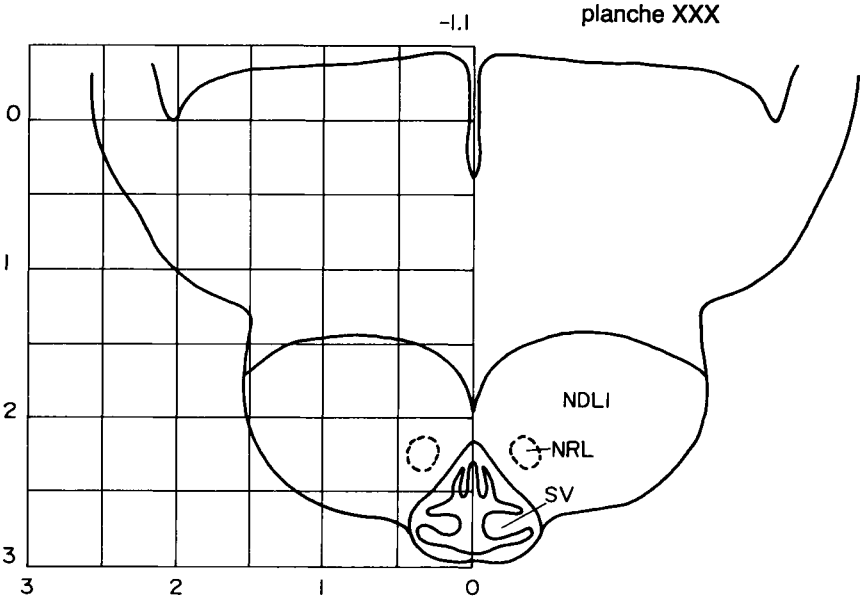

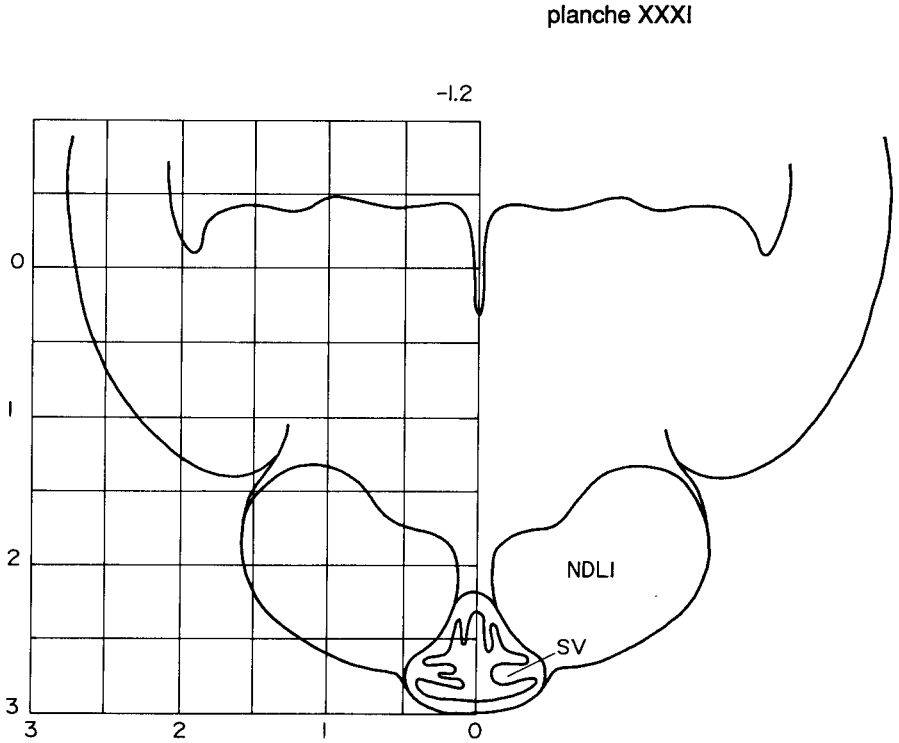

Plates I–XXXI. Atlas of the nuclei of the diencephalon of Atlantic salmon parr. The number at the top of each drawing gives the distances in mm anterior (+) or posterior (–) of the zero point (0.0). The lines of the grid were drawn to scale 0.5 mm apart. The numbers on the vertical (left) side of each grid give the distances down from the horizontal zero point, as measured at 0.0 at the anterior mid margin of the posterior commissure (PC). The numbers on the bottom side of each grid give the distances from the midline. For key to abbreviations, see table I.

margin of PC, thereby defining the anterior-posterior and horizontal zeros, the electrode was lifted clear of the brain and moved forward, backward, or laterally, as required for the desired target. The electrode was then returned to the horizontal zero position determined previously, the vertical distance to the target read on the drawing and the electrode moved accordingly. The coordinates for placement and the distances in mm were expressed as follows: 0.0, or + or – for distances anterior or posterior, respectively from 0.0; midline (M), or distance right (R) and/or left (L) from M; depth (D). All values are expressed to the nearest 0.1 mm.

Nomenclature of diencephalic nuclei

The nomenclature used for the diencephalic nuclei of the Atlantic salmon was the same as that previously used for goldfish (Peter and Gill, 1975), killifish (Peter *et al,* 1975) and rainbow trout (Billard and Peter, 1982), with some revisions according to Bradford and Northcutt (1983). A suprachiasmatic nucleus was recognized in the goldfish by Bradford and Northcut (1983) on the basis of innervation of the area by fibers from the optic tracts. Consistent with this observation, we have also designated a nucleus suprachiasmaticus, although we have made no attempt to trace optic fibers to this area. Bradford and Northcutt (1983) also renamed most other preoptic nuclei, frequently using size of cell bodies and supposed functional divisions as justification. Such a scheme can be problematic when comparing specimens of different age within a species, or when attempting to develop a nomenclature system that can be applied to several species. Functional subdivisions have already been recognized in the proposed *nucleus suprachiasmaticus* in addition to the input of optic fibers; to illustrate, cell bodies immunoreactive for salmon gonadotropin-releasing hormone have been localized in this region in goldfish (Kah *et al,* 1986) and rainbow trout (Schafer *et al,* 1989), and a dopaminergic group of cell bodies has also been reported in this area in goldfish (Kah *et al,* 1984). Given that there is a continuum of periventricular cell bodies in the preoptic region, and that multiple functional zones will likely be identified, we prefered to maintain the relatively simple nomenclature for preoptic nuclei originally described for goldfish (Peter and Gill, 1975), killifish (Peter *et al,* 1975) and rainbow trout (Billard and Peter, 1982).

Bradford and Northcutt (1983) recommended renaming large portions of the nucleus lateralis tuberis. We have not followed their proposed scheme, because the terminology of nucleus lateralis tuberis is very widely recognized. Also, a number of functional zones have already been defined for the nucleus lateralis tuberis, some of which overlap (Peter and Fryer, 1983), and it is not clear how any nomenclature scheme can recognize these subdivisions.

In accordance with the recommendations of Bradford and Northcutt (1983), nuclei identified in goldfish (Peter and Gill, 1975), killifish (Peter *et al,* 1975) and rainbow trout (Billard and Peter, 1982), have been renamed as follows: nucleus anterioris hypothalami to nucleus preglomerulosus anterior, nucleus preglomerulosus pars lateralis to nucleus preglomerulosus lateralis, nucleus preglomerulosus pars medialis to nucleus preglomerulosus medialis, nucleus pregomerulosus pars medialis commissuralis to nucleus preglomerulosus commissuralis, nucleus cerebellosus hypothalami to nucleus subglomerulosus, nucleus rotundus to nucleus pretectalis rotundus, nucleus corticalis to nucleus pretectalis centralis, and nucleus lateralis geniculatus to nucleus pretectalis superficialis. Renaming

of some other nuclei was also suggested by Bradford and Northcutt (1983) for a variety of reasons; however, we do not agree with these suggestions for reasons similar to those indicated above for preoptic and ventral hypothalamic nuclei.

DISCUSSION

The stereotaxic atlas and technique described here for Atlantic salmon parr has been used for placement of cocoa butter pellets containing sex steroids in the preoptic region and hypothalamus (Crim and Peter, 1978), and electrodes for lesioning of the diencephalon (Dodd *et al*, 1978; Crim *et al*, unpublished results). The average success for placements in this work was 80%, which is similar to the success of placements in the diencephalon of goldfish (Peter and Gill, 1975) and killifish (Peter *et al*, 1975). Although the size range of Atlantic salmon parr indicated for the sterotaxic technique was 20–30 g, we have applied the technique to parr ranging from 10–40 g with an overall success rate of about 60%. We hope that this stereotaxic atlas and technique will help to stimulate further research on this species.

ACKNOWLEDGMENTS

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