

Localization and source of γ aminobutyric acid immunoreactivity in the isthmic nucleus of the frog *Rana esculenta*

Edit Pollák,¹ Gyula Lázár,^{2*} Róbert Gábrriel¹ and Shou-Rong Wang³

¹Department of General Zoology and Neurobiology, Janus Pannonius University, Ifjúság, Hungary; ²Department of Human Anatomy, University Medical School, Szigeti, Hungary; and ³Laboratory for Visual Information Processing, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

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ABSTRACT: The distribution of γ -aminobutyric acid (GABA)-containing neurons and nerve fibers was studied in the isthmic nucleus of the frog *Rana esculenta* using light and electron microscopical immunohistochemical techniques. Approximately 0.5% of isthmic cells showed GABA immunopositivity, and the majority of these cells was found in the anterior one-third of the nucleus. A meshwork of GABA-immunostained fine beaded axons filled the entire isthmic nucleus. The GABA-immunoreactive terminals formed pericellular basket-like structures around a few cells both in the medulla and the cortex of the isthmic nucleus. To determine the source of GABA-positive fibers in the isthmic nucleus lesion experiments were carried out. After unilateral tectal ablation no change was observed in GABA immunoreactivity. Hemisectioning the tegmentum close to the anterior border of the isthmic nucleus, transection of the caudal tectal commissure and decussatio veli, or electrical lesioning of the anterodorsal tegmental nucleus all resulted in a moderate decrease in the density of GABA-positive fibers. Our results suggest that the majority of GABA-positive fibers derives from local GABA-positive cells, but some GABAergic afferents seem to arise in the tegmentum. © 1999 Elsevier Science Inc.

KEY WORDS: Inhibitory interneurons, Tegmental lesions, Synapses, Electron microscopy.

INTRODUCTION

The isthmic nucleus (IN) is “a prominent part of the frog’s visual system” [30] in the caudal midbrain. It consists of a central group of cells embedded in neuropil called the medulla. This portion is surrounded by the cortex, which contains two-thirds of the isthmic cells. The IN receives afferents from the ipsilateral optic tectum and projects to both tecta [7–9]. It is now generally accepted that the IN is the relay that mediates the indirect ipsilateral retinotectal projections, first described in the frog by Gaze and Jacobson [6]. Though non-tectal connections of the IN were mentioned in early descriptions [5,17], their existence has only been proven recently: a bilateral tegmento-isthmic pathway was found to originate from the anterodorsal tegmental nucleus [30,34]. Furthermore, the co-

chlear nucleus, the superior olive and three subnuclei of the torus semicircularis are reciprocally connected with the IN [15,16,23]. Electrical stimulation applied to the ipsilateral optic tract elicited excitatory postsynaptic potentials (EPSPs) in 75% of the recorded cells in the ipsilateral IN, whereas in the contralateral IN 92% of the cells received inhibitory input. Comparing the latencies of EPSPs and inhibitory postsynaptic potentials (IPSPs) the inhibitory effects were proposed to be indirect, while the excitatory input was found to derive directly from the optic tectum [33]. These experiments indicate that the IN receives multiple afferents, a notion which is further supported by the presence of several neuropeptides in the IN [12,18,19]. Earlier investigations identified some γ -aminobutyric acid (GABA)-immunoreactive neurons and many terminals in the IN [20]. In another study it was found that the number of cells labelled by retrograde filling in the contralaterally projecting area of the IN was less than the total number of neurons here [29]. Taking into account the discrepancy in the number of total versus labelled neurons, we suppose the existence of a local circuit neuron system, and the GABA-immunoreactive neurons in the IN may therefore be interneurons. The GABA-immunoreactive fiber network may belong to these cells, but may also originate from other brain areas. One candidate is the optic tectum, since one-third of the neurons here show GABA immunoreactivity [2]. This study is an attempt to characterize the light microscopic and ultrastructural features of the GABA-positive elements in the IN and to ascertain the possible extrinsic sources of GABAergic innervation to the IN.

MATERIALS AND METHODS

Animals

Sixty adult frogs (*Rana esculenta*) of both sexes were purchased from a fish breeding farm in Szarvas (Hungary) and were kept under standard laboratory conditions. Different kinds of surgery, as will be described later below, were performed on 50 animals. Combined lesions and long postoperative times caused the loss of 26 frogs. In three animals, the lesions were in the wrong

* Address for correspondence: Dr. Gyula Lázár, Department of Human Anatomy, H-7643 Pécs, Szigeti út 12, Hungary. Fax: +36-72-326244; E-mail: lazargy@apacs.pote.hu

TABLE 1
TYPES OF LESIONS INTERRUPTING POSSIBLE ISTHMAL AFFERENT TRACTS

Operation	Disrupted Tract	Number of Animals	Tectum Opticum		Tegmental Nuclei		Principal Nucleus of TS		Cochlear Nucleus	
			C	I	C	I	C	I	C	I
Tectal ablation	Tecto-isthmal	6	+	+	-	-	-	-	-	-
Transection rostral to the IN	Tegmento-isthmal connections	3	-	-	+	+	+	+	-	-
Electrolytic lesion rostral to the IN	Tegmento-isthmal connections	3	-	-	+	+	+	+	-	-
Midline transection in the caudal tectal region	Commissura tecti, decussatio veli	6	-	-	+	-	+	-	+	-
Transection behind the IN	Ascending lateral	3	-	-	-	-	-	-	-	+
Lesion in unwanted place		3								
Operated animals died		26								

TS, torus semicircularis; C, contralateral; I, ipsilateral; IN, isthmic nucleus; +, refers to the destruction of fibers deriving from the mentioned sources; -, no selective fibre decrease.

place, therefore these brains were not used. The present description is based on 21 successfully operated frogs (Table 1). Four intact animals were used for mapping GABA-positive neurons in the IN; these animals served also as normal unoperated controls. The IN of 6 other animals were used for electron microscopic investigations. *Principles of the Laboratory Animal Care* (NIH publication No. 86-23, revised 1985) were followed. All the antisera and the chemicals for the immunohistochemical procedure were purchased from Sigma (St. Louis, MO, USA).

Mapping of GABA-Immunoreactive Cells in the IN

Before starting this series of experiments we compared the effect of immersion and perfusion fixation on GABA immunostaining and the preservation of ultrastructure. We did not find any advantage of perfusion over immersion fixation if the small piece of brain we investigated had been dissected immediately and immersed in a cold (4°C) fixative. Therefore, we used this technique in this study. Four intact animals were deeply anaesthetized in tricaine methane sulphate and were then decapitated. The brains were removed, the caudal half of the mesencephalon was dissected, immersed into a fixative containing 2% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), and fixed overnight at 4°C. The specimens then were dehydrated and embedded in Durcupan (Fluka). Serial 8 µm-thick sections were cut from the epoxy-resin embedded blocks, dried on hot plate onto gelatin-coated slides, and processed for GABA immunoreactivity. The resin was etched in ethanolic sodium-hydroxyde, the samples were rehydrated, and then the three-step avidin-biotin-horseradish peroxidase method was employed. Following preincubation in a solution containing 0.1% bovine serum albumin and 0.01% Triton-X in 0.1 M phosphate-buffered saline (PBS, pH 7.4) for 30 min, the slides were covered with rabbit anti-GABA primary

serum (1:1000) and kept in a wet chamber overnight. After thorough washing in PBS six times for 10 mins, biotinylated goat anti-rabbit IgG (1:50) was layered on the slides for 1 h, followed by incubation with extrAvidin peroxidase (1:50) for 1 h. To detect immunoreactive structures, a solution containing 0.05% diaminobenzidine (DAB) and 0.01% H₂O₂ in 0.1 M Tris-HCl buffer (pH 7.6) was layered on the slides for 10 min and precipitation of DAB was controlled under a light microscope. Slides were dehydrated and coverslipped in Canada balsam.

Characterization of GABA-Positive Structures in the IN

Brains dissected from three of the six intact frogs designated for electron microscopic investigations and 21 operated animals were fixed in the mixture of 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer at 4°C overnight. After thorough rinsing in PBS, 30–40 µm-thick vibratome sections were cut in the transverse, horizontal, and sagittal planes.

To show GABA-immunoreactive structures on the free-floating vibratome sections the three-step avidin-biotin-horseradish peroxidase complex immunohistochemical technique was used. The sections were kept in a solution containing 0.1% bovine serum albumin and 0.01% Triton-X in PBS for 30 min, and were then incubated overnight in rabbit anti-GABA primary serum diluted 1:2500 in the same solution used for preincubation. After incubating the sections with biotinylated anti-rabbit IgG (1:85) for 10 h, extrAvidin peroxidase in a dilution 1:50 in PBS was applied for 10 h. Between each step sections were washed six times in PBS. Immunoreactive structures were visualized in a solution containing 0.05% DAB and 0.01% H₂O₂ in 0.1 M Tris-HCl buffer (pH 7.6). The sections were mounted on gelatin-coated slides, dehydrated, and coverslipped.

Before coverslip, the samples deriving from the three intact

animals were selected: the IN was dissected from the best sections, postfixed in 1% OsO₄ in 0.1 M phosphate buffer and embedded in Durcupan for electron microscopical investigations of structures marked by DAB reaction.

Characterization of GABA-Immunoreactive Synaptic Profiles

Samples deriving from the other three of the six intact animals designated for electron microscopic investigations were fixed in the mixture of 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer overnight at 4°C. Then the brains were cut on vibratome, the IN was dissected from the sections, osmicated in 1% OsO₄ at room temperature for 1 h in the dark and embedded into Durcupan. Ultrathin sections of the IN were cut on Reichert ultramicrotome, collected on nickel grids, and an immunogold reaction was performed according to the method of Somogyi and Hodgson [28]. Sections on the grids were treated with 1% periodic acid, and then placed face down on a drop of 1% sodium periodate to remove osmium. After several washes in distilled water and TBS (Tris-buffered saline 0.1 M, pH 7.4) and preincubation with normal goat serum for 20 min, grids were put on rabbit anti-GABA serum drops (1:750) overnight in wet chamber. After several washes in TBS the grids were transferred to a solution of goat anti-rabbit IgG (diluted 1:20) in TBS conjugated with 10 nm colloidal gold. Sections were washed in distilled water and counterstained with uranyl acetate and lead citrate or potassium permanganate. Controls were also performed omitting the primary antibody or replacing it with non-immune sera. No staining was detected in these samples.

Surgical Procedures

The possible extrinsic sources of GABA-immunoreactive fibers were studied in lesion experiments as shown in Table 1. Frogs were anesthetized with 10% urethane (2 g/100 g b. wt.) intraperitoneally.

In one group of animals, we studied the effect of elimination of tectal afferents to the isthmic nucleus. The most effective method for this is the removal of the optic tectum. For this operation the midbrain was exposed from above by drilling a hole in the skull. After removing the meninges the left tectum was sucked out with a vacuum pump. Postoperative survival time was 5 weeks for three animals and 12 weeks for another three animals. Since the tecto-isthmic projection is strictly unilateral, the contralateral isthmic nuclei of tectum ablated animals served as control. Using the same approach, the decussatio veli between the two IN and the most posterior part of the tectal commissure was transected using a fine pair of scissors (survival times: 3 weeks for three animals and 10 weeks for three animals).

To interrupt non-tectal connections to the IN we hemisected the ventral mesencephalon just rostral or caudal to the nucleus (survival time: 7 weeks). This operation may interrupt all connections that reach the IN from rostrally or caudally located sources. For more restricted direct lesioning of tegmental nuclei, supposedly projecting to the IN, they were electrolytically destroyed using a glass-insulated silver wire electrode. The skull was opened from above and the electrode was introduced into the tegmentum through the optic tectum with the aid of a stereotaxic apparatus, and 0.2 mA cathodal DC current was passed for 2 min. The parameters for the lesions were as follows: 100 μm from the rostral edge of the tectal commissure caudally, 900 μm from the midline, depth 1000–1100 μm from the surface of the tectum (survival time: 7 weeks). The place and extent of the lesions were verified during the histological processing.

Data Analysis and Documentation

The GABA-positive neurons were mapped in camera lucida drawings of 8 μm serial sections of the brains of intact animals. The number of GABA-labelled cells was compared to the total number of isthmic cells (9400) of *Rana esculenta* obtained in an earlier investigation [29]. The Abercrombie's formula was used for mathematical correction of counting [1].

Microphotographs were taken with a Nikon FXA microscope. Ultrathin sections were viewed and photographed by a Jeol 1200 EX electron microscope.

RESULTS

Light Microscopic Observations: GABA-Positive Neurons and Fibers in the Isthmic Nucleus

The density of GABA-positive neurons varies along the rostrocaudal axis of the IN. The total number of the GABA-positive cells was 48 ± 3 ($n = 4$). The majority of stained neurons was located in the rostral portion of the IN. Sections cut in three different planes showed grouping of cells in the anterior non-rim cortex and the neighboring rim cortical area (Figs. 1, 2a). Immunoreactive cells were rarely found in transverse sections cut through the middle third of the nucleus. The caudal region was almost free of stained cells and only a small group of less than 10 GABA-positive neurons occurred in the ventrolateral and ventral cortical region in the caudal third. GABA-positive cortical neurons were located mainly at the outer edge of the cortex, but occasionally some medullary cells also showed GABA reactivity (Fig. 2b). Usually only perikarya were stained, although in some cases the primary dendrites were also visible. Figure 2c shows a cell and its main dendrite as it comes through the cortex and forms a wide-spread arborisation in the rim neuropil along the line of cortical neurons.

GABA-positive fine beaded axons could be seen throughout the IN. They were evenly distributed in the neuropil and among the medullary cells (Fig. 3b). Several fibers invaded cortical areas and GABA-positive beads could be seen among the tightly packed cortical cells (Fig. 2d). In the medulla varicose thin axons showed basket-like arrangements around perikarya (Fig. 2e). Many axon segments gave origin to several short side-branches. GABA-positive axons close to GABA-containing perikarya were rarely seen (Fig. 2f). The density of GABA-immunoreactive fibers did not show any obvious regional differences within the IN. The only exception was an immunoreactive plexus in the dorsomedial part of the nucleus, slightly denser than in other parts of the IN.

Lesion Experiments

Tectal ablation was never complete. A small part anterolaterally or posteromedially remained intact, but more than 80% of the tectum was removed. Ablation of the optic tectum resulted in massive degeneration of tectal afferents accompanied by a shrinkage of the IN within 5 weeks after the operation. Cortical cells were more closely packed and the density of medullary cells increased (Fig. 3 a,b). GABA-immunoreactive neurons were present both in the cortex and the medulla. The density of immunoreactive beads and fibers was slightly increased (Fig. 3c), when compared to the IN on the intact side (Fig. 3d).

Hemisecion close to the rostral border of the IN reached the bottom of the optic ventricle, thus the fibers running to the hilus of the IN were interrupted. This lesion caused shrinkage of the rim neuropil, therefore, the originally loosely arranged medullary neurons got closer to each other. The density of immunoreactive beads decreased in the rim neuropil (Fig. 3e), when compared to an intact IN (Fig. 3d).

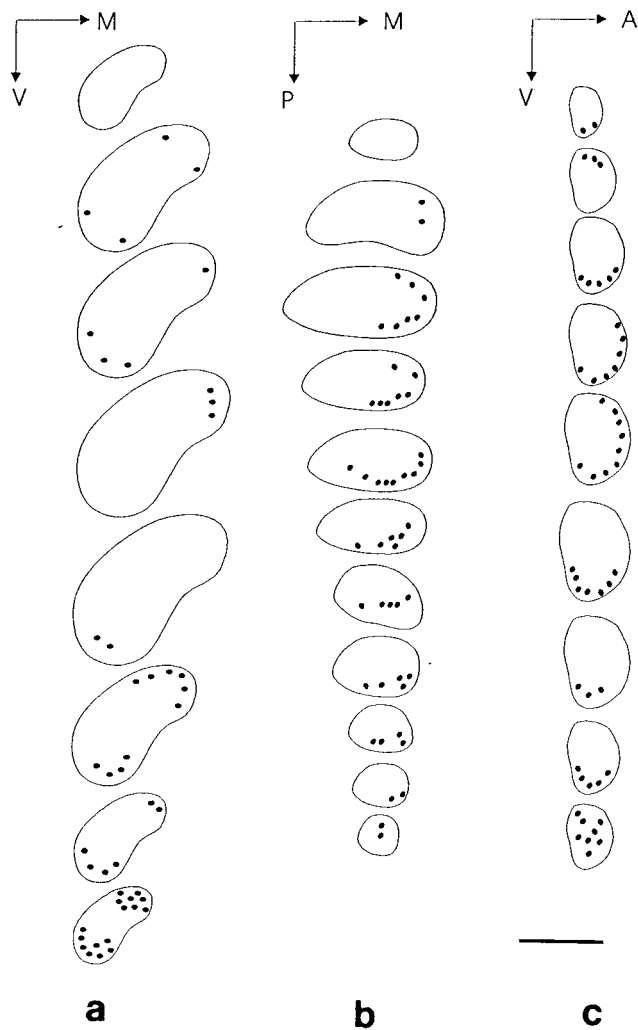


FIG. 1. Distribution of the γ -aminobutyric acid-positive neurons in the isthmic nucleus. Camera lucida drawings of serial 8 μm -thick sections. (a) Transverse, (b) horizontal, (c) sagittal plane. In each plane of the drawing, cells of two neighboring sections were plotted. Each dot is equivalent to one neuron. The first section on top of the series is the most posterior in (a), the most dorsal in (b), and the most medial in (c). Abbreviations: A, anterior; M, medial; P, posterior; V, ventral. Scale bar: 250 μm .

Electrolytic lesions destroyed the anterodorsal tegmental nucleus (AD), the larger part of the nucleus profundus mesencephali (NPM) and the dorsalmost part of the anteroventral tegmental nucleus (AV) (Fig. 4). These lesions also brought about a decrease in the density of GABA-immunoreactive beads in the rim neuropil and this was similar to that found after hemisectioning the tegmentum rostral to the IN.

Transection of the decussatio veli separated the IN of the two sides and unavoidably disrupted the caudal tectal commissure. By the third postoperative week the density of GABA-positive terminals decreased in the medial neuropil of the rostral IN. By the 10th postoperative week the density of labelled fibers was further reduced not only in the medial, but also in the ventrolateral neuropil of the rostral and caudal portions of the IN. The degree of reduction in the density of GABA-positive fibers was somewhat smaller than in the cases of tegmental lesions.

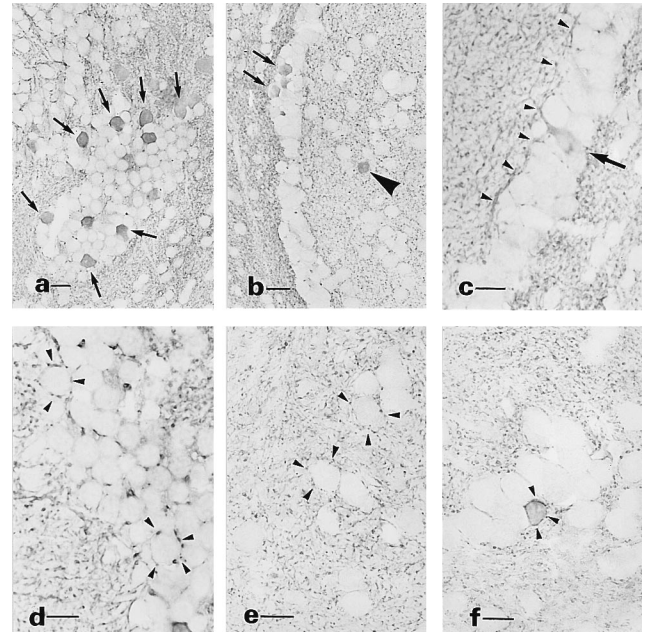


FIG. 2. Gamma aminobutyric acid (GABA)-immunoreactive structures in the isthmic nucleus. Light microscopic preparations. (a) GABA-immunoreactive neurons in the rostral portion of the nucleus. Note a group of positive cells (arrows). (b) Cortical (arrows) and medullary (arrowhead) GABA-positive neurons in the isthmic nucleus. (c) Large pyriform GABA-positive neuron (arrow) in the rim cortex. Note the considerable extent of two thick labelled dendrites along the rim cortex (arrowheads). (d) Basket-like arrangements of GABA-positive fibers around perikarya in the rim cortex (arrowheads) and (e) in the medulla of the nucleus (arrowheads). (f) GABA-positive beaded axons surround a GABA-positive perikaryon in the cortex of the isthmic nucleus (arrowheads). All photographs were taken from 8 μm -thick sections. GABA immunoreactivity was visualized by diaminobenzidine reaction. Scale bars: 10 μm .

Hemisectioning the medulla just caudal to the IN did not cause any obvious change either in the volume and cortical neuronal organization or in the density of immunoreactive beads compared to the control samples.

Ultrastructural Observations

The GABA-positive neurons could also be identified by electron microscopy. The immunolabelled cells had round or slightly oval perikarya. The cytoplasmic rim around the nucleus was narrow and no indentation was detected on the nucleus, nor did we find a myelin sheath around the somata of positive neurons.

Two kinds of GABA-immunoreactive presynaptic profiles were identified by electron microscopy. Type A profiles were small and contained both round dense core vesicles and round clear vesicles of medium size ($43 \pm 6 \text{ nm}$) (Fig. 5a). In many cases, vesicles were loosely scattered in the presynaptic profile. These profiles established symmetrical synapses with unlabelled smaller or medium-size dendrites. Postsynaptic crescents or spines were rarely seen. Type B presynaptic terminals were large and contained round clear vesicles of the same size as type A profiles and did not contain dense core vesicles (Fig. 5b). They also formed symmetrical synapses. Grouping of vesicles along the presynaptic density or close to it was often found, and several profiles were densely packed with vesicles and mitochondria. Postsynaptic dendrites often bore synaptic crescent. GABAergic fibers regularly

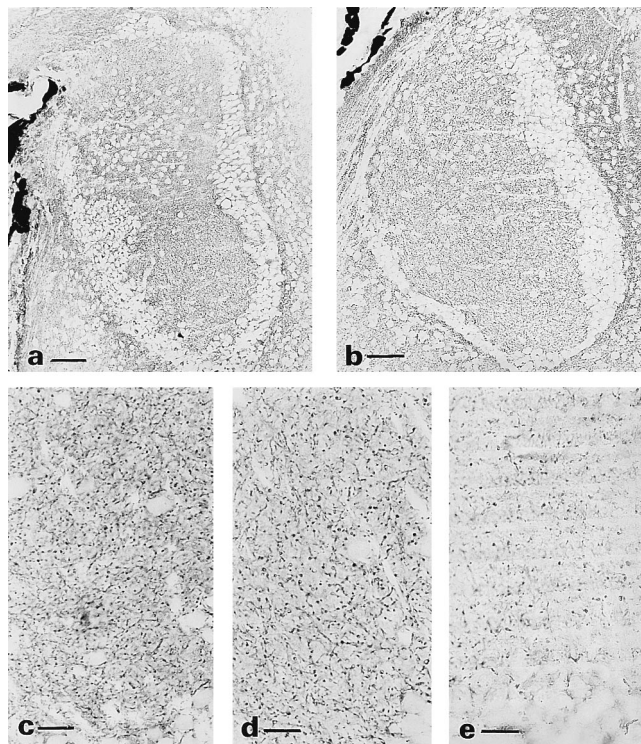


FIG. 3. Light microscopic preparations showing γ -aminobutyric acid (GABA)-positive structures in the isthmic nucleus of an intact animal (b,d) and after two kinds of lesions (a,c,e). (a) shows the effect of tectum ablation on the distribution of GABA-positive elements: cortical neurons are more closely packed and the density of GABA-immunoreactive beads and fibers is slightly increased in the medulla. (c) High power magnification of the neuropil after tectal ablation showing higher density of beads compared to the intact side (d). (e) High power magnification of the neuropil after electrolytic lesion of tegmental nuclei just rostral to the isthmic nucleus. The picture was taken from that part of the neuropil where the reduction of density of the GABA-immunoreactive beads and fibers is obvious compared to (d). Scale bars: 50 μ m in (a) and (b), and 10 μ m in (c,d,e).

had pericellular basket-like arrangements. Some axosomatic synapses of both types were also observed on both medullary and cortical neurons (Fig. 5c). Synapses between GABA-immunoreactive structures were very rare (Fig. 5d).

DISCUSSION

In the IN a small population of GABA-immunoreactive cells of definite number and localization was described. In most brain areas GABAergic cells are intrinsic neurons. We suppose that this is the case also in the IN. Comparing the number of GABA-positive neurons and the density of GABA-containing axons one may conclude that there are more fibers than could be expected on the basis of the number of cells. Theoretically there are two possibilities to explain this discrepancy: (1) The axons of GABA-positive isthmic cells extensively arborize. (2) GABAergic axons from extrinsic sources also contribute to the intrinsic network. We found evidence in favor of both possibilities. Since GABAergic cells are generally regarded as inhibitory, this effect may be exerted on isthmic cells both by the local GABAergic system or by GABAergic afferents. In an earlier electron microscopic investigation one type of profiles containing flattened vesicles and forming symmet-

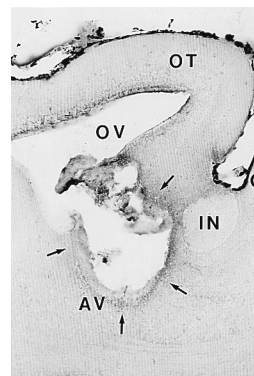


FIG. 4. Location and maximal extent of an electrolytic lesion (arrows) in the tegmentum mesencephali of the frog *Rana esculenta* in a sagittal section. Abbreviations: AV, anteroventral tegmental nucleus; C, cerebellum; IN, isthmic nucleus; OT, optic tectum; OV, optic ventricle.

rical synapses was identified [24]. It is generally accepted that in many brain areas these profiles form inhibitory synapses, which are mainly GABAergic. Therefore one goal of our electron microscopic study was to verify the possible GABAergic nature of these profiles. In the present electron microscopic study we identified two types of GABA-positive axon terminals forming symmetrical synapses. However, both of them contained round synaptic vesicles. One type of these GABA-positive profiles may belong to intrinsic cells, and the other to extrinsic afferents. We did not attempt to determine the place of origin of these two types of axons. The synapses between GABA-positive cells and GABA-positive axons suggest the existence of disinhibitory circuits that may have some physiological significance despite their very low number.

Possible Origin of Extrinsic GABAergic Innervation

The most probable candidate for extrinsic GABAergic innervation of the IN is the optic tectum, because it is the main source of isthmic afferents [9,14,17,26], and many cells in the sixth layer of the optic tectum that comprise the tecto-isthmic projection neurons [10] are GABAergic [2]. If GABA-positive neurons projected to the IN, one would expect an obvious reduction in the density of GABA-immunoreactive fibers here following ipsilateral tectal removal. However, we found the opposite. For the interpretation of this finding, structural changes in the IN following tectal lesion should be considered. By the 12th postoperative week tectal afferents degenerated and were removed from the IN. The linear shrinkage of the nucleus may be more than 20%. The apparent increase in the density of immunoreactive profiles may be the result of shrinkage of the nucleus, therefore the number of beads remained the same as before operation. Thus, the tectum can be excluded as the source of the GABA-positive fibers projecting to the IN.

In species other than frogs non-tectal inhibitory inputs to the IN have been described. In a teleost the nucleus pretectalis projects to the IN. The pretecto-isthmic terminals contain pleomorphic vesicles suggesting the inhibitory nature of this pathway [13]. In reptiles the nucleus profundus mesencephali has a possible inhibitory connection with the IN [27,32]. In birds the magnocellular IN sends GABAergic axons to the parvocellular IN [cit. 27]. In frogs, Wu and Wang [33] showed that stimulation of the optic tract evoked inhibition in the IN through a relay nucleus, presumably the AD. Recently, Xiao and Wang [35] have shown that inhibition

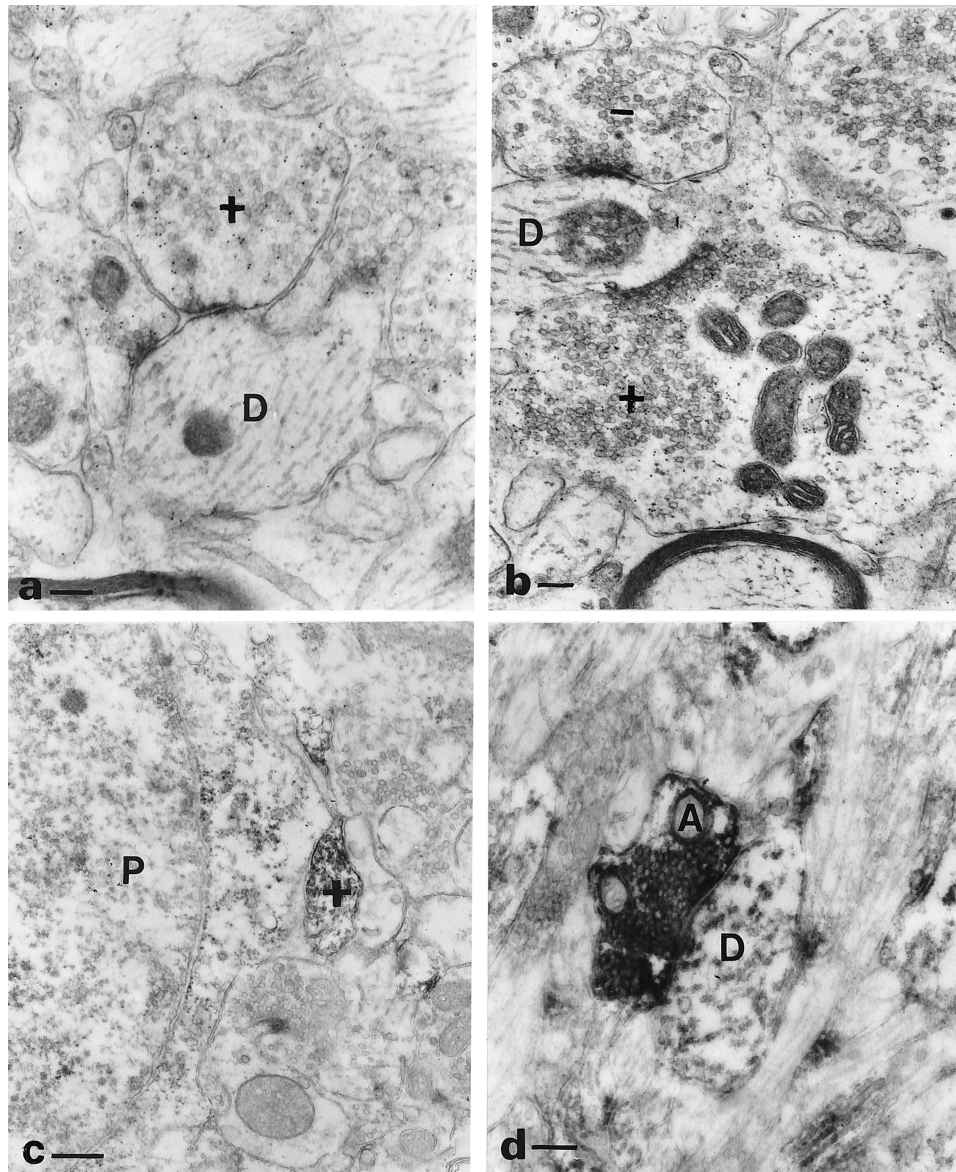


FIG. 5. Electron microscopic preparations showing γ -aminobutyric acid (GABA)-immunoreactive structures in the isthmus nucleus. (a) GABA-immunogold labelling: synapse of a type A GABA-immunoreactive terminal (+) on a GABA-negative dendrite (D). Note small, scattered electrondense gold particles indicative of GABA immunoreactivity in the axon profile (+). (b) Synapse of a type B GABA-positive profile (+) and a GABA-negative axon (-) on a GABA-negative small dendrite (D). (c) GABA-immunoreactive axon terminal (+) forming synapse on a GABA-negative perikaryon (P). Note electrondense clumps of diaminobenzidine (DAB) precipitate. (d) Synaptic contact of a GABA-positive axon (A) and a GABA-positive dendrite (D). Note heavy DAB precipitate in A and scattered clumps of DAB in (D). Scale bars: 200 μ m.

occurs in most isthmus cells following electrical stimulation to the decussatio veli. It has been demonstrated that tegmental nuclei including the AD [30], NPM and the superficial isthmus reticular nucleus [34] project to the IN, and tegmento-isthmus fibers extensively arborize within this nucleus [30,34]. Fibers of passage reach the contralateral IN through the decussatio veli. These observations were recently confirmed by Kulik and Matesz [15]. These latter authors could retrogradely fill some 40 cells in the ipsilateral AD, and AV from the IN. Considering the results of the above-mentioned hodological studies and our observation that in the AD

and the NPM some GABA-immunoreactive neurons occur in the frog, we were led to perform experiments in which the tegmento-isthmus afferents were interrupted, or part of their sources destroyed. Since these lesions brought about some decrease in the density of GABA-immunoreactive fibers in the same part of the IN where the tegmento-isthmus afferents terminate, and some of the destroyed cells showed GABA immunoreactivity, we can conclude that a part of the GABA-positive fibers in the IN originate from the AD and the NPM. The slight reduction in the density of GABA-immunoreactive fibers in the dorsomedial part of the IN following

the transection of the decussatio veli supports the conclusion that these fibers originate from the contralateral tegmentum [30,34]. This arrangement of afferents may explain the bilateral inhibition shown in the IN when one optic tract was stimulated [33]. The recently identified very few afferents originating from the cochlear nucleus [16] do not contribute to the GABA-immunoreactive fiber network of the IN since GABA-containing neurons were not found in the cochlear nucleus. In electrophysiological experiments responses to auditory stimuli could not be recorded in the IN [4].

Functional Considerations

Comparing the results of physiological experiments with the anatomical data, widespread inhibition after stimulation of the visual pathway is difficult to explain. The intrinsic inhibitory effect is probably mediated by the local GABA-positive isthmic cells, and the GABA-positive fibers which very densely arborize within the IN may be the anatomical basis of the inhibitory effect. Inhibition through an extrinsic pathway may originate from tegmental nuclei, most probably from the AD and NPM. These nuclei receive afferents from both optic tecta [22]. Since tegmental afferents terminate throughout the IN [34] and their axons are extensively arborized [30,34], it would explain why inhibitory responses could be recorded throughout the IN [33,35]. On the other hand, some, if not all, GABA-containing isthmic cells may project to the tectum, thereby electrical stimulation of the IN could exert inhibition on tectal cell [31,36]. This inhibition is mediated by GABA_A receptors. However, since such a small population of GABA-positive neurons could not satisfactorily explain strong isthmotectal inhibition, intrinsic GABAergic cells within the amphibian tectum may also play important roles in isthmotectal inhibition.

Summarizing our results, the existence of an intrinsic interneuron system consisting of GABA-containing cells and a weak extrinsic GABAergic system originating from the AD and the NPM was verified. The presence of these inhibitory systems along with several neuropeptides [12,18,19] and transmitter-containing elements [3,11,21,25] suggests that the IN is not a simple relay between the two optic tecta.

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