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Anatomy and Embryology

Optic synapses are found in diencephalic neuropils before development of the tectum in *Xenopus*

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Summary. The position of the earliest optic synapses in *Xenopus* and the stage at which they developed were studied with the electron microscope after labelling of optic axons with horseradish peroxidase. In addition, tritiated thymidine autoradiography and bromodeoxyuridine immunohistology were used to identify the birth dates of cells in the regions where the synapses had been found. The earliest mature optic synapses were found in the mid-diencephalic region, where the major diencephalic optic neuropils were beginning to develop. These synapses were seen at stage 35/36, before cells in the tectal precursor region had become postmitotic. In other animals labelling with tritiated thymidine or bromodeoxyuridine showed that cells in the diencephalon, close to where the synapses had been seen, were becoming postmitotic at the time the earliest optic axons arrived. The first optic synapses to form in the developing *Xenopus* visual system thus appeared to do so in the neuropil of Bellonci and the rostral visual nucleus.

Key words: Optic synapses – Diencephalon – Development – *Xenopus*

Introduction

The amphibian retinotectal fibre projection provides a detailed and orderly map of the retina across the surface of the contralateral optic tectum, and has been used in numerous studies of the development and regeneration of nerve connections. There are retinal projections to various diencephalic visual relays as well as to the tectum, and those which have been investigated also show retinotopic ordering (Lazar 1971; Scalia and Fite 1974; Montgomery and Fite 1989). The development of the projection to the optic tectum has been extensively studied in *Xenopus* by physiological (Gaze et al. 1974; Holt and Harris 1983) and anatomical (Holt and Harris 1983; O'Rourke and Fraser 1986, 1990) means, but little is known about the development of the retinodiencephalic projections.

The axons which form the retinotectal projection have to pass through the diencephalon to reach the tectum. It has recently been argued, however, that when the earliest retinotopic distribution of optic axon terminals is found in *Xenopus*, in the vicinity of the di-mesencephalic junction, the neurons which will later form the tectum do not yet exist; and that the earliest ordering of the fibre projection is likely to relate to the optic tract and diencephalic neuropils rather than to the tectum (Gaze and Grant 1992). To investigate further this possibility we have determined the birthdates of cells in the rostral visual nucleus, one of the diencephalic visual relays, and of cells which send processes into the adjacent neuropil of Bellonci and the corpus geniculatum thalamicum (also diencephalic visual relays) and we show that there are postmitotic neurons in the region of this complex of structures from the earliest stages of retinal axon ingrowth. We have also investigated the position and time of appearance of the earliest optic synapses in *Xenopus*; and we find that optic axons synapse with mid-diencephalic neuropils in this same region before any of the axons have reached the site of the future tectum.

Materials and methods

Animals

Xenopus laevis embryos were bred in the laboratory by standard methods using chorionic gonadotrophin. Animals were staged according to the Normal Table of Nieuwkoop and Faber (1967). Embryos up to stage 45 were kept in aerated 1:10 Niu-Twitty (NT) solution. Thereafter tadpoles were kept in continuously aerated tap water at 20° C, in a 12 h light-dark regime. Tadpoles were fed on nettle powder (P. Harris) mixed with Complan (Farley Health Products).

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Labelling of optic axons

Horseradish peroxidase labelling. Embryos were anaesthetised in 1:4500 ethyl-m-aminobenzoate (MS222, Sandoz) in 66% NT solution and placed, left side up, on a piece of damp tissue. Tungsten needles were used to open the ectoderm covering the eye and to remove the lens. The retina was gently scratched and a small piece of recrystalized horseradish peroxidase (HRP) with 0.1% Nonidet P40 (BDH) was placed on it. The embryo was left in a moist box for 5 min to allow for uptake of the HRP and then returned to 66% NT solution. After a further 10–20 min the embryo was re-anaesthetised and then fixed in 2.5% glutaraldehyde and 5% sucrose in 0.1 M phosphate buffer. After 3–4 h the brains were dissected, demembranated and reacted with diaminobenzidine. Brains were then post-fixed in 1% osmium tetroxide, dehydrated and embedded in Araldite. For electron microscopic (EM) examination, 70-nm sections were collected every 20 or 40 μm depending on the stage of the embryo, stained with lead citrate and uranyl acetate and examined in a Philips 300 EM.

Proline labelling. Retinal ganglion cells and their axons were labelled with tritiated proline in two tadpoles of stage 54. The animals were anaesthetised in MS222 (1:1500) and 0.25 μCi of tritiated proline [^{3}H]-P; specific activity 4.0 TBq/mmol (108 Ci/mmol) and radioactive concentration 37.0 MBq/ml (1 μCi/μl), obtained from Amersham was injected slowly into an eye. After a further 24 h the animals were re-anaesthetised and fixed in formol-saline.

Cell labelling. The time of origin of cell groups in the diencephalon was investigated by immunohistological detection of previously administered bromodeoxyuridine or by autoradiography following the administration of tritiated thymidine, both of which label newly formed DNA.

Embryos were anaesthetised in 1:4500 MS222 and placed in shallow depressions in the wax base of an operating dish, under anaesthetic solution. The labelling solution was pressure-injected by hand, via a hand-drawn, calibrated micropipette. This permitted easily controlled injections of amounts down to 50 nl. Injections were given slowly, into the belly region, via the dorsum of the animal. Injected animals were then allowed to recover for an hour in full strength NT solution before being transferred to the rearing solution (1:10 NT).

Thymidine labelling. [*Methyl*-^{3}H]-thymidine ([^{3}H]-T) was obtained from Amersham. Animals of st. 26 (10) and st. 27/28 (8) each received 50 nCi [^{3}H]-T and animals of st. 40-42 (24) each received 100 nCi [^{3}H]-T, specific activity 925 GBq/mmol (25 Ci/mmol) and radioactive concentration of 37.0 MBq/ml (1.0 μCi/μl).

Bromodeoxyuridine labelling. The 5-bromo-2'-deoxyuridine (BrdU) was obtained from Sigma. Animals at st 27/28 (2) each received 50 nl of 0.5 mmol BrdU made up in NT solution. Animals of st. 25/26 (12), 28 (5), 29/30 (2), 31/32 (8) and 32/33 (10) were given 100 nl of 0.5 mmol BrdU. Animals of st. 54 (10) received 0.5 μl of 20 mmol BrdU.

Thymidine autoradiography

At various intervals after the administration of [^{3}H]-T, animals were killed with an overdose of anaesthetic, and then fixed in Smith's solution overnight. Heads were embedded in paraffin and serial 5-μm transverse sections were mounted on slides, dewaxed in xylene, taken down to water and dipped in Ilford K2 emulsion, diluted 1:1 with 1% glycerol. Slides were dried and exposed in light-proof boxes containing silicone gel at 4° C for 10–14 days. They were then developed in Kodak D19 developer. Sections were lightly counterstained with cresyl fast violet.

Proline autoradiography

The animals were re-anaesthetised 24 h after eye-labelling and fixed in formol-saline. Transverse 10-μm wax sections were coated with Ilford K2 emulsion and exposed as above, for varying periods. Sections were then developed and counterstained as above.

Immunohistology

At various intervals after the administration of BrdU, animals were killed with an overdose of anaesthetic and then fixed in 2% trichloracetic acid at room temperature (RT) for 2 h. Heads were embedded in paraffin and serial 5 μm transverse sections were mounted on slides in small groups. The sections were reacted with anti-BrdU (mouse IgG, Beckton-Dickinson) according to the following schedule:

1. Sections were dewaxed in xylene and rehydrated through graded ethanols to phosphate buffered saline (PBS) at pH 7.4 with 3.0 ml/l triton X-100 (PBS/Tx). Slides were washed several times in PBS/Tx before being treated with 2N HCl/Tx for 30 min at RT, then washed several times in PBS/Tx.
2. Sections were covered with 10% sheep serum in PBS/Tx for 30–60 min at RT.
3. After removal of excess sheep serum, sections were treated with anti-BrdU, 1:20 in PBS/Tx with 0.5% bovine serum albumin (BSA). Sections were left thus for 30 min at RT, or overnight at 4° C, in a moist chamber. Thereafter they were washed several times in PBS/Tx.
4. Sections were then treated with HRP-conjugated sheep anti-mouse IgG (Scottish Antibody Production Unit), diluted 1:40 with PBS/Tx containing 0.5% BSA, for 30 min at RT in a moist chamber. Slides were then washed several times in PBS/Tx.
5. Sections were then treated with diaminobenzidine to reveal HRP, counterstained with 1% methyl green and mounted in DPX.

Brain reconstructions

Brains from embryos in which optic axons had been labelled with HRP were reconstructed from serial transverse 1-μm resin sections. From each brain every fifth section (or some other number, depending on the total number of relevant sections) was drawn on tracing paper, through a camera lucida attachment, at a magnification of 300. Every 20–40 μm, depending on the stage of the embryo, thin sections were taken as previously described, for ultrastructural examination. Any labelled synaptic profiles seen were then entered at the corresponding position on the drawing of the appropriate adjacent 1-μm section. When few profiles were present, all were marked. When many were present, representative profiles were marked so as to identify their distribution. The individual drawings were then digitised with a Complot digitiser. Each section was

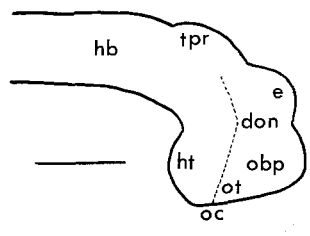

Fig. 1. Diagrammatic view of the brain of a stage 37/38 *Xenopus* embryo, traced from a photograph. Rostral is to the right, dorsal upwards. *hb*, hindbrain; *tpr*, tectal precursor region (part of midbrain); *e*, epiphysis; *obp*, olfactory bulb precursor region; *don*, site of developing diencephalic optic neuropils; *ht*, hypothalamus (these last four structures are all parts of the forebrain); *oc*, optic chiasma; *ot*, optic tract. Bar 250 μm

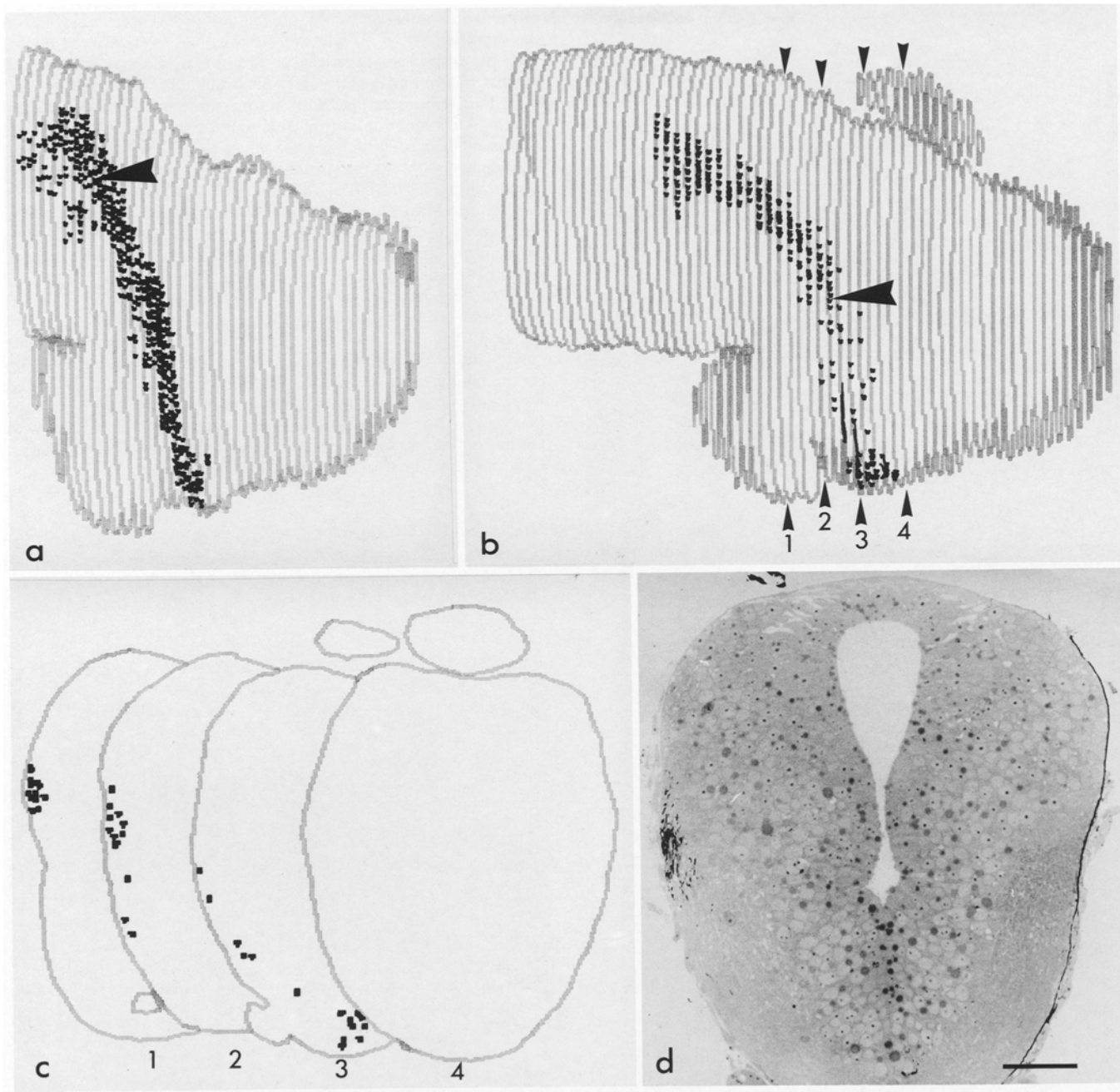

Fig. 2a–d. Optic synapses in stage 39 embryos. **a** Reconstruction of the rostral end of a brain, from serial 1-μm resin sections. Rostral is to the right, dorsal upwards. *Dots* indicate positions at which HRP-filled axonal profiles were seen by light microscopy. Every 10th section was used for the preparation of the brain reconstruction, whereas the position of the optic tract is indicated by the distribution of HRP-labelled profiles taken from every 5th section over the relevant part of the brain. The position of the labelled synapse shown in Fig. 3 is indicated by a large *arrowhead*. The method of reconstruction in **a** and **b** (and in Fig. 4) is shown here in **b, c** and **d**. **b** Reconstruction of another brain showing the position of labelled synapses (*large arrowhead*) found in the mid-dorsoventral part of the tract. Rostral is to the right, dorsal upwards. Sections are shown with the correct distance between them, relative to the size of the reconstruction, but are rotated through 85° clockwise from the plane of the section, to give a lateral view of the brain. (The sections indicated by the *small arrowheads* numbered *1–4* are also shown in **c**.) **c** The four numbered sections from **b** are shown here with their rostrocaudal separation greatly increased and after rotation through only 20° clockwise, so that the distribution of labelled profiles round the edge of each section can be seen. Where many profiles were seen the dots are intended to give an impression of the distribution. **d** Photomicrograph of one of the sections used to produce the reconstruction in **b**. This is adjacent to section 2 in **b** and **c** and also to the position where the synapses were seen (*large arrowhead* in **b**). HRP-labelled profiles are grouped midway up the right optic tract (left in photograph). Bar 50 μm

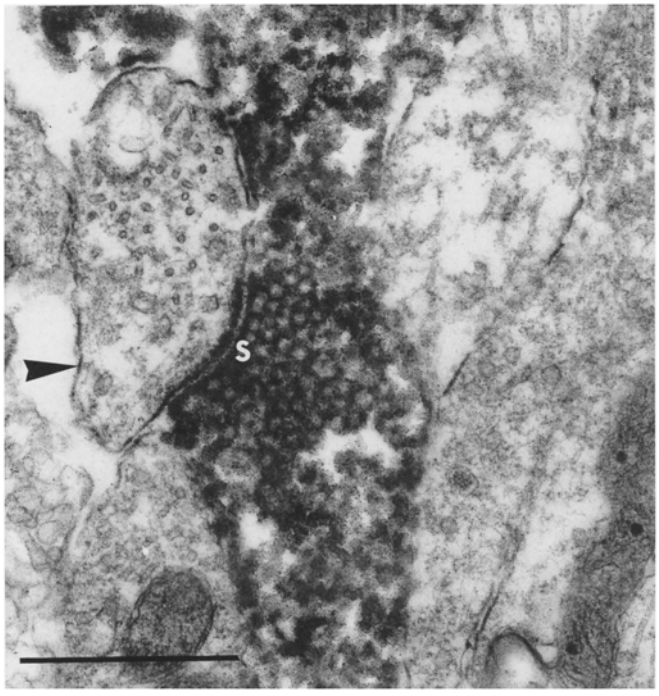

Fig. 3. Stage 39 embryo. Labelled synapse (*s*) contacting a dendritic process (*arrowhead*), from the position shown in Fig. 2a. The postsynaptic process contains more microtubules than that in the stage 35/36 embryo (see Fig. 5). Bar 0.5 μm

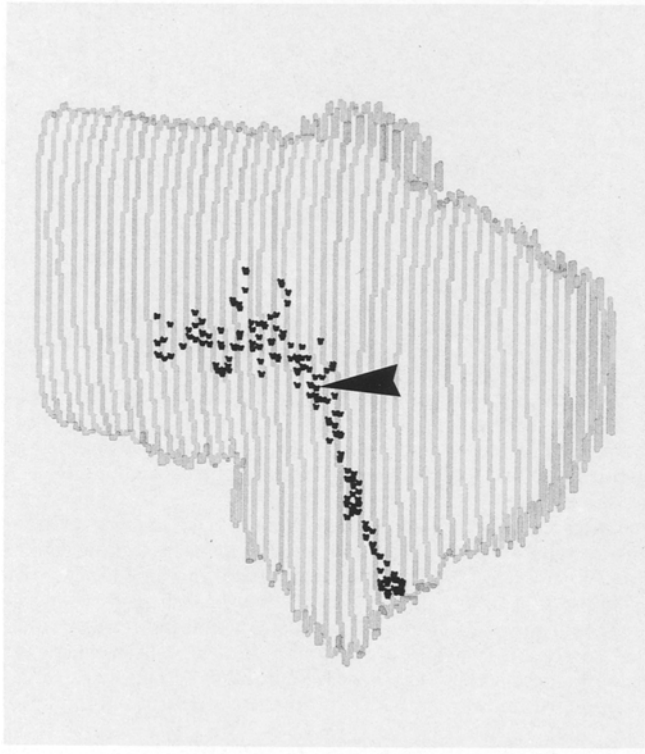

Fig. 4. Reconstruction of brain and tract from an embryo at stage 35/36, showing the position (*large arrowhead*) at which the labelled synapse shown in Fig. 5 was found. Rostral is to the right, dorsal upwards

centred and oriented by eye in relation to the previous one on the digitiser. When all relevant sections had been entered in this way, the entire array was used to produce a pseudo-3-dimensional reconstruction of the brain and optic tract in the preferred orientation. For demonstrating the position of the optic tract, as determined by the distribution of labelled profiles, a right lateral view was used. If necessary, the array of sections was further oriented and aligned within the computer so as to produce a reconstruction which resembled as closely as possible the shape of the brain as determined from photographs of other embryos. Figure 1 shows the appearance of an embryonic *Xenopus* brain, oriented in the same way as the reconstructions in Figs. 2a, b and 4. In addition, part of the diencephalon from a stage 54 tadpole, in which one eye had been labelled with [^{3}H]-P, was reconstructed in a similar way from serial transverse 10-μm paraffin sections. In this case the result was presented as a stereo pair, giving a view along the rostrocaudal axis of the brain, to show the relationship between the incoming optic axons, the rostral visual nucleus (also called the posterior entopeduncular nucleus; see Levine 1980) and the neuropil of Bellonci (Fig. 7). All reconstructions were made with the aid of a series of programmes (SSRCON) written by J. Green (National Institute for Medical Research, London).

Anatomical terminology

CGT, corpus geniculatum thalamicum; NB, neuropil of Bellonci; RVN, rostral visual nucleus.

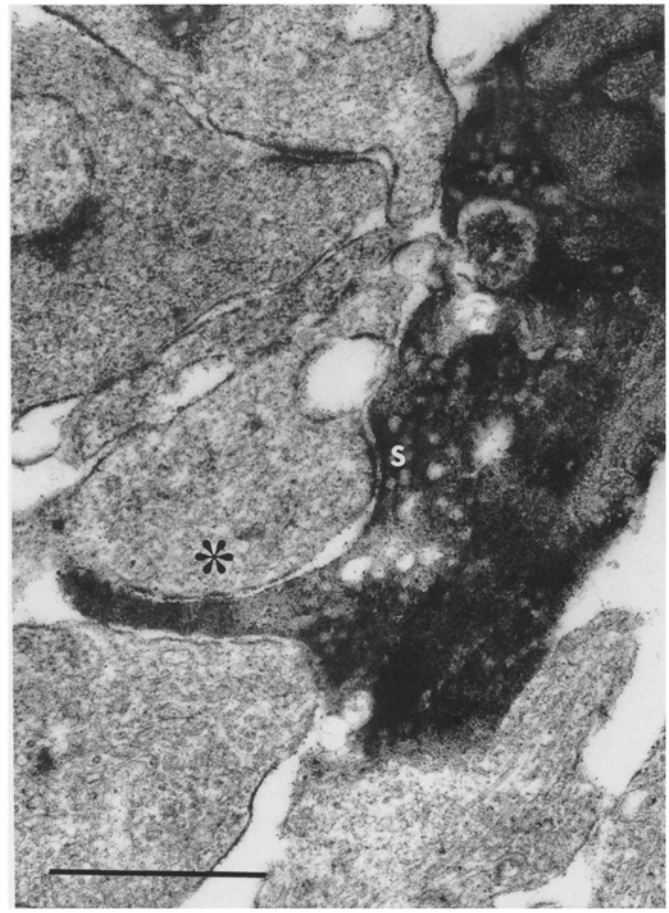

Fig. 5. Stage 35/36 embryo. Labelled synapse (*s*) from the position indicated in Fig. 4. The synapse is close to the paraventricular cell mass (*). Bar 0.5 μm

Welfare of experimental animals

The operative procedures described in this paper were performed with the animals under appropriate anaesthesia. On recovery from the anaesthetic the animals did not appear to be suffering discomfort. When surviving for more than 24 h the larval animals fed well.

Results

The EM observations reported here were based on the examination of 15 embryos; 4 at st. 39, 2 at st. 37/38, 3 at st. 35/36 and 6 at st. 33/34.

At stage 39 HRP-labelled optic axons formed synapses with dendrites of unidentified cells in two regions of the brain. Synapses were found dorsally, where retinal axons were forming terminal arbors (Figs. 2a, 3), and halfway up the dorsoventral extent of the diencephalon (Fig. 2b, c, d). In this latter case no filled synaptic profiles were seen further caudally in the tract.

At stage 37/38 synapses (not illustrated) were seen at and just dorsal to the geniculate bend (see below) in the optic tract. In the ventral half of the tract structures resembling synaptic vesicles were often found in the deeper growth cones, but were absent from those found more peripherally.

At stage 35/36 filled synapses were formed with profiles showing a cytoplasmic composition which resembled that of the deeper-lying cells. These synapses were only found halfway up the dorsoventral extent of the diencephalon, in the optic tract where the fibres turned caudally (Figs. 4, 5). The synapses were mature in that they had postsynaptic membrane thickenings, synaptic vesicles, varying amounts of electron-dense material in the synaptic cleft and sometimes obvious striations within the cleft (Hayes and Roberts 1974). In addition, at stage 35/36, other vesicle-filled HRP-labelled structures were seen within the optic tract. These were not associated with any membrane specialisations on neighbouring profiles. Several such vesicle-containing profiles were found deep in the lateral diencephalic neuropil, close to the cells, and some were also seen more ventrally in the optic tract. These structures may have been growth cone profiles.

At stage 33/34 no labelled profiles were seen in the mid-diencephalon. However, labelled profiles were

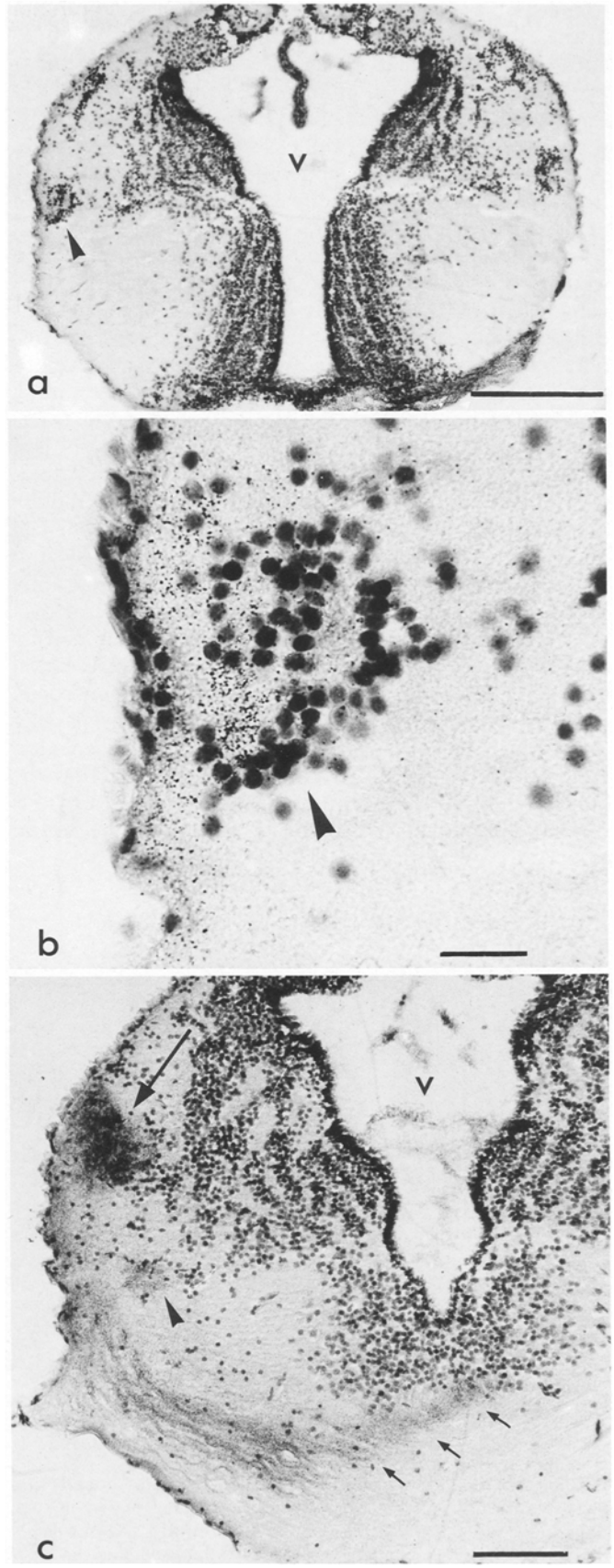

Fig. 6a–c. The optic tract at stage 54 in relation to the rostral visual nucleus and the neuropil of Bellonci. In this animal the optic axons from the left eye had been labelled with [^{3}H]-P. Photomicrographs are from transverse 10-μm paraffin sections. **a** The diencephalon at the level of the rostral part of the optic chiasma. The right RVN (left in photograph) is marked by an *arrowhead*. *v*, ventricle. Dorsal is upwards. The right RVN is shown at higher magnification in **b**. Bar 200 μm. **b** Higher magnification of the right RVN shown in **a**. Autoradiographic grains are distributed at the most peripheral margin of the tract and throughout the RVN (*arrowhead*). Bar 20 μm. **c** Section through the diencephalon approximately 120 μm caudal to **a**. The Bellonci neuropil (*large arrow*) is now visible, as is the caudal neuropil of the RVN (*arrowhead*). The labelled optic tract is shown sweeping up round the side of the diencephalon, with the deeper fibres (*small arrows*) beginning to pass dorsolaterally, near the paraventricular cell mass. *v*, ventricle. Dorsal is upwards. Bar 100 μm

found as the optic axons entered the diencephalon and in the chiasma. Some of these resembled growth cones and contained vesicles. The consistent finding of similar profiles in a number of animals suggests that they represent some form of specialized contact. Unfortunately, the poor preservation of the tissues at these early stages in embryos which have undergone histochemical reaction for HRP during EM processing, made identification of the adjacent profiles impossible.

The optic synapses found in mid-tract at st. 35/36 were in a region where the tract turns caudally. This geniculate turn is well seen in Figs. 4 and 2b. In embryos of st. 39 this change of direction of the optic axons may be seen to occur at the position where the developing visual relays, the RVN, NB and CGT, are becoming visible in whole mounts of HRP-labelled optic tracts (Easter and Taylor 1989; Taylor 1990). The RVN is a conical structure with its cellular base close to the lateral wall of the diencephalon. The nucleus extends caudally and medially to an apex which abuts the central cell mass of the diencephalon (see Fig. 7). The RVN and the NB are well shown by [^{3}H]-proline autoradiography following labelling of the retina. The rostrolateral part of the RVN starts as a rounded structure, bounded by cells, and close to the lateral wall of the diencephalon (Fig. 6a). This structure receives input from superficial (i.e. the most recently developed) optic axons passing up round the outermost part of the diencephalon (Fig. 6b). Further caudally the RVN diverges from the lateral edge of the diencephalon and loses its obvious cellular components as it comes to lie close to the central diencephalic cell mass and close to the apex of the NB (Fig. 6c). At this position the caudal part of the RVN shows up as an autoradiographically labelled area of neuropil which may easily be shown, from serial sections, to be continuous with the rostral cellular part of the nucleus. At this caudal extremity of the RVN, the deepest (i.e. the oldest) of the incoming optic axons may be shown to pass through or close to the neuropil. A few micrometres more caudally, the deepest optic axons pass also through the medial extremity of the NB. Figure 7 shows the three-dimensional structure of this part of the visual system, reconstructed from the same preparation shown in Fig. 6.

It seemed likely that the early optic synapses found in the mid-thalamic region at stage 35/36 might involve cells related to the RVN and the NB, in which case we could expect to find postmitotic cells in the appropriate region at that stage. We therefore attempted to identify the birthdates of cells in this part of the brain, using [^{3}H]-T and BrdU. In this we were only partly successful, because we were unable to determine which cells contributed dendrites to the neuropil containing these synapses. It was possible to show, however, that cells labelled at stages 26 to 33 were heavily labelled (and thus close to their terminal mitoses at the time of injection), both at stage 54 and after metamorphosis, in positions near the deepest optic axons in the tract, and in the appropriate dorsoventral region of the diencephalon (Fig. 8). The deepest axons in the tract are the surviving members of the first group of optic axons to pass up the tract in early development, and thus in-

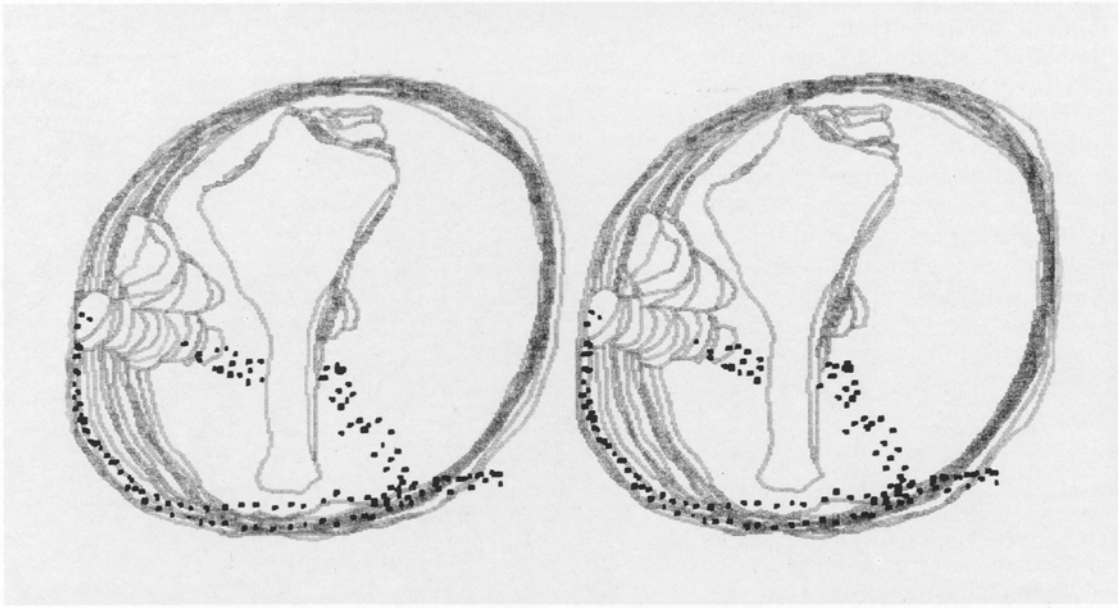

Fig. 7. Stereo pair showing a reconstruction, from serial 10-μm paraffin sections, of the thalamus shown in Fig. 6. Inside each thalamic outline the ventricle is shown centrally and the RVN and the Bellonci neuropil are at the left lateral edge. Bellonci is above and RVN below. The ventricle, RVN and Bellonci have been reproduced in solid outline to facilitate viewing. The entry of the left optic nerve is shown at lower right. The courses of the most superficial and of the deepest fibres of the optic tract are shown as series of *dots*. All the rest of the optic tract, which extends as a sheet of axons joining the superficial and deep fascicles shown, is omitted for clarity. The most rostral fibres of the optic tract pass up the outer margin of the diencephalon and traverse the rostral end of the RVN (see Fig. 6b). Some of the deeper fibres are seen to pass dorsocaudally, then turn laterally towards the RVN/Bellonci complex. The rostrocaudal extent of this reconstruction is approximately 230 μm

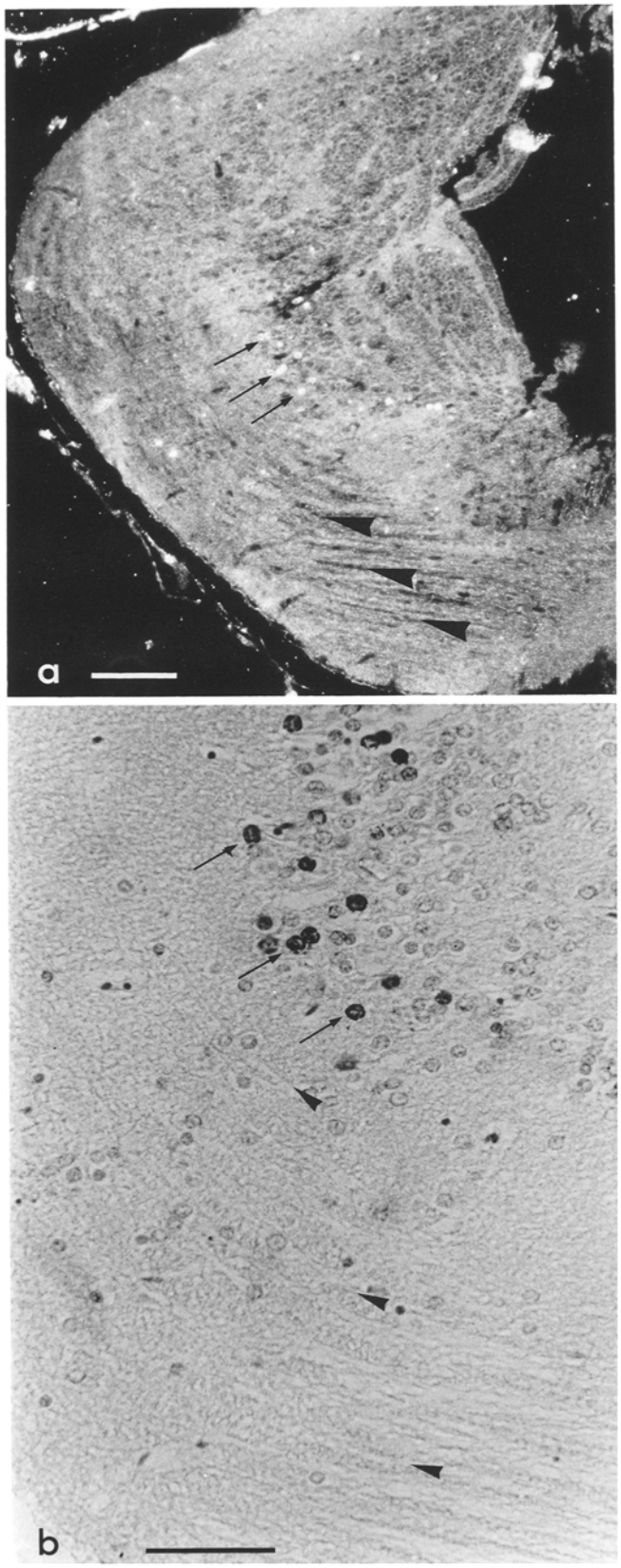

Fig. 8a, b. The positions of the deepest optic axons in the tract after metamorphosis, in relation to diencephalic cells which were undergoing their final mitoses when these axons grew in. In this animal BrdU had been administered at st. 31/32 and the label revealed immunohistologically after metamorphosis. **a** Dark-field micrograph of the right side of the diencephalon to show the position of the optic tract (*arrowheads*) and of labelled cells (*arrows*).

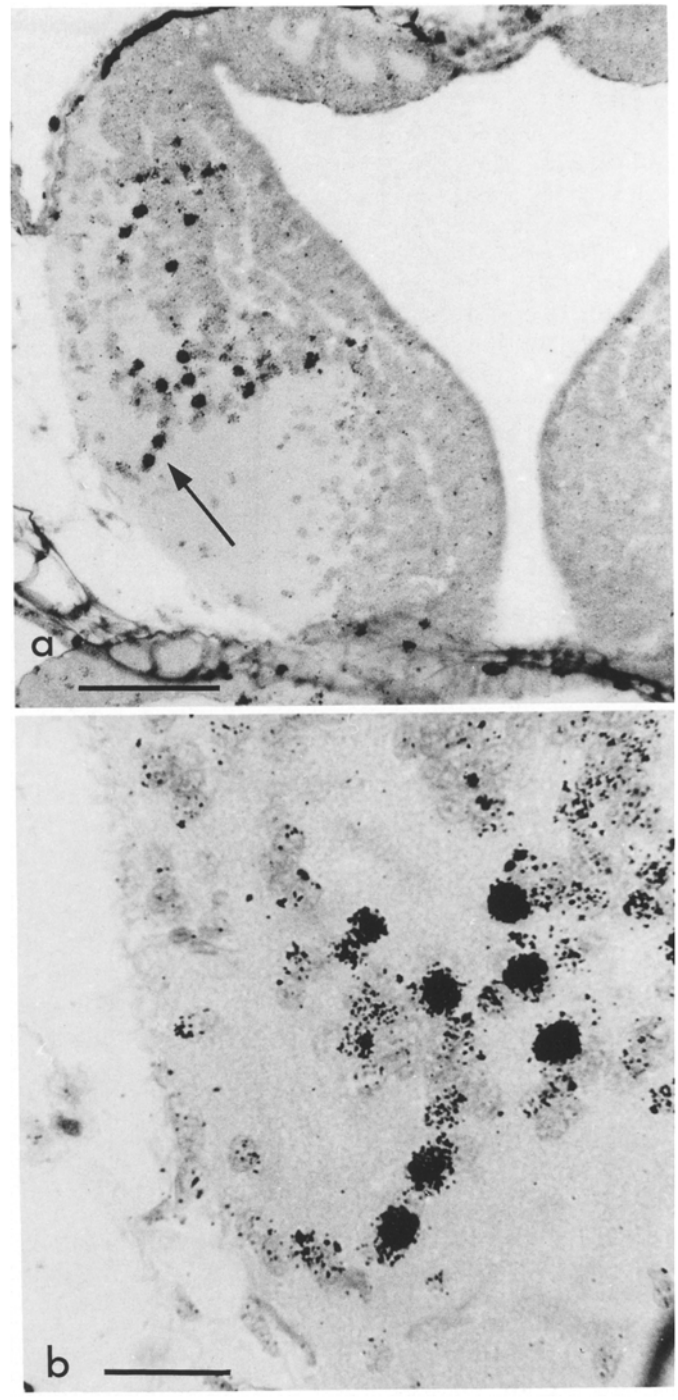

Fig. 9a, b. The time of origin of cells in the lateral part of the RVN. **a** Transverse section through rostral diencephalon from an animal in which [^{3}H]-T had been administered at stage 41/42 and autoradiography performed at st. 51. The ring-like cellular structure half way up the diencephalic margin (*arrow*) is the RVN. Bar 100 μm. **b** Higher magnification of the RVN. Some medial cells in the ring are heavily labelled, whereas more lateral cells are only lightly labelled. Bar 25 μm

Bar 100 μm. **b** Bright-field micrograph of part of the section shown in **a**. BrdU-labelled cells (the same as in **a**) are indicated by *arrows*. The deep fascicles of the optic tract are unstained but can be seen (*arrowheads*). Labelled cells lie close to the deepest of the optic fascicles. The cells are heavily labelled, and thus were at or approaching their terminal mitosis when the label was given. Bar 50 μm

clude any survivors of the group of axons giving synapses in this region at st. 35/36. We propose, therefore, that the synapses we have identified in this mid-diencephalic region at st. 35/36 were formed with the earliest generated neurons of the RVN-NB complex.

The rostrolateral part of the RVN, which forms the base of this conical nucleus, does not exist at this early stage. The diencephalon expands radially during growth and also grows from caudal to rostral (Tay and Straznicky 1982). The base of the RVN thus comes to lie eventually both rostral to the apex of this nucleus and at the lateral border of the diencephalon, in a position which only develops considerably later. Accordingly, the cells of the rostrolateral part of the RVN were only found to be heavily labelled when [^{3}H]-T or BrdU was administered at stage 41 or later (Fig. 9).

Discussion

In addition to the main (tectal) termination site for fibres of the Anuran retinofugal projection, various regions of the diencephalon also receive input from optic axons. For *Rana*, the retinorecipient regions have been described by Knapp et al. (1965), Scalia et al. (1968) and Scalia and Gregory (1970). The retinotopic arrangement of the inputs to some of these regions has been discussed by Lazar (1971), Scalia and Fite (1974) and by Montgomery and Fite (1989). The comparable retinorecipient thalamic regions in *Xenopus* have been investigated by Levine (1978, 1980) and are, in general, similar to those in *Rana*. In *Xenopus* the main thalamic termination areas for optic axons are the RVN (which is partly cellular and partly neuropil), the CGT (a neuropil), the NB and two regions in caudal thalamus called the thalamopretectal field and the uncinate field (Levine 1980).

The first optic axons in *Xenopus* leave the eye at stage 30–31 (Grant and Rubin 1980), pass the chiasma by stage 32 (Grant et al. 1980) and thereafter grow up round the outer edge of the diencephalon to form the contralateral optic tract. In the present experiments the earliest optic synapses were found in the diencephalon, halfway up the optic tract at st. 35/36. The position at which these synapses were found was appropriate for the NB-RVN complex, but at st. 35/36 these structures are not yet recognisable. The NB and the caudomedial part of the RVN are neuropil structures. Optic axons synapsing in these neuropils do so on dendrites from cells outside the structures themselves. Most of the cells identifiable as post-synaptic elements in these neuropils, according to Scalia and Gregory (1970), were situated close to the neuropils and medial to them, in cell groups identified (in *Rana*) as the nucleus rotundus, the lateral geniculate nucleus, the posterocentral and posterolateral nuclei and the ventrolateral area. These nuclear regions invest the central ends of the neuropils mentioned. Because of the "tree-ring" mode of growth of the diencephalon and diencephalic optic tract in *Xenopus* (Gaze and Grant 1978; Gaze 1978) the axons forming the earliest optic synapses found in these parts of the diencephalon, while near the lateral diencephalic margin at st. 35/36, later become buried deep in the neuropil by the serial addition of newly arriving retinal (and other) axons external to them. These earliest synapses should thus have been formed on processes extending from cells which are located in one or other of the nuclei mentioned above, and which were postmitotic at that time. The present investigation shows that cells which became postmitotic at around that time can indeed be found just medial to the relevant areas of neuropil.

The present results are relevant to earlier work on the retinotopic arrangement of optic axons in the thalamic neuropils. Lazar (1971) suggested that the periphery of the retina in *Rana* projects mainly to the lateral parts of the lateral geniculate body (here called the CGT) and the NB, whereas the centre of the retina projects to the medial parts of these neuropils. On the other hand, Scalia and Fite (1974) maintained that, since the dendrites of the postsynaptic thalamic neurons extend through the CGT from its medial to its lateral surface, one would expect that a single map point (i.e. fibres from one particular point on the retina) should extend, in columnar fashion, from the lateral to the medial surface of the neuropil; and their experiments indicated that this was likely to be so.

The observations reported here accord with the suggestion of Lazar (1971). The earliest optic synapses in *Xenopus* are found in a region which will later form the caudomedial part of the RVN-NB complex, and the axons involved come from what will later be central retina. At this early stage of development neither the rostrolateral part of the RVN-NB complex nor the peripheral part of the retina have yet been formed. We also show that much later in midlarval life, at stage 54, the most superficial of the optic axons in the tract pass through the rostrolateral end of the RVN and the NB. We do not know whereabouts in these neuropils any of these later axons synapse, but it seems likely that, at least in the RVN, they may do so on processes of the RVN cells which invest this part of the neuropil. And we show here that some of these cells are becoming postmitotic at stage 41/42 or later, when the most recently arrived optic axons at those stages are growing through the neuropil.

In a previous paper it was argued that the initial ordering of optic axons, seen near the di-mesencephalic junction as the axons first grew in, was a manifestation of their ability to position themselves in relation to the optic tract and diencephalic neuropils rather than to the (as yet undeveloped) tectum (Gaze and Grant 1992). The work presented in this paper shows that the earliest ingrowing optic axons make synapses as soon as they reach the developing diencephalic visual neuropils; and that cells, presumably neurons, which probably contribute the postsynaptic elements of these synapses, are newly postmitotic when the axons arrive.

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