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Projection of visuotopically organized afferents to the dorsal thalamus in the opossum, *Monodelphis domestica*

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Abstract. Retrogradely transported dyes, Fluorogold and Fast Blue were injected into both sides of the dorsal thalamus in the *Monodelphis* opossum. Projection of the presumed primary visual cortical area, superior colliculus and parabigeminal nucleus to the dorsal lateral geniculate nucleus and the lateral posterior - lateral intermedius nuclear complex were described. They show close similarities to the homologous projections in the North American Opossum, insectivores and some rodents. In comparison with rat, cortico-thalamic and tecto-thalamic projections in the *Monodelphis* are less numerous. The peculiarity of cytoarchtitectonics of cortical layer 6 is described and discussed.

INTRODUCTION

In recent years the Brazilian short-tailed opossum (*Monodelphis domestica*) is gaining interest as a new laboratory animal (Fadem et al. 1982, Dzięgielewska et al. 1989, Saunders et al. 1989, Zou et al. 1991). Its easy management, prolific breeding and birth at a stage when most of its central nervous system, and especially its cerebral cortex is only starting to develop make it a very useful object of developmental studies in neurophysiology (Nicholls et al. 1990, Stewart et al. 1991, Balslev et al. 1992). However, data on the anatomy and connections of the brain in *Monodelphis* are as yet scarce, especially in comparison with the volume of information on the anatomy of the brain of albino rat (for review see Paxinos et al. 1985), the most common subject of developmental investigations. The only central nervous structures of *Monodelphis* described in some detail at present are the olfactory bulb (Brunjes 1992, Philpot 1994), neocortex (Saunders et al. 1989), hypothalamus (Rivkees et al. 1988, Fox et al. 1990, Kuehl-Kovarik et al. 1993), cerebellar vermis (Dore et al. 1990) and spinal cord (Nicholls et al. 1990, Stewart et al. 1991).

The current study is of the anatomy of connections of the visual system in this species which has also allow some phylogenetic comparisons to be made. For this purpose we decided to investigate the afferentation of the dorsal thalamic nuclei (that are known to be a part of the visual system in the *Didelphis* opossum - Benevento and Ebner 1970, 1971, Royce et al. 1976) by the visuotopically organized brain structures. We have labelled separately the projections to the dorsal lateral geniculate nucleus (DLG) and two lateral nuclei: the lateral posterior nucleus (LP) and the lateral intermediate nucleus (LI), which is probably homologous to the Eutherian lateral dorsal nucleus (LD, Oswaldo-Cruz and Rocha-Miranda 1967, Benevento and Ebner 1971). LP and LI will be described together, as the projections of the visuotopically organized structures to both these nuclei seem to be similar in the *Didelphis* (Benevento and Ebner 1970, 1971) and not different from projections to LP in other mammals (Erickson et al. 1967, Niimi and Kuwahara 1973, Takahashi 1985).

For this purpose we injected two fluorescent, retrogradely transported dyes, each into opposite sides of the dorsal thalamus in the opossums and looked for the retrogradely labelled neurones in the posterodorsal region of cerebral cortex, where the striate and extrastriate cortex was identified in the *Didelphis* (Bodian 1935, Benevento and Ebner 1971), in the superior colliculus (CS) and the parabigeminal nucleus (PBG). These visuotopically organized structures are known to project to DLG and two of them (cortex and CS) to LP in the *Dydelphis* (Martin 1969, Benevento and Ebner 1970, 1971), rat (for review see Sefton and Dreher 1985) and other mammals (Giolli and Pope 1971, Benedek et al. 1983, Garey et al. 1991, Harting et al. 1991a, b). The pretectal projection that in the *Didelphis* ends in the LI and LP (Benevento and Ebner 1970) has not been analyzed. Borders of this small region with the posterior part of LP and LI are not very clear-cut (Oswaldo-Cruz and Rocha-Miranda 1967, Linden and Rocha-Miranda 1981) and we could not exclude the possibility of small spills of the injected dyes in the pretectum. The ventral geniculate nucleus (VLG) was always injected together with DLG and therefore will not be analyzed separately.

Experimental procedures of the present study are the same as those used in the investigations of afferents of the dorsal thalamus in the rat (Turlejski et al. 1993, 1994). This allows for direct comparisons between the two species. Some other comparisons will be also attempted on the basis of the results obtained.

METHODS

Three young adult male *Monodelphis* (weight 70-80 g, age about 4 months) were used. They were anaesthetized with 2% halothane in 70%:30% NO_2-O_2 gas mixture and placed in the Kopf rat stereotaxic apparatus with a modified tooth bar. The animals were placed in the stereotaxic apparatus with the tooth bar placed in the diastema between

incissors and canines. The bar was moved vertically to a position when the inferior orbital ridge was at the same horizontal level as the middle of the ear bar. A mask loosely fitted over the snout supplied the halothane mixture during surgery and injections. Small openings in the bone and dura were made and the glass micropipettes with the tip broken to the external diameter of 50-60 µm were glued to the needles of 1 µl syringes and filled with the 0.2-0.3 µl of 2% Fluorogold (Fluorochrome Inc., Schmued and Fallon 1986) and 3% Fast Blue (Dr Illing GMBH and Co. KG, Bentivoglio et al. 1980). The tips of the pipettes were stereotaxically lowered to the points defined by two-third of the Lambda-Bregma distance counting from the Bregma, 2.0-2.5 mm laterally from the midline on each side and 3.0-3.5 mm down from the surface of the cortex. The dyes were pressure injected in the thalamus and the pipettes left in place for a few minutes. Then the pipettes were removed, lidocaine applied to the wounds and the skin sewn together. The animals received intramuscular injections of long-lasting antibiotics.

After three days' survival the animals were killed with an overdose of Nembutal and perfused through the heart with 4% paraformaldehyde solution in 0.1 M phosphate buffer, pH 7.4. The brains were kept in 30% sucrose for 3 days and cut on freezing microtome into 40 µm sections. Every section of the tegmentum and every second section of the more rostral parts of the brain were collected on gelatinized slides, air dried and kept in darkness in the freezer until inspected with the Nikon Optiphot - 2 fluorescent microscope, photographed and drawn. The sections were later Nissl stained and the same areas were photographed again in the transmitted light.

Monodelphis and *Didelphis* are closely related (Corbet and Hill 1991), therefore we made the assumption that the thalamic nuclei in *Monodelphis* are basically homologous to those in *Didelphis* (Oswaldo-Cruz and Rocha-Miranda 1967, 1968) and decided to use the names of the nuclei established for *Didelphis*.

RESULTS

Injections

As there is no established stereotaxic atlas for *Monodelphis*, we had to use a few slightly different coordinates of injections, which caused the differences in placement of the centres of injections. In addition, the small size of the thalamus in *Monodelphis* did not allow for placing the injections into separate nuclei, even though the volumes of injected dyes were small.
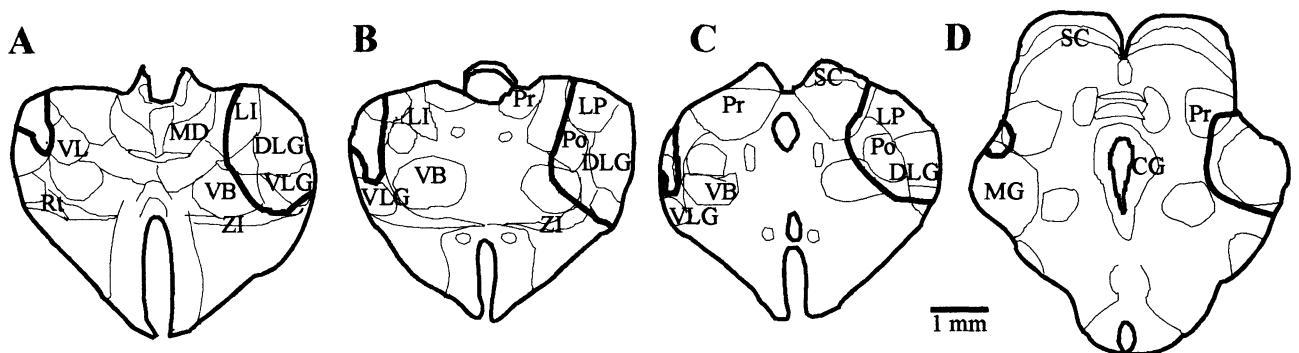

Fig. 1. Reconstruction of injection sites in the opossum O-1. Sections at the level of characteristic structures were chosen. The parts of thalamus and midbrain surrounded by the thick line were permeated by the spreading dye. Fluorogold was injected on the right side and Fast Blue on the left side. Abbreviated names of the nuclei: LI, lateral intermediate nucleus; LP, lateral posterior nucleus; DLG, dorsal lateral geniculate nucleus; VLG, ventral lateral geniculate nucleus; Rt, reticular thalamic nucleus; MD, mediodorsal nucleus; VI, ventral intermediate nucleus; VB, ventrobasal nucleus; ZI, zona incerta; SC, superior colliculus; Pr, pretectum; Po, posterior thalamic nucleus; MG, medial geniculate nucleus; CG, central grey.

Out of the six injections the most selective was the Fast Blue (FB) injection in the animal O-1, encompassing almost exclusively the visual nuclei of the dorsal thalamus - DLG, VLG and the lateral parts of LP and LI (Fig. 1). In addition, a small piece of the medial geniculate nucleus (MG) and the pretectal nucleus of the optic tract (OT) was covered by the dye. Two injections were too medial to include the whole DLG, but they included most of the LP and LI: injection of Fluorogold (FG) in the animal O-3 was centred in the ventral posterior nucleus (VP) and medial geniculate body (MG), involving most of the LP and LI and a small medial part of DLG and VLG; injection of FB in the animal O-2 was centred in LP and LI and did not spread to DLG or VLG but involved also the dorsal part of VB and nucleus posterior (Po) and the rostral part of pretectum.

Two other injections (FG injection in O-1 and FB injection in O- 3) were large. The dyes spread along most of the dorsolateral thalamus, including DLG, LI and LP, but also dorsal parts of the ventrolateral nucleus (VL), VB and MG (Fig.1). Differential evaluation of the results of all those injections was taken into account in this description of the central visual projections to DLG and LP - LI. A small injection of FG in the animal O-2 was centred in the hypothalamus and was excluded from the present analysis.

Thalamic projection of the visual cortex

The neocortex in *Monodelphis* has a similar thickness to that in mouse (1.0-0.8 mm) whereas the neocortex of rat is much thicker (1.6-1.4 mm). It has a proportionally thick layer 1, very dense layer 2 and characteristic parallel rows of cells in the layer 6. All cortical layers are easily recognizable. White matter of the internal capsule is very thin (see Fig. 2A). Both layer 1 and the white matter contain a sparse neuronal population (Fig. 2A).

Injection of FB in the animal O-1 involved selectively DLG and lateral parts of LI and LP. These nuclei are the target of the cortico-thalamic projection of the striate and peristriate cortex in the *Didelphis*

(Benevento and Ebner 1970, 1971) and rat (for review see Sefton and Dreher 1985). Retrograde labelling resulting from this injection was found exclusively in cortical layers 5 and 6, and in some sections exclusively in the layer 6 (Fig. 2B). In the layer 5 all of the labelled neurones were of the pyramidal type, with the cell body size of 8-10 μm (Fig. 2B and C). In the layer 6 the majority of the retrogradely labelled neurones were pyramids positioned in 3-5 horizontal rows occupying the upper part of the layer (Fig. 2B and C). Very few of the labelled neurones in this layer were placed above the rows and only 20-30% of them were found below the rows, where a mixed population of pyramidal, inverted pyramidal and fusiform neurones was present (Fig. 2A, B and C). Labelled neurones were not found in the thin layer of white matter below layer 6, even though on the Nissl stained preparations some cells of neuronal morphology were visible there. Judging by the distribution of the retrograde labelling of cortical neurones resulting from this injection, primary visual cortex in *Monodelphis* is located in the posterior, latero-dorsal part of neocortex (Fig. 3). This is the thickest area of the *Monodelphis* cerebral cortex.

Injection of FB in the animal O-2, where the dye spread in the LP, LI and VB and the injection of FG in the O-3 that covered LP, LI, VP and MG showed strikingly different results: in the dorsal and posterior part of the cerebral cortex (in the area where the retrograde labelling was found in layer 6 after the previously described injection) the retrogradely labelled neurones were found exclusively in layer 5. Again, the topography of this area corresponded with the topography of striate cortex in *Didelphis* (Oswaldo-Cruz and Rocha-Miranda 1967, Donaghue and Ebner 1981) and hedgehog (Kaas et al. 1970, Gould et al. 1978). In the more rostral and lower regions of cortex (where the peristriate, but also somatosensory and auditory areas are most probably placed) labelled neurones were found exclusively in layer 6. The border between the two different projections was very sharp (Fig. 3). One possible interpretation of this abrupt change is that it may show because of the different projection pat-
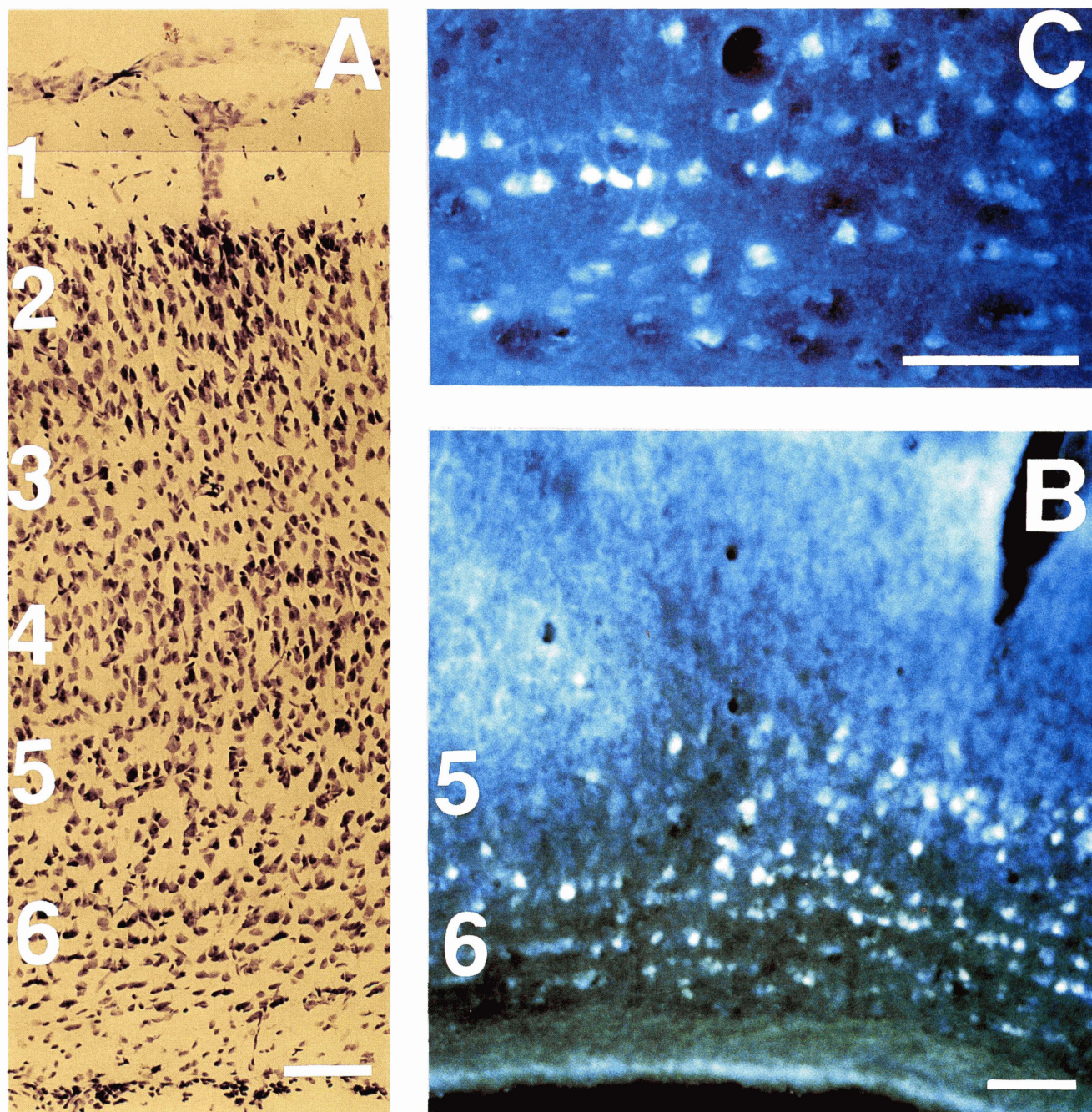
Fig. 2. Photomicrographs of coronal sections of the cerebral cortex. A, Nissl stained section showing the layers of the *Monodelphis* cortex. The photomicrograph was taken at the presumed border of the striate cortex and the peristriate belt, exactly in the same place as the centre of (B). B, cortical neurones retrogradely labelled with the Fast Blue injected in DLG and LP. Note that the labelling on the right side of the photographed area is placed in layers 5 and 6, whereas on the left side it is found only in layer 6. C, higher power photomicrograph of the retrogradely labelled neurones in the cortical layer 6. Note the rows of pyramids in layer 6a and a mixed population of the labelled neurones in the layer 6b. All scale bars equal 100 μm.

terns of various cortical areas to the same thalamic nuclei, and in this case it shows the border between the primary and the secondary visual areas (cf.Fig. 4 and Benevento and Ebner 1971). If this interpretation is true, then it would mean that the thalamic projection of layers 5 and 6 of the striate cortex in
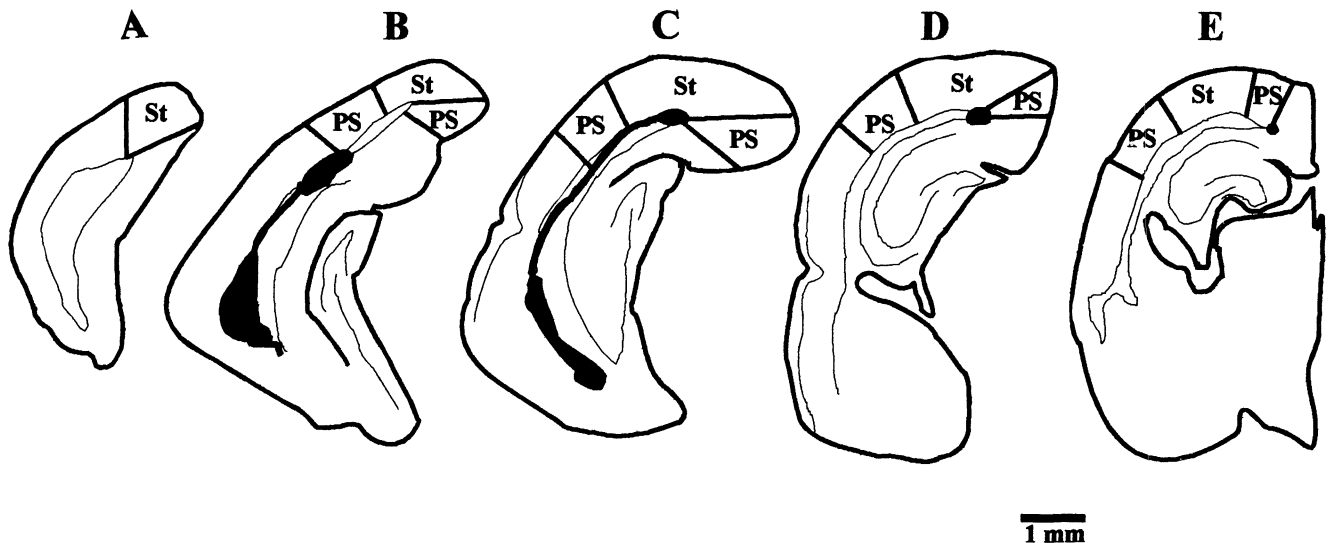
Fig. 3. Drawings of 40 μm coronal sections through the visual cortex of *Monodelphis*, showing the placement of the primary visual cortex (St) and the peristriate belt (PS), as it was defined by the projections of layers 5 and 6 to the thalamus (for details - see Results). The series progresses from caudal (A) to rostral (E).

Monodelphis reaches different nuclei, as it is also true in the rat (Mason and Groos 1981, Sefton and al. 1981, Hubner and Bolz 1988, Turlejski et al. 1993).

The two large injections (FG in O-1 and FB in O-3) resulted in massive labelling of neurones in the layers 5 and 6 in most of the region of cortex investigated, including the postero-dorsal area. No labelling was ever observed in any other cortical layer. At the border of layers 5 and 6 labelled neurones were very scarce (Fig. 2B). This pattern of thalamic projections is strikingly similar to the one found in rat (cf. Sefton and Dreher 1985, Turlejski et al. 1994). One difference that we observed was the density of the labelled neurones, which was much lower in *Monodelphis* than in rat, even following our largest injections. This did not seem to depend on the lower density of neurones in the cortex (Fig. 2A), which on the Nissl stained sections was comparable to the one in rat.

Superior colliculus

The superficial gray layer of the superior colliculus (CS) of *Monodelphis* is well developed and sep-

arated from the lower layers by the optic nerve layer (Fig. 4B). The selective FB injection in the DLG/LP in the opossum O-1 resulted in the retrograde labelling almost exclusively limited to the zonal (Zo) and superficial gray (SuG) layers of the ipsilateral superior colliculus (Figs. 4A and 5). Even large thalamic injections labelled only a few neurones in the superficial gray and optic nerve layers of the contralateral side. However, the density of the labelled neurones in CS was low. In animals with the large injections, on a section through the central part of CS we could count about 100 labelled neurones in the superficial layers of CS, whereas comparable injections in rat yielded a few hundred labelled neurones per section (Turlejski et al. 1994).

Parabigeminal nucleus (PBG)

This nucleus is known to project to DLG and CS (Baleydier and Magnin 1979, Menendez-Otero et al. 1980, Sefton and Martin 1984, Baizer et al. 1991, Harting et al. 1991b). Our description of this projection is based on the results of the three injections that covered whole DLG: both injections in the O-1 and the FB injections in O-3. These were the
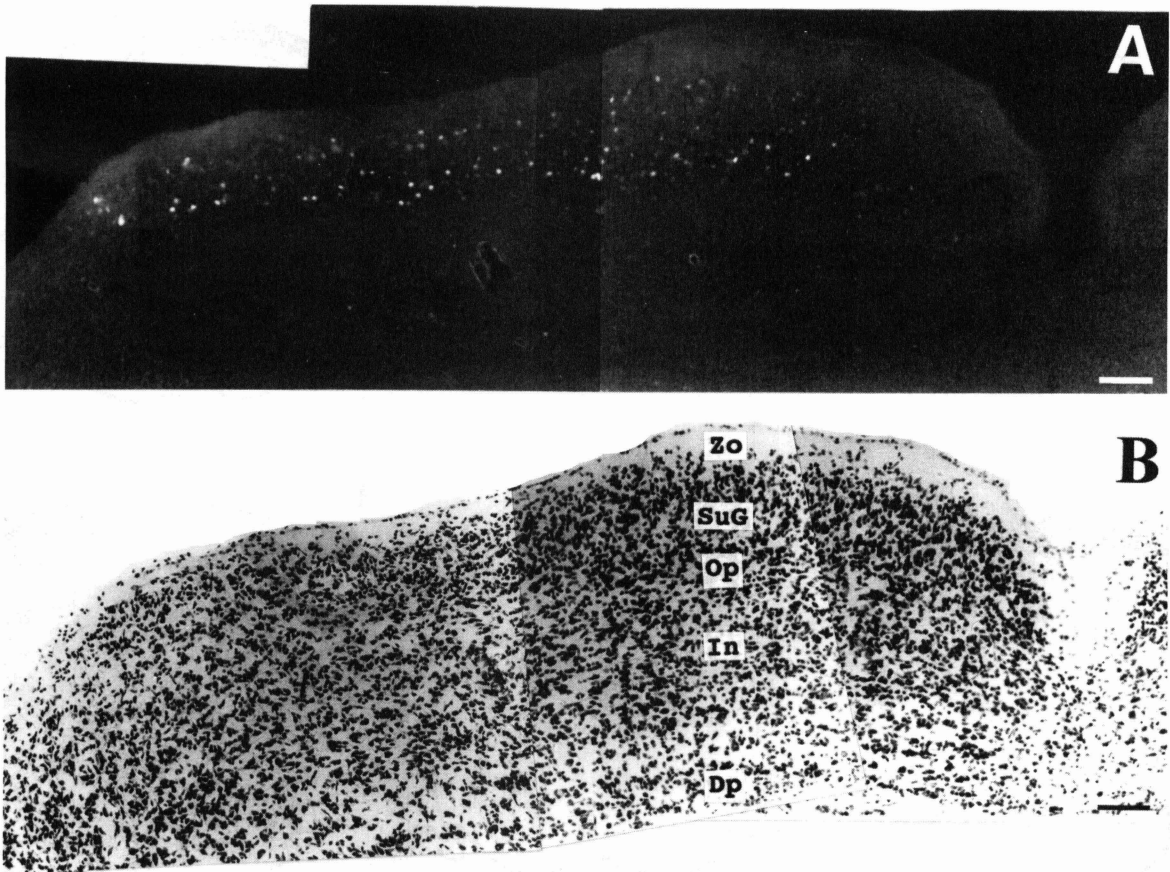
Fig. 4. Photomicrographs of a section through the centre of the left superior colliculus. A, cells retrogradely labelled with Fast Blue are placed in the superficial gray, and to a lesser extent in the zonal layers of CS. B, CS on the same section photographed after Nissl staining. Zo, zonal layer; SuG, superficial gray layer; Op, optic nerve layer; In, intermediate layer; Dp, deep layer. Scale bars equal 100 µm.

only injections that resulted in labelling neurones in the PBG. The retrogradely labelled neurones of PBG constituted a clearly demarcated group in the lateral wall of tegmentum (Figs. 5 and 6), with no other labelled neurones in their vicinity (Fig. 6A).

The rostro-caudal extent of PBG was calculated first by counting the number of sections on which the retrogradely labelled projection of PBG to DLG was visible (3 cases) and then again by counting the Nissl stained sections on which we could delineate it (Fig. 6B). The differences of calculations with the two methods were from 0 to 2 sections. Both measures showed the extent of the PBG to be 920-1,000 µm, which was similar to that in the rat (Turlejski et al. 1993). Rostrally PBG started sharply, reaching sizable dimensions in the first 100 µm.

(Fig. 6C). Its largest cross-section was observed about 300 µm from the rostral end. The size of the nucleus slowly decreased for the next 300 µm and then was quickly reduced to a narrow "tail" that occupied the last 300-400 µm. Altogether from 700 to 800 neurones retrogradely labelled from DLG were counted in the contralateral PBG. The rostral 600 µm (the "body") contained more than 90% of those neurones and all of them projected exclusively contralaterally. In the "tail" the number of the labelled neurones was very low, but some of them were labelled from the ipsilateral DLG. On the few caudal-most transections of PBG only the ipsilaterally labelled neurones were found. Altogether, the ipsilaterally projecting neurones constituted 4% of the labelled population. No double-labelled (and there-
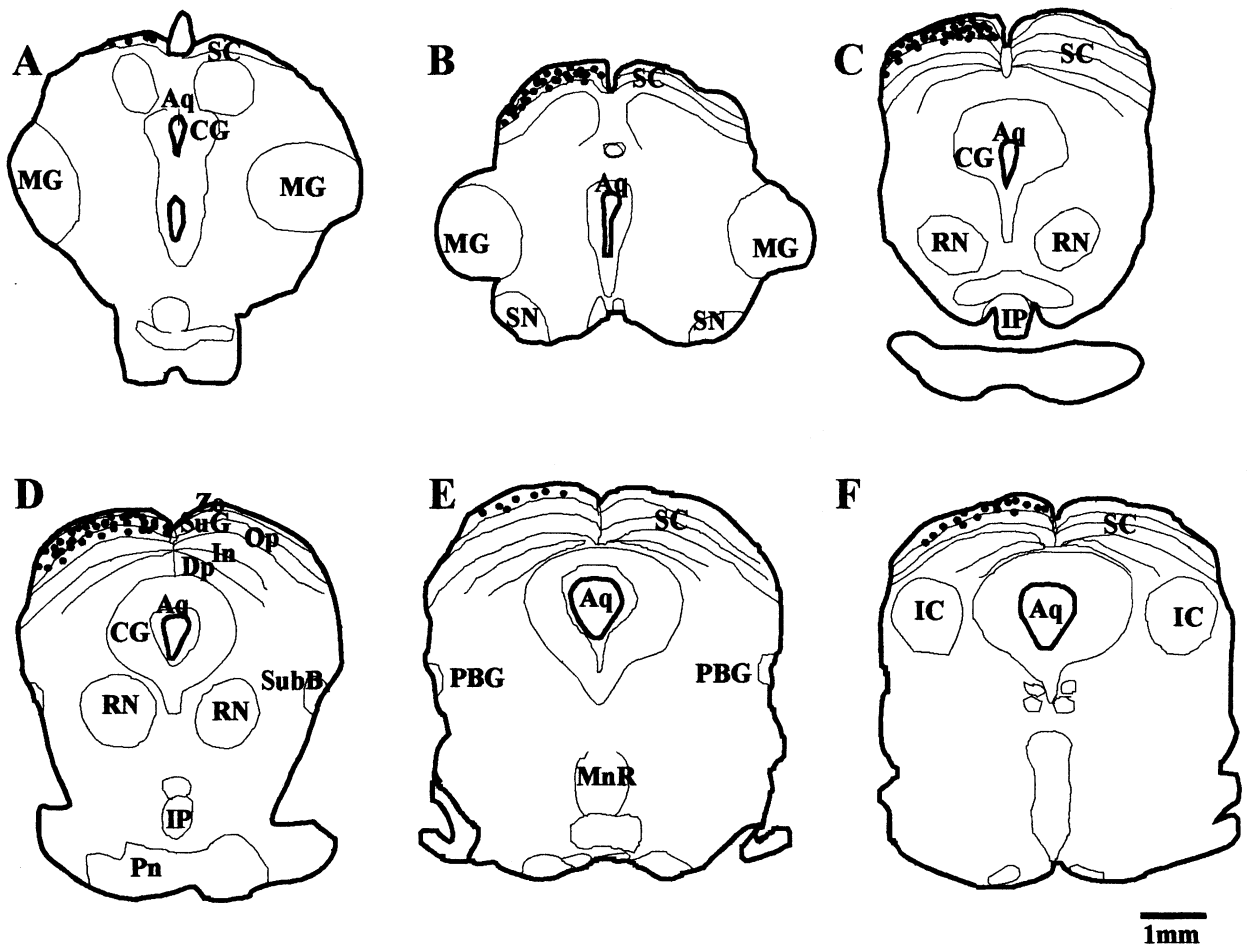
Fig. 5. Drawings of coronal sections showing the retrogradely labelled neurones in the superior colliculus of the opossum O-3. They were found only in the superficial layers of the superior colliculus ipsilateral to the Fast Blue injection. Every dot represents two labelled neurones. The series proceeds from rostral (A) to caudal (F). In the contralateral parabigeminal nucleus (section E) 35 FB labelled neurones were found. These could not be marked due to the small size of the nucleus. Aq, aqueduct; CG, central gray; Dp, deep layers; IC, inferior colliculus; In, intermediate layers; IP, interpeduncular nucleus; MG, medial geniculate nucleus; MnR, median raphe nucleus; PBG, parabigeminal nucleus; Pn, pontine nucleus; RN, red nucleus; SC, superior colliculus; SN, substantia nigra; SubB, subbrachial nucleus; SuG, superficial gray layer; Zo, zonal layer.

fore bilaterally projecting) PBG neurones were observed. On every section there were also PBG neurones that were not labelled by our injections. Therefore, it is probable that the projection of the PBG to CS is derived (at least in a significant part) from a different subset of PBG neurones.

DISCUSSION

These preliminary data on the central afferents of the thalamic visual nuclei in the marsupial *Mono-*

delphis domestica show striking hodological similarities with the homologous connections in both Metatherian (Menendez-Otero et al. 1980, Harting et al. 1991a,b) and Eutherian mammals, especially insectivores and rodents (cf. Hall and Diamond 1968, Sefton and Dreher 1985, Garey et al. 1991, Harting et al. 1991a,b, Turlejski et al. 1993).

Visual cortex

The presently described differential projection of layers 5 and 6 of the posterodorsal part of neo-
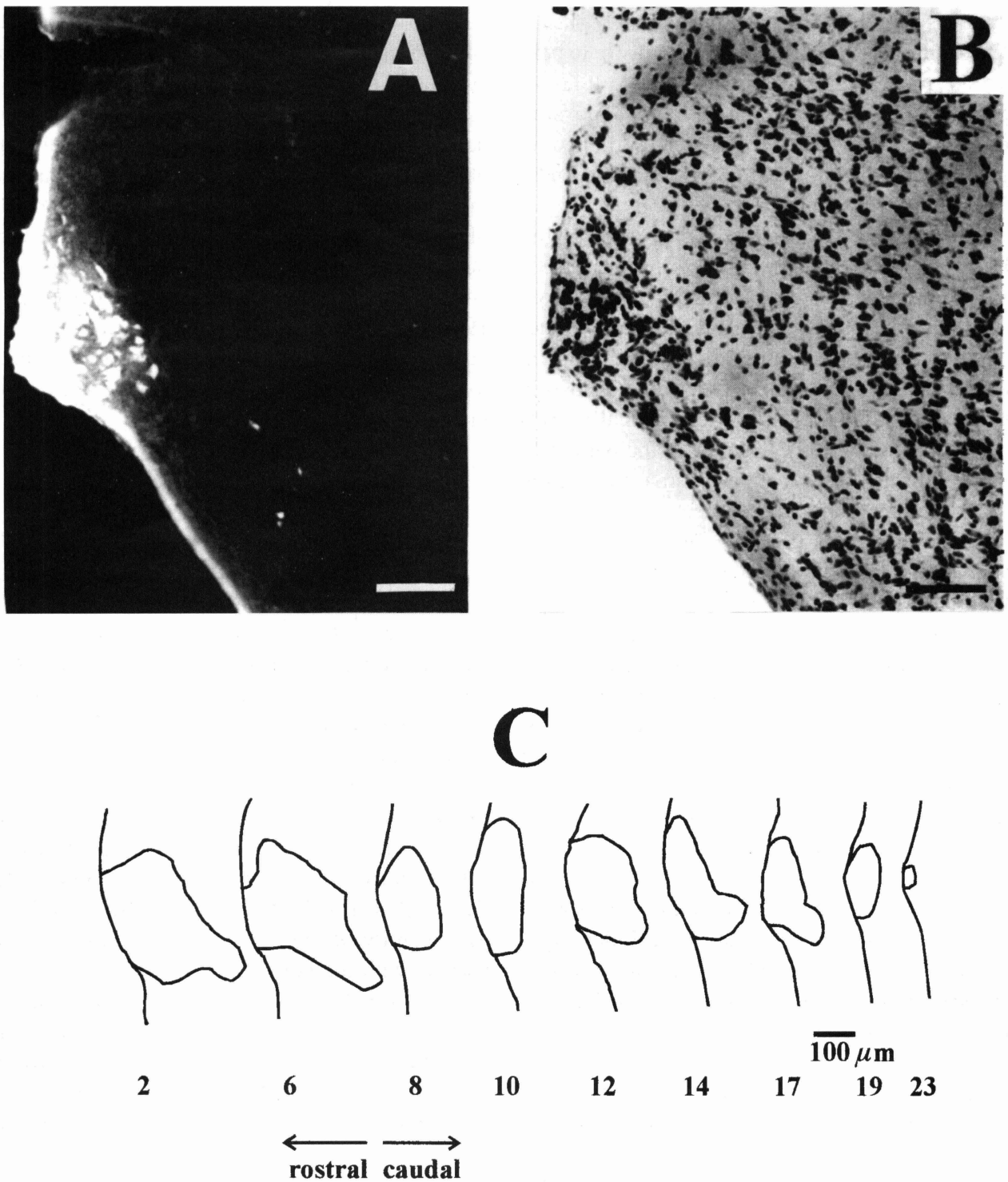
Fig. 6. Parabigeminal nucleus. A, neurones in PBG retrogradely labelled with the contralateral thalamic injection of Fluorogold. B, the same section photographed after the Nissl staining. C, serial reconstruction of the PBG nucleus. Numbers under drawings show the number of consecutive 40 μm section, counting from the rostral, on which the PBG was localized. All scale bars equal 100 μm.

cortex in *Monodelphis domestica* is a partial proof that the visual cortical area in this mammal consists of the primary, striate area and the extrastriate belt. They are placed in the region similar to that occupied by visual cortex in the *Didelphis* opossum (Bodian 1935, Benevento and Ebner 1970), hedgehog (Hall and Diamond 1968) mouse (Caviness 1975) or rat (Zilles and Wree 1985).

Thalamic projections of the layers 5 and 6 of visual cortex in *Monodelphis* reach different targets. They are interspersed with a narrow band of very sparse thalamic projection. Such a band was described in the rabbit (Reblet et al. 1992) and rat (Turlejski et al. 1993) and showed to contain mainly neurones of the ipsilateral cortico-cortical projection (Reblet et al. 1992). Therefore, the pattern of projection of the infragranular layers is uniform in widely different mammalian orders.

One major peculiarity of the cerebral cortex in *Monodelphis* is the cytoarchitectonics of its layer 6, which in most of the temporal and occipital cortex consists of parallel rows of neurones. Our investigations of both the stratification of retrograde labelling and the cytoarchitectonics of the Nissl stained cortex led us to the conclusion that the rows of neurones constitute the layer 6a, as just above them there is the zone of a sparse thalamic projection characteristic for the border of layers 5 and 6 (Reblet et al. 1992, Turlejski et al. 1993) and just below them there is a thin layer where normal pyramids, fusiform neurones and inverted pyramids are found. This composition is characteristic for cellular population derived from the subplate, remnants of which form the sublayer 6b in rodents (Tömbol 1984, Ferrer et al. 1986, Reep and Goodwin 1988, Valverde et al. 1989). This population is significantly older than the population forming sublayer 6a (Marín-Padilla 1978, Valverde et al. 1989, Woo et al. 1991). Therefore, we postulate that the layer 6 of cerebral cortex in the *Monodelphis* opossum may be divided into the sublayers a and b, which both project to the DLG.

Similar cytoarchitectonics of the layer 6 is visible in the cortex of another marsupial, the Tammar Wallaby. It was first described in the develo-ping brain of this species (Reynolds et al. 1985), but it is also visible in the temporal cortical region of the adult animals (Mayner 1989). The tendency to form horizontal rows of neurones in the cortical layer 6a may be also observed in rats during the first postnatal week (Turlejski and Djavadian, unpublished observations). In rat these rows later disappear; most probably cell bodies of the neurones are displaced "out of register" by the developing neuropil, but the cell death may be a complementary mechanism (Al-Ghoul and Miller 1989, Ferrer et al. 1990, Spreafico et al. 1994). Therefore, the horizontal rows of cells in the layer 6 may be an ancient cytoarchitectonic feature of the cerebral cortex, preserved in *Monodelphis*.

Superior colliculus

The colliculo-thalamic projection in *Monodelphis* was shown to follow a typical mammalian pattern (Diamond 1973, Sefton and Dreher 1985, Hartinng et al. 1991a), with a sparse, strictly ipsilateral projection of the superficial layers of SC to DLG and much more numerous projection of both superficial and deeper layers to the LD and LI. This pattern of colliculo-thalamic projection is homologous to that found in the *Didelphis* opossum (Rafols and Matzke 1970). However, the contralateral colliculo-thalamic projection in rat seems to be more substantial (Perry 1980, Mason and Groos 1981, Reese 1984, Takahashi 1985, Turlejski et al. 1993). The scarcity of the collicular projections in comparison with the homologous projection in the rat (Turlejski et al. 1994) is worth mentioning, together with the sparse cortico-thalamic projection. It may mean that the central control of the visual information flow throught DLG to the cortex is much more rudimentary and less specific in *Monodelphis* than in rat.

Parabigeminal nucleus

The pattern of PBG projection to DLG is similar to the homologous projection in *Didelphis* (Harting et al. 1991b) and several rodents (Harting et al.

1991b, Turlejski 1993) and is characteristic for mammals with the laterally positioned eyes. It is also similar, although not identical, to the parabigemino-collicular projection in the *Didelphis* (Menendez-Otero et al. 1980), where the ipsilateral component is larger and some of the ipsilaterally projecting neurones were found in the "body" of the nucleus, although the majority of them was positioned in the "tail". Therefore, we postulate that the PBG in the *Monodelphis* is organized visuotopically, at least to some extent, with the representation of the central field of view (in *Monodelphis* perceived by the posterior, temporal rim of the retina) in the caudal part of the nucleus.

General remarks

The central visual system of *Monodelphis domestica* seems to consist of nuclei and areas that are typical for all mammals. Their connections and placement are also typical for animals with lissencephalic brains and poorly developed visual system (Kaas et al. 1980, Ferrer et al. 1986). Therefore, *Monodelphis* is a suitable species for investigations of the early phases of development of central nervous system and cortical conectivity in particular. It may be easily compared with the rat, but accessibility of the pups of *Monodelphis* at a much earlier stage of development opens new possibilities for experimental manipulations and testing of some general hypotheses (Dreher and Robinson 1988). However, further investigations are needed to find the exact homologies of cortical areas and layers, their connections and phases of development between the Metatherian and Eutherian clads.

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