

## Short Communications

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### New thalamic visual nuclei in lizards

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As many as 5 distinct, dorsal thalamic cell populations receive retinal projections in birds<sup>25,32</sup>. At least 4 and perhaps all 5 of these cell populations project to the visual Wulst in the telencephalon<sup>19,21,25,26,31</sup>. However, most retinal projection studies in reptiles, utilizing the anterograde degeneration technique, have identified only a single target in the dorsal thalamus<sup>8,16</sup>. These studies would suggest that the avian visual thalamus can be characterized by an increase in the number of visual nuclei. However, as more recent studies have indicated that more than one dorsal thalamic retinal target may also be present in reptiles<sup>2,4,27–29</sup>, a re-examination of the retinal projections in two lizards, utilizing anterograde axonal transport of tritiated proline, was undertaken. A preliminary report of these data has been published elsewhere<sup>10</sup>.

Two juvenile specimens of *Iguana iguana* (12 and 15 cm snout-vent length) and three adult specimens of *Gekko gekko* (12–14 cm snout-vent length) received intra-ocular injections of 40–60  $\mu\text{Ci}$  of L-[4,5-<sup>3</sup>H]proline, 9.4  $\mu\text{Ci}/\mu\text{l}$ , under sodium pentobarbital anesthesia (20 mg/kg, i.p.). Injections were carried out with a 10  $\mu\text{l}$  Hamilton syringe and 26-gauge needle fitted with a polyethylene sleeve which limited penetration into the eye to 3 mm. Aqueous radioactive solutions were evaporated to dryness with nitrogen to remove <sup>3</sup>H<sub>2</sub>O and redissolved in 0.86% saline immediately before use. Following postoperative survival times of 1 and 6 days for the iguanas and 1–3 days for the geckos at 28 °C, the animals were perfused with AFA (90 ml of 80% ethanol, 5 ml formalin, and 5 ml glacial acetic acid). The brains were removed and stored in AFA for at least one week prior to dehydration and embedding in paraffin. The brains were then cut at 15  $\mu\text{m}$  in the transverse plane.

Autoradiographic procedures followed those described by Kopriwa and Leblond<sup>24</sup>. Kodak NTB3 nuclear track emulsion was diluted 1:1 with distilled water at 40 °C, and the slides were dipped into the emulsion and dried for approximately 1 h. Following an exposure time of 20 days, the slides were developed in Kodak Dektol and stained with cresyl violet.

With the autoradiographic method, the pattern of retinal projections to the

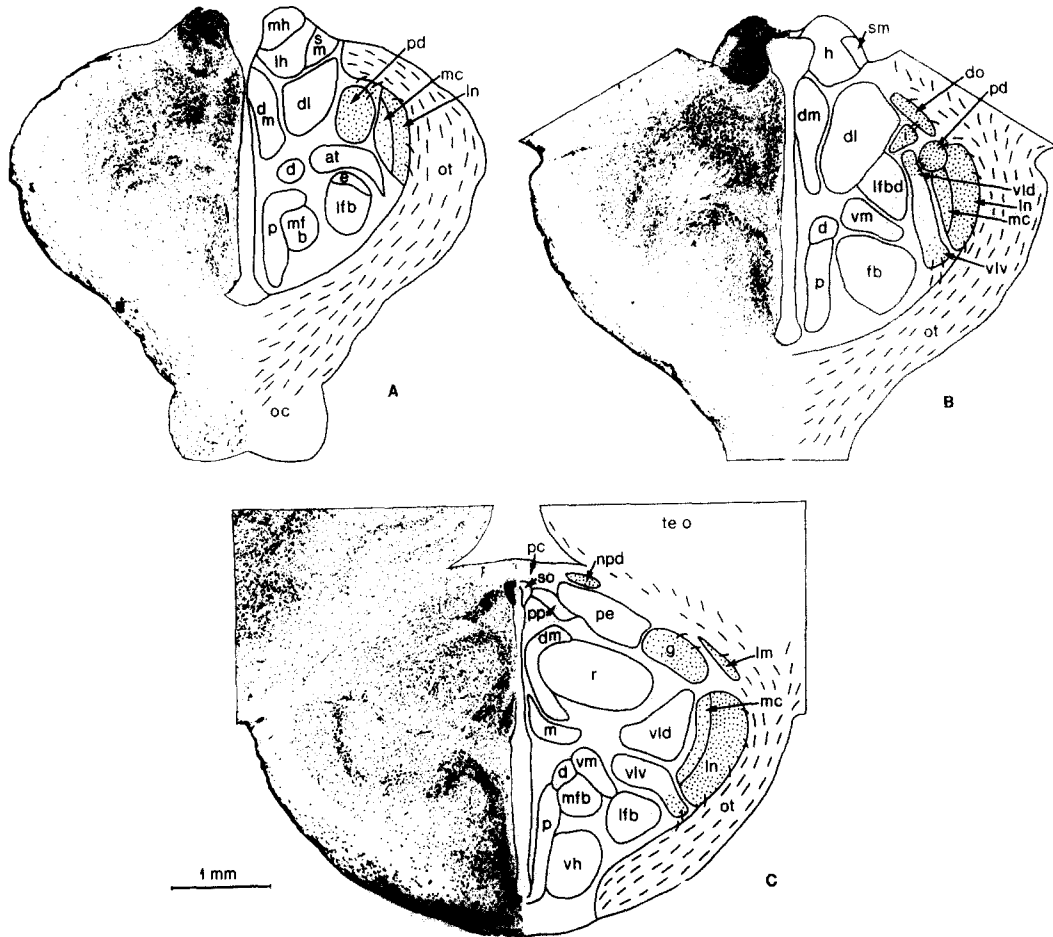


Fig. 1. Nissl photomicrographs of transverse sections (left) and chartings (right) of the contralateral component of the retinofugal projections to the thalamus and rostral pretectum in *Iguana iguana*. Fibers are indicated by dashes and areas of grain density interpreted to be over terminal fields by stippling. The sections are through the levels of the rostral thalamus (A), the rostral thalamus slightly caudal to that in A (B), and the rostral pretectum (C). Projections to the tectum, previously published<sup>8</sup>, have not been illustrated in C. Abbreviations: at, area triangularis; c, nucleus dorsocentralis; d, dorsolateral hypothalamus; dl, nucleus dorsolateralis; dm, nucleus dorsomedialis; do, dorsal optic nucleus; e, nucleus entopeduncularis; f, fasciculus retroflexus; fb, forebrain bundles; g, nucleus geniculatus prepectalis; h, habenula; i, nucleus intercalatus thalami; lfb, lateral forebrain bundle; lfbd, dorsal peduncle of lateral forebrain bundle; lfbv, ventral peduncle of lateral forebrain bundle; lh, lateral habenula; lm, nucleus lentiformis mesencephali; ln, lateral neuropil of nucleus geniculatus lateralis pars ventralis; m, nucleus medialis; mc, medial cell plate of nucleus geniculatus lateralis pars ventralis; mfb, medial forebrain bundle; mh, medial habenula; mp, nucleus medialis posterior; npd, nucleus posterodorsalis; oc, optic chiasm; ot, optic tract; p, nucleus periventricularis; pc, posterior commissure; pd, nucleus geniculatus lateralis pars dorsalis; pe, nucleus lentiformis thalami pars extensa; pp, nucleus lentiformis thalami pars plicata; r, nucleus rotundus; sm, stria medullaris; so, subcommissural organ; te o, optic tectum; vh, ventral hypothalamus; vl, nucleus ventrolateralis; vld, nucleus ventrolateralis pars dorsalis; vlv, nucleus ventrolateralis pars ventralis; vm, nucleus ventromedialis.

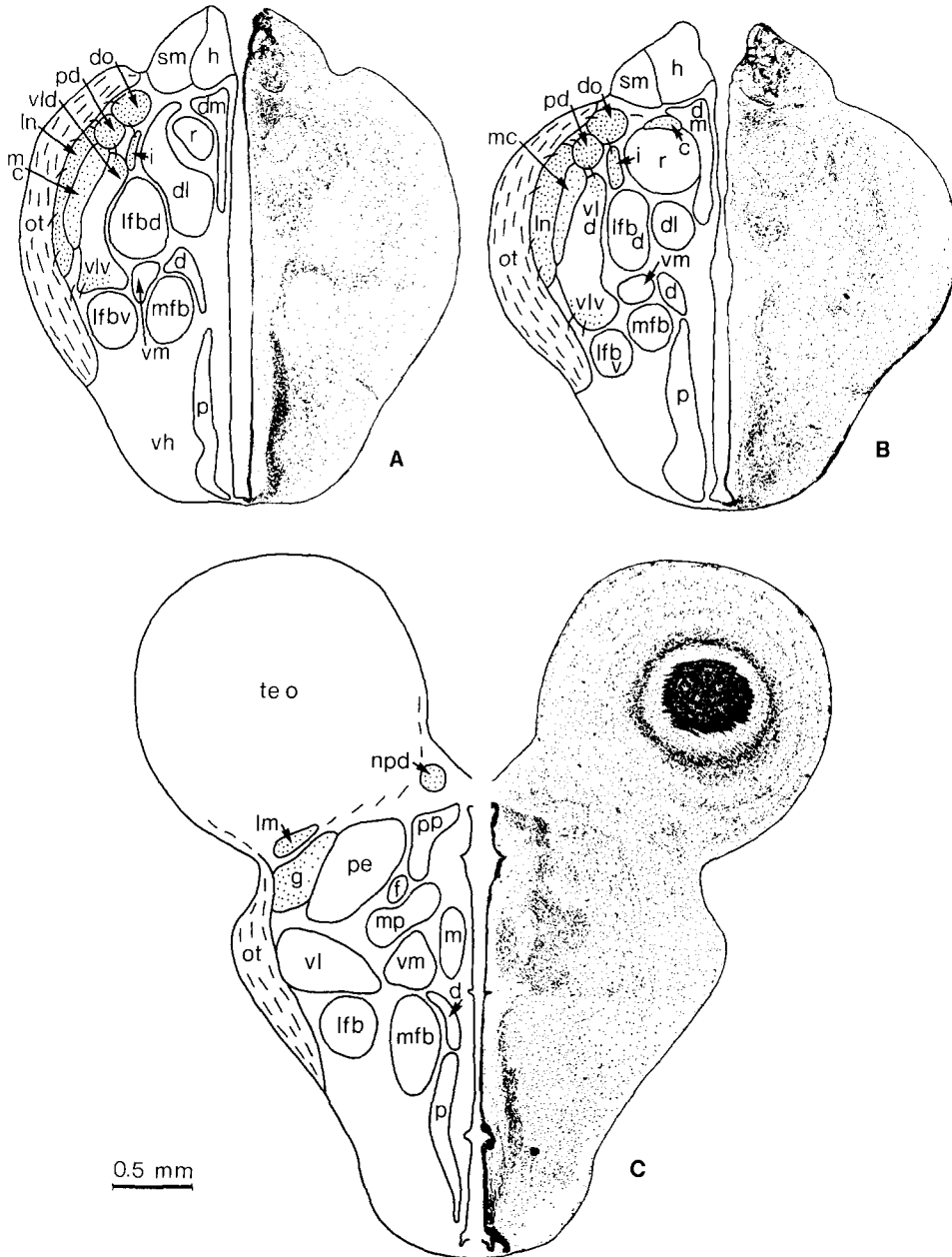


Fig. 2. Nissl photomicrographs of transverse sections (right) and chartings (left) of the contralateral component of the retinofugal projections to the thalamus and rostral pretectum in *Gekko gecko*. Fibers are indicated by dashes and areas of grain density interpreted to be over terminal fields by stippling. The sections are through the levels of the rostral thalamus (A), the thalamus at the level of nucleus rotundus (B), and the rostral pretectum (C). Projections to the tectum, previously published<sup>29</sup>, have not been illustrated in C. Abbreviations as in Fig. 1.

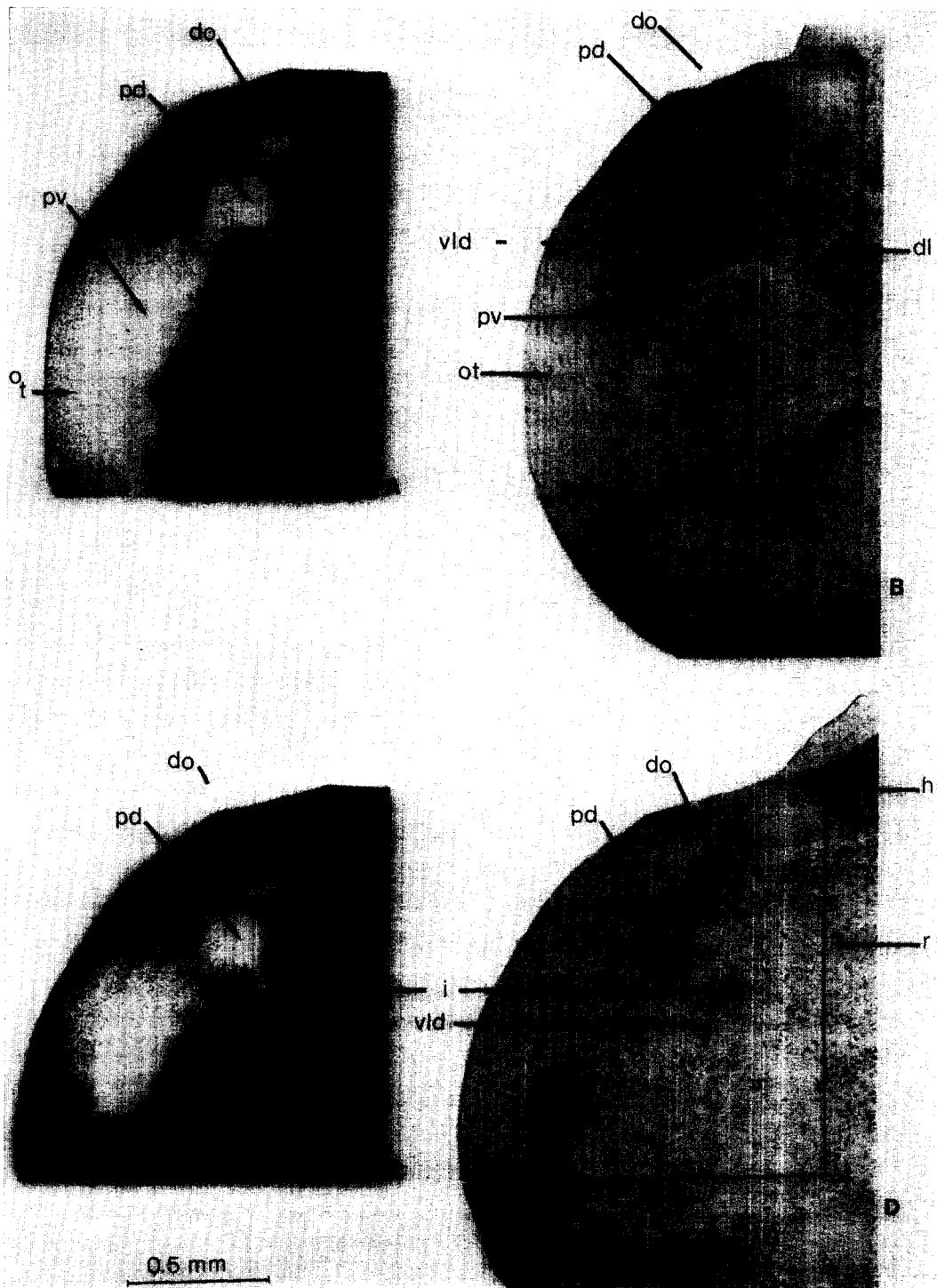


Fig. 3. Photomicrographs of contralateral retinofugal projections to the thalamus in *Gekko gecko* in transverse sections. A and B: dark-field and bright-field photomicrographs, respectively, through the rostral thalamus. Note particularly the intense density of grains over the dorsal optic nucleus (do), the pars dorsalis (pd), and the dorsal part of nucleus ventrolateralis (vld). The large cells of nucleus dorsolateralis (dl) do not appear to receive a retinal projection. C and D: dark-field and bright-field photomicrographs, respectively, through the rostral thalamus, at a level slightly caudal to that in A and B. Note particularly the intense density of grains over the dorsal optic nucleus (do), the pars dorsalis (pd), nucleus intercalatus thalami (i), and the dorsal part of nucleus ventrolateralis (vld).

thalamus could be more finely resolved than in our previous anterograde degeneration studies<sup>8,29</sup>. In particular, several distinct additional targets were found, and this pattern of projections was similar in both Iguana and Gekko. The contralateral components of the projections are illustrated in Figs. 1–3. Fibers course dorsally in the optic tract (ot), and turn medially to terminate in the ventral part of nucleus ventrolateralis (vlv), the medial cell plate (mc) and lateral neuropil (ln) of nucleus geniculatus lateralis pars ventralis (pv), and nucleus geniculatus lateralis pars dorsalis (pd), as previously described<sup>8,29</sup>. Fibers also terminate in a distinct region dorsal to the pars dorsalis, the dorsal optic nucleus (do). This region was originally included in our chartings of degeneration in Iguana<sup>8</sup> and Natrix<sup>30</sup> as part of the pars dorsalis, and we also charted degeneration here, dorsal to the pars dorsalis, in Gekko<sup>29</sup> and Xantusia<sup>6</sup>.

Retinal fibers then pass medially, through pars dorsalis, to terminate in the dorsal part of nucleus ventrolateralis (vld). Other fibers traverse the dorsal optic nucleus to reach two additional targets — nucleus intercalatus thalami (i) and nucleus dorso-centralis (c). The latter nucleus is very small and forms a dorsal cap to nucleus rotundus (r); it is shown only in Fig. 2B.

From this analysis, the retinofugal thalamic targets can be divided into outer and inner tiers. The outer tier is comprised of nucleus ventrolateralis pars ventralis, nucleus geniculatus lateralis pars ventralis and pars dorsalis, and the dorsal optic nucleus. These nuclei all receive *bilateral* retinal projections. The inner tier is comprised of nuclei ventrolateralis pars dorsalis, intercalatus thalami, and dorso-centralis, and these nuclei receive only *contralateral* retinal projections.

The pattern of terminations in the pretectum, tectum, and nucleus opticus tegmenti was almost identical to that previously described<sup>8,29</sup> and will thus not be discussed in detail here. The only difference was that nucleus lentiformis mesencephali (lm, Figs. 1C and 2C) was found to occupy a more restricted region in the lateral aspect of the pretectum than described before in Iguana<sup>8</sup>.

The problem of transneuronal transport of tritiated proline, particularly to the inner tier group of nuclei, was considered and is argued against by several factors. First, the survival times (1–3 days for most animals) are shorter than times needed to demonstrate transneuronal transport in other non-mammalian vertebrates<sup>33</sup>. Second, no known secondary visual targets, such as nucleus rotundus or nucleus isthmi<sup>7,9,14</sup>, contained grains above background. Third, the grain densities over the inner and outer tiers are uniformly much higher than over the optic tract, suggesting that all of the tier sites are terminal fields. Finally, re-examination of anterograde degeneration material revealed the presence of sparse terminal argyrophilia in all of these regions.

We thus feel confident that there are indeed multiple visual targets in the thalamus of these lizards. These data, in addition to similar recent findings in other reptiles and birds, cited above, raises questions concerning the evolution of the visual thalamus within the sauropsid radiation.

Data from initial studies<sup>16,17,22,31</sup> suggested that reptiles and birds have retinothalamic and thalamotelencephalic projections which are homologous to the geniculostriate visual system in mammals. The present data do not contradict this hypothesis, but do indicate that the visual projections in these animals are more complex than previously realized.

In the side-necked turtle, Podocnemeis, Knapp and Kang<sup>23</sup> recognized 3 subdivisions of the ventral geniculate nucleus and also described a dorsal geniculate nucleus which probably corresponds to the dorsal optic nucleus discussed here. In the garter snake, *Thamnophis*, Halpern and Frumin<sup>18</sup> described a dorsomedial part of the dorsal geniculate nucleus which may correspond to the dorsal optic nucleus. Multiple retinal targets in the thalamus, including the dorsal optic nucleus, have been found in the rhynchocephalian *Sphenodon*<sup>28</sup>, the turtles *Caretta*<sup>4</sup> and *Chrysemys*<sup>2</sup>, and the crocodile *Caiman*<sup>5</sup>. A number of studies in birds have also demonstrated multiple, discrete retinofugal thalamic targets<sup>20,21,25,32</sup>.

Telencephalic projections of these thalamic retinal targets are similarly complex. In birds, a number of them project to the visual Wulst, and the individual projections are differentially ipsilateral, contralateral, or bilateral<sup>19,21,25,26</sup>. Recent electrophysiological studies in both the lizard *Gekko*<sup>1</sup> and the turtle *Chrysemys*<sup>3</sup> have also revealed the presence of multiple visual projection sites in the telencephalon.

In view of these findings of more than one thalamic visual target in reptiles and birds, it is interesting to note that two targets — LGNa and LGNb — were described in monotremes by Campbell and Hayhow<sup>12,13</sup>. LGNa lies in the ventral thalamus in the echidna<sup>12</sup> but is in the dorsal thalamus in the platypus<sup>13</sup>, and LGNb lies dorso-rostral to LGNa<sup>12,13</sup>. The phylogenetic position of the monotremes in relation to both reptiles and therian mammals is still unclear<sup>12</sup>, and direct comparisons of LGNa and LGNb with nuclei seen in the thalamus of reptiles and birds cannot, at present, be made. However, we can observe that the character of multiple visual thalamic sites is common to both monotremes and members of the reptilian lineage.

Within the mammalian radiation, Campbell<sup>11</sup> has demonstrated that discrete laminae within the dorsal lateral geniculate nucleus have evolved independently in a number of orders of mammals. It is possible that the retinal targets in the dorsal thalamus of reptiles and birds may represent similar, independently evolved subdivisions of the dorsal geniculate nucleus. A second possibility is that some of these retinal targets do not correspond to any nuclear regions in mammals but are, rather, unique to reptiles and birds. Thirdly, additional retinofugal targets may exist in mammals which have not yet been discovered.

Thus, while one can make general comparisons of thalamofugal visual pathways in reptiles and birds with the mammalian geniculostriate system<sup>15,21</sup>, the connections of the multiple thalamic visual nuclei in these animals require more detailed study. It would appear that the organization of the visual pathways in reptiles and birds is far more complex than originally thought, and that the visual system in these animals cannot therefore serve as a simple model of the primitive mammalian condition. Rather, the organization and evolution of visual pathways in therian mammals can only be understood in a larger context by comparisons with evolutionary trends which have occurred in reptiles and birds, monotremes, and the anamniote vertebrate radiations.

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