

## BRAIN ORGANIZATION AND RETINAL PATHWAYS IN THE SLEEPY LIZARD, *TILIQUA RUGOSA*

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### Abstract

Brain structure and retinal pathways to the brain of the sleepy lizard *Tiliqua rugosa* were described, with this species identifiable as a type II lizard according to brain organization. The retinal pathway appeared entirely crossed to the opposite side of the brain with termination of retinal fibres observed in the optic thalamus, pretectum, tectum and brainstem tegmentum.

KEY WORDS: *Tiliqua rugosa*; sleepy lizard; brain organization; retinal pathways.

### Introduction

The sleepy lizard *Tiliqua rugosa* Gray has been the subject of considerable study, including investigations extending for around 30 years on the ecology and behaviour by Bull and co-workers (Bull 1995), and on melatonin and pineal rhythms by Firth and co-workers (Firth *et al.* 1979). The importance of vision and light for *T. rugosa* have been demonstrated in studies which showed that light influences the plasma melatonin rhythm (Firth & Kennaway 1989), that lizards appear to be able to use visual cues for recognition of shelter sites (Zuri & Bull 2000), that colour vision is important for visual recognition of food (Wohlfeil 2008), and that polarized light is important for homing behaviour (Freake 1999) with the parietal eye implicated in this (Freake 2001). Knowledge of the organization of the brain and visual pathways in *T. rugosa* are clearly significant in describing the anatomy on which the physiology and behaviour of light and vision in this species depend. The anatomy of the pineal complex (Teo *et al.* 1993) and aspects of eye structure (Braekvelt 1989) have been reported. Recently New (2008) has described an all cone visual cell layer in *T. rugosa* and has estimated an overall panoramic field of view of 265° in this species with a binocular field of 15–17°. Here we provide information on overall brain structure and the organisation of the main visual pathways from the lateral eyes.

### Materials and Methods

Brain sections from six lizards were originally prepared in 1978 (Mayner 1978), collected under permit from the South Australian National Parks and Wildlife Service. At that time (1978), which was prior to the 1985 Animal Welfare Act, the School of Biological Sciences had a general ethics approval for animal research and it took oversight of projects which were being done under its authority to ensure that animals were treated humanely. The oversight included consultation with researchers in some cases to get modification of procedures deemed to be unacceptable. This project raised no particular concerns.

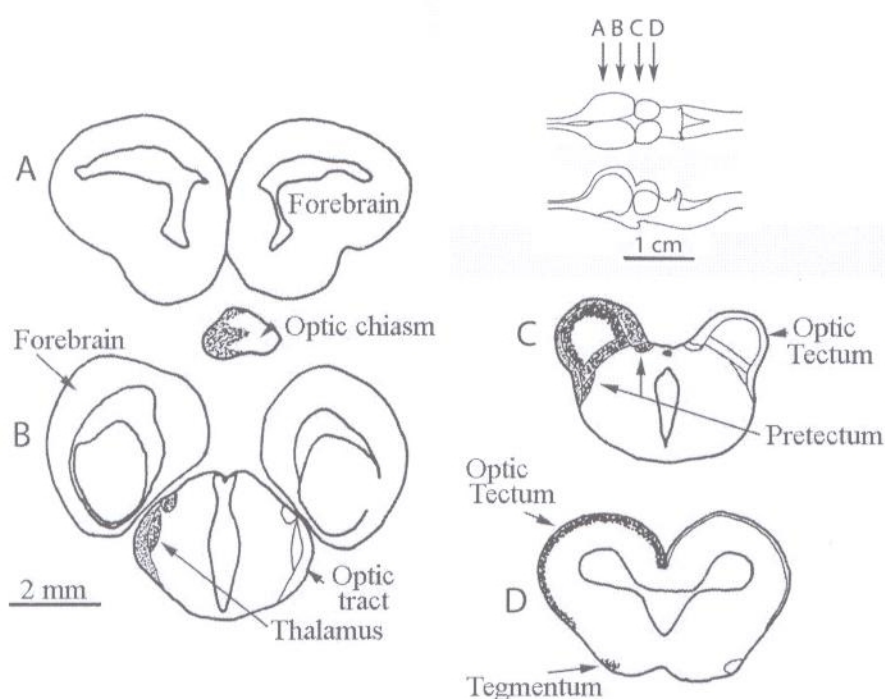
The lizards were anaesthetised by cooling the animals, and perfused transcardially with formalin to fix the brains. The brains were dissected out, embedded in paraffin wax, sectioned in the frontal plane at 10 µm,

mounted on slides and most were stained with cresyl violet to show cell bodies. Retinal pathways were demonstrated in two lizards which received eye injections of 30  $\mu\text{Ci}$  of [ $^3\text{H}$ ] proline, a radioactive tracer, delivered in a volume of 1–5  $\mu\text{l}$  of 0.9% saline into the vitreous of the eye via a 5  $\mu\text{l}$  SGE syringe, and survived one or two days before brain fixation. The brain sections for autoradiography were mounted on slides, dipped in nuclear research emulsion (Ilford K2 or Kodak NTB2), exposed in the dark for 4–8 weeks at 4°C, developed in Kodak D-19 developer and counterstained with cresyl violet before microscopic examination with dark- and bright-field optics. In 2004, when we re-examined this material we had available to us brain sections from three lizards and photographs showing brain regions labelled with [ $^3\text{H}$ ] proline following retinal injection. Additional photographs of the normal histology of the brain were taken at selected levels through the brain (forebrain, optic tectum and cerebellum) with a Nikon Coolpix Digital Camera or an Olympus DP70 Camera attached to an Olympus Microscope.

## Results

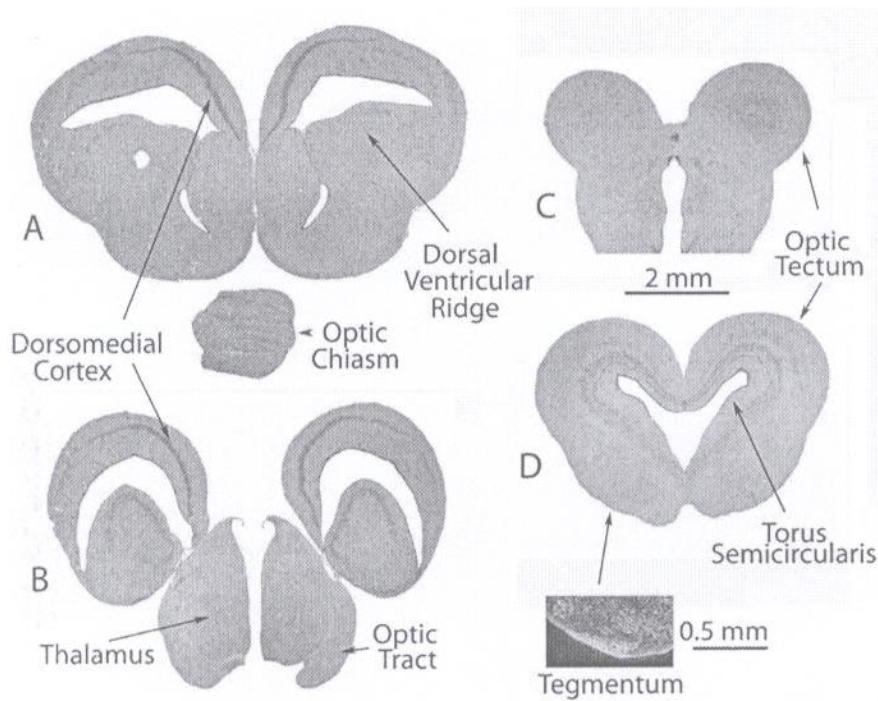
Fig. 1 shows drawings of frontal sections through the brain of a lizard in which the retinal pathways were traced with [ $^3\text{H}$ ] proline, showing primary retinal targets in the optic thalamus, pretectum, optic tectum and tegmentum, together with an outline drawing of the brain of *T. rugosa*, showing the levels of the frontal sections. The olfactory bulbs were not successfully extracted during brain dissection, so are not shown in the diagram. In outward morphology the brain appears reasonably similar in structure to what is seen in some other lizards (eg *Lacerta sicula*, Butler and Hodos 1996 Fig. 3-2). Obvious brain features in the diagrams include the forebrain, the midbrain (including the optic tectum) and the spinal cord.

The retinal pathway appeared to be entirely crossed (going from an eye to the opposite side of the brain), probably related to the lateral eye position in *T. rugosa*, with a small binocular field of view (15–17°) present (New 2008). The pattern of projection from the retina to its targets in the brain is generally similar to that seen in other lizards, though with very laterally placed eyes *T. rugosa* did not appear to have the uncrossed retinal projection that has been described in most other lizards (Butler & Northcutt 1971; Repérant *et al.* 1978, Dunlop *et al.* 2000), some of which are predatory and have a significant binocular field of view. In addition we did not find a retinal input to the hypothalamus, though it is often small in size and may have been difficult to detect. A photo of the retinal projection to the tegmentum appears in Fig. 2.



**Figure 1.** Line drawings of frontal sections through the brain of *T. rugosa* showing primary retinal targets labelled by an eye injection of [ $^3\text{H}$ ] proline, with the levels of the sections indicated on outline drawings of dorsal and lateral views of the brain.

Fig. 2 shows a series of digital photographs of frontal sections through the brain of *T. rugosa*, corresponding approximately to the levels shown in Fig. 1, but taken from a different animal to the one which provided the data in Fig. 1. These sections through the brain of *T. rugosa* appear reasonably similar to sections at similar levels in other lizards (see Butler & Hodos 1996 Figs. 17-13 and 18-28 optic tectum of *Tupinambis nigropunctatus* and *Iguana iguana*), with obvious features including dorsomedial cortex and dorsal ventricular ridge (DVR) in the forebrain, and many layers in the optic tectum. On the basis of differences in DVR organization, lizards have been divided into two groups, type I and type II (Northcutt 1972). Type I lizards have a DVR core nucleus surrounded by a band of cells, whereas type II lizards have a DVR composed of a number of nuclear groups (see Northcutt & Butler 1974 Fig. 1), with *T. rugosa* identifiable as a type II lizard (personal communication from Prof. A. Butler, who viewed our digital photographs of *T. rugosa* material). Type II lizards are typically diurnal in habit (Northcutt & Butler 1974), as is *T. rugosa*.



**Figure 2.** Photographs of frontal sections through the brain of *T. rugosa*, corresponding approximately to the levels shown in Fig. 1, but taken from a different animal. A small ventral part of sections at levels B and C is missing. The sections were stained with cresyl violet, apart from the optic chiasm (level A) which was stained with a fibre stain. The inset (below level D) is a darkfield photograph of a section from an animal which received 3H proline labelling, and shows the retinal projection to the tegmentum (see Fig. 1) at higher magnification, with the approximate location of the tegmentum in level D indicated. Significant features in the brain of *T. rugosa* referred to in the text are labelled. The optic tectum receives a major visual input, while the torus semicircularis is an auditory relay station.

### Discussion

The results described here present basic information on brain structure and visual pathways in the sleepy lizard, *T. rugosa*, an animal whose ecology and behaviour has been much studied. The primary visual pathways provide significant crossed retinal input to the optic tectum, pretectum, thalamus and tegmentum (Fig. 1), which provide the neural substrate for visual recognition demonstrated in this species (Zuri & Bull 2000). The apparent lack of any uncrossed retinal projection is interesting, considering that there is a small binocular field of view. However New (2008) has noted that there is no temporally placed fovea in this species, which could allow both eyes to obtain a high acuity view within the binocular field of view, and stereopsis has not been demonstrated within this species. Studies in other reptiles (summarised in Manger *et al.* 2002) have shown that there is visual input to the dorsal cortex and dorsal ventricular ridge of the telencephalon (Fig. 2), where the visual recognition and memory probably occurs (Day *et al.* 2001).

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## References

- Braekvelt, C.R. (1989) Retinal pigment epithelial fine structure in the bobtail goanna (*Tiliqua rugosa*). *Histology and Histopathology* **4**: 295–300.
- Bull, C.M. (1995) Population ecology of the sleepy lizard, *Tiliqua rugosa*, at Mt Mary, South Australia. *Australian Journal of Ecology* **20**: 393–402.
- Butler, A.B. & Hodos, W. (1996) 'Comparative Vertebrate Neuroanatomy. Evolution and Adaptation' (Wiley-Liss, New York).
- Butler, A.B. & Northcutt, R.G. (1971) Retinal projections in *Iguana iguana* and *Anolis carolinensis*. *Brain Research* **26**: 1–13.
- Day, L.B., Crews, D. & Wilczynski, W. (2001) Effects of medial and dorsal cortex lesions on spatial memory in lizards. *Behavioural Brain Research* **118**: 27–42.
- Dunlop, S.A., Tran, N., Tee, L.B.G., Papadimitriou, J. & Beazley, L.D. (2000) Retinal projections throughout optic nerve regeneration in the ornate dragon lizard, *Ctenophorus ornatus*. *Journal of Comparative Neurology* **416**: 188–200.
- Firth, B.T. & Kennaway, D.J. (1989) Thermoperiod and photoperiod interact to affect the phase of the plasma melatonin rhythm in the lizard, *Tiliqua rugosa*. *Neuroscience Letters* **106**: 125–130.
- Firth, B.T., Kennaway, D.J. & Rozenbils, M.A.M. (1979) Plasma melatonin in the scincid lizard, *Trachydosaurus rugosus*: diel rhythm, seasonality, and the effect of constant light and constant darkness. *General and Comparative Endocrinology* **37**: 493–500.
- Freake, M.J. (1999) Evidence for orientation using the e-vector direction of polarised light in the sleepy lizard, *Tiliqua rugosa*. *Journal of Experimental Biology* **202**: 1159–1166.
- Freake, M.J. (2001) Homing behaviour in the sleepy lizard (*Tiliqua rugosa*): the role of visual cues and the parietal eye. *Behavioral Ecology and Sociobiology* **50**: 563–569.
- Manger, P.R., Slutsky, D.A. & Molnár, Z. (2002) Visual subdivisions of the dorsal ventricular ridge of the iguana (*Iguana iguana*) as determined by electrophysiologic mapping. *Journal of Comparative Neurology* **453**: 226–246.
- Mayner, L. (1978) Transport of tritiated proline in the central nervous system. Honours Thesis, Flinders University, Adelaide, South Australia.
- New, T.D. (2008) Vision in the sleepy lizard (*Tiliqua rugosa*): retinal organisation, visual acuity and ecological constraints. Masters Thesis, Flinders University.
- Northcutt, R.G. (1972) The teiid prosencephalon and its bearing on squamate systematics. *Program and abstracts of American Society of Ichthyologists and Herpetologists* pp 75 & 76.
- Northcutt, R.G. & Butler, A.B. (1974) Evolution in reptilian visual systems: Retinal projections in a nocturnal lizard, *Gekko gekko* (Linnaeus). *Journal of Comparative Neurology* **157**: 453–466.
- Repérant, J., Rio, J.P., Miceli, D. & Lemire, M. (1978) A radioautographic study of retinal projections in type 1 and type 2 lizards. *Brain Research* **142**: 401–411.
- Teo, E.H., Carati, C., Firth, B.T., Barbour, R.A. & Gannon, B. (1993) Vascularization of the pineal complex in the lizard *Tiliqua rugosa*. *The Anatomical Record* **236**: 521–536.
- Wohlfeil, C.K. (2008) Cues associated with foraging mode in the sleepy lizard, *Tiliqua rugosa*. Honours Thesis, Flinders University, Adelaide, South Australia.
- Zuri, I. & Bull, C.M. (2000) The use of visual cues for spatial orientation in the lizard, (*Tiliqua rugosa*). *Canadian Journal of Zoology* **78**: 515–520.