A 3D MRI-based atlas of a lizard brain

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Abstract

Magnetic resonance imaging (MRI) is an established technique for neuroanatomical analysis, being particularly useful in the medical sciences. However, the application of MRI to evolutionary neuroscience is still in its infancy. Few magnetic resonance brain atlases exist outside the standard model organisms in neuroscience and no magnetic resonance atlas has been produced for any reptile brain. A detailed understanding of reptilian brain anatomy is necessary to elucidate the evolutionary origin of enigmatic brain structures such as the cerebral cortex. Here, we present a magnetic resonance atlas for the brain of a representative squamate reptile, the Australian tawny dragon (Agamidae: Ctenophorus decresii), which has been the subject of numerous ecological and behavioral studies. We used a high-field 11.74T magnet, a paramagnetic contrasting-enhancing agent and minimum-deformation modeling of the brains of thirteen adult male individuals. From this, we created a high-resolution three-dimensional model of a lizard brain. The 3D-MRI model can be freely downloaded and allows a better comprehension of brain areas, nuclei, and fiber tracts, facilitating comparison with other species and setting the basis for future comparative evolution imaging studies. The MRI model and atlas of a tawny dragon brain (Ctenophorus decresii) can be viewed online and downloaded using the Wiley Biolucida Server at wiley.biolucida.net.

KEYWORDS

brain organization, columnar, evolution, magnetic resonance imaging, neuromeric, prosomeric, reptile

1 | INTRODUCTION

Squamate reptiles (lizards and snakes) comprise the second largest group of terrestrial vertebrates, with more than 10,000 species (Pyron, Burbrink, & Wiens, 2013; Reeder et al., 2015; Uetz & Hošek, 2017). Due to the extent to which they occupy diverse ecological niches, squamates have been recognized as an ideal group for comparative studies of brain evolution and the evolution of brain-behavior relationships (Hoops, 2018). For example, they are an optimal group with which to study comparative cognition (Clark, Amiel, Shine, Noble, & Whiting, 2013; Leal & Powell, 2012; Northcutt, 2013) and its relationship with the evolution of sociality (Whiting & While, 2017). Furthermore, interest in the neurobiology of squamates is increasing, including both single-species studies (Amiel, Bao, & Shine, 2017; Day, Crews, & Wilczynski, 1999; LaDage et al., 2013; LaDage, Riggs, Sinervo, & Pravosudov, 2009; Lutterschmidt & Maine, 2014)
Comparative studies on brain anatomy and evolution would be greatly facilitated if the animal’s nervous system could be rapidly visualized in an intact head, and even in live specimens (Corfield, Wild, Cowan, Parsons, & Kubke, 2008). Magnetic resonance imaging (MRI) is a noninvasive technique that allows for such visualization. This technique is particularly useful in the case of endangered or protected species, and when working with precious museum specimens that would be destroyed in the process of extracting the brain. From a practical perspective, MRI is advantageous because it does not require any of the labor-intensive tissue processing necessary for histology. The resulting image can be viewed in any plane, allowing for brain regions and fiber tracts to be viewed from multiple orientations throughout their rostral-caudal extent. In addition, MRI can facilitate both inter- and intraspecific comparisons as measurements can be semi-computer-automated (e.g., Lerch et al., 2008).

In order for MRI to facilitate comparative neuroscience, however, MRI atlases must be available for a diversity of animal species. Such atlases are available for brains from most major vertebrate lineages, including bony fishes (Kabli, Alia, Spaink, Verbeek, & De Groot, 2006; Ullmann, Cowin, & Collin, 2010a; Ullmann, Cowin, Kurniawan, & Collin, 2010b), cartilaginous fishes (Yopak & Frank, 2009), birds (Poirier et al., 2008; Vellema, Verschueren, Van Meir, & Van der Linden, 2011), and mammals (Dorr, Lerch, Spring, Kabani, & Henkelman, 2008; Ullmann et al., 2012; Ullmann, Watson, Janke, Kurniawan, & Reutens, 2013a; Ullmann et al., 2013b). To our knowledge, only one published study has used MRI to image the brain of a reptile, the garter snake (Thamnophis sirtalis; Anderson, Kabalka, Layne, Dyke, & Burghardt, 2000); however the resolution was not sufficient to distinguish most structures. Developing an MRI atlas of a reptilian brain would be the first step in conducting broad-scale comparative analyses both within reptiles and across all the major vertebrate clades.

Here, we present a detailed description of the brain of an agamid lizard, the Australian tawny dragon (Ctenophorus decresii, Duméril & Bibron, 1837; Reptilia: Agamidae), using high-resolution MRI. In the
atlas we identify nuclei, fiber tracts, and other structures throughout the brain in coronal, sagittal, and horizontal orientations. Furthermore, we describe our MRI data with reference to the neuromeric/prosomeric model, in addition to the traditional columnar model, since the former is more natural as it relates to the fundamental divisions of the brain that are shared by all vertebrates (Puelles, 2009; Puelles & Rubenstein, 2015; Puelles, Harrison, Paxinos, & Watson, 2013). This atlas therefore provides a new means of understanding the structure and connectivity of the reptile brain.

2 | METHODS

2.1 | Specimen acquisition

Sixteen male tawny dragons were collected from the southern Flinders Ranges, South Australia. We euthanized each lizard with an injection of 100 mg/kg sodium pentobarbital and an equal volume of 2 mg/mL lignocaine. Each lizard was then intracardially perfused following Hoops (2015). Magnevist was added to the fixative perfusate (4% paraformaldehyde) to maximize image contrast in magnetic resonance imaging (Ullmann, Cowin, & Collin, 2010a). The brains were stored at 4°C in a solution of 0.1% Magnevist and 0.05% sodium azide in phosphate-buffered saline until imaging. The Australian National University’s Animal Experimental Ethics Committee approved all research under protocol number A2011-49.

2.2 | Magnetic resonance imaging

Whole-brain images of 13 tawny dragon brains (e.g., Figure 1a) were acquired using a Bruker Avance 11.74 Tesla wide-bore spectrometer (Ettlingen, Germany) with a micro-2.5 imaging probe capable of generating magnetic gradients of 1.50 T/m. Brains were immersed in Fomblin (perfluoropolyether, Grade Y06/6, JAVAC, Sydney, Australia) and placed in a 10 mm diameter Wilmad tube using a custom-built plastic holder (Hyare et al., 2008). Parameters used in the scans were optimized for gray-white matter contrast in the presence of Magnevist. We used a 3D fast gradient-echo sequence (FLASH; T2*-weighted), with repetition time = 40 ms, echo time = 8 ms, field-of-view = 11 × 11 × 16 mm, and matrix size = 110 × 110 × 160, producing an image with 50 μm³ isotropic voxels.

For comparison, two brains were embedded in agarose and vibratome-sectioned at 70 μm. Brain sections were stained for 5 min using the DNA-binding stain SYBR-green (Life Technologies Australia, Melbourne, Australia), rinsed in phosphate-buffered saline, mounted in Fluoro-Gel (ProSciTech, Brisbane, Australia), and imaged using Olympus fluorescence light microscopes.

2.3 | Model generation and analysis

To ensure consistent measures of brain morphometry all images were first manually masked such that consistent coverage of brain structures and nerve endings was achieved. In the tawny dragon the olfactory bulbs are small and separated from the brain on long stalks (Figure 2) and we were unable to stabilize their location in the Wilmad tube. Therefore, the olfactory bulbs were included in the masked regions. The manually masked areas were then set to the background value such that they were not included in subsequent calculations.

Thirteen brain image datasets of 50 μm³ resolution were first reoriented to standard rostro-caudal orientation. All images were then corrected for B0 intensity inhomogeneity using the N3 algorithm (Sled, Zijdenbos, & Evans, 1998). An image with a good signal to noise ratio and no obvious artifacts was then manually selected from the group to create an initial model by blurring. All images were then recursively matched to this evolving model of average structure to create a minimum deformation average with a resulting resolution of 20 μm³ (Figure 1b). The details of the model creation process can be found in Janke and Ullmann (2015). The fitting stages in this case started at a resolution of 1.28 mm and finished with a resolution of 80 μm³. The model finished with a resolution of 20 μm³.

To compare the natural orientation of the tawny dragon brain to the orientation of our model, a representative scan was acquired of a brain within a fully intact tawny dragon head. The brain was automatically segmented from this scan using a combination of registration to
## TABLE 1  Legend of abbreviations used in Figures 4–39 in alphabetical order. As per convention, nuclei, areas and other structures are capitalized, while fibre tracks not (Paxinos & Franklin, 2013; Paxinos & Watson, 2013)

| Abbreviation | Brain Region | Coronal Figures | Horizontal Figures | Sagittal Figures |
|--------------|--------------|-----------------|--------------------|-----------------|
| *            | Blood Vessel | 8               |                    |                 |
| 3n           | Oculomotor Nerve | 14, 24, 25, 31, 32 |                 |                 |
| 3N           | Nucleus of the Oculomotor Nerve | 14, 25, 26 |                 |                 |
| 3ND          | Nucleus of the Oculomotor Nerve, dorsal part | 15 |                 |                 |
| 3NV          | Nucleus of the Oculomotor Nerve, ventral part | 15, 25 |                 | 32 |
| 3V           | Third Ventricle | 8, 9, 10, 11, 12, 13, 23, 24 |                 |                 |
| 4n           | Trochlear Nerve | 15, 16, 26, 27, 31, 32, 33, 34 |             |                 |
| 4N           | Nucleus of the Trochlear Nerve | 15, 16 |                 | 26, 31, 32      |
| 4V           | Fourth Ventricle | 16, 17, 18, 19, 20 |                 | 26, 27          |
| 5d           | Descending Tract of the Trigeminal Nerve | 18, 19, 20 |                 |                 |
| 5DM          | Dorsal Motor Nucleus of the Trigeminal Nerve | 17 |                 | 33 |
| 5DN          | Descending Nucleus of the Trigeminal Nerve | 18, 19, 20, 25, 26, 27 | 34 |
| 5me          | Trigeminal Mesencephalic Tract | 16 |                 |                 |
| 5n           | Trigeminal Nerve | 16, 25, 26, 33, 34, 35 |             |                 |
| 5Pr          | Principal Nucleus of the Trigeminal Nerve | 17 |                 | 34 |
| 5Sp          | Spinal Nucleus of the Trigeminal Nerve | 32 |                 |                 |
| 5VM          | Ventral Motor Nucleus of the Trigeminal Nerve | 17, 25 | 34 |
| 6n           | Abducens Nerve | 17, 18, 24, 25, 32 |             |                 |
| 6N           | Nucleus of the Abducens Nerve | 17, 18 | 26 |
| 7DM          | Dorsal Motor Nucleus of the Facial Nerve | 18 |                 |                 |
| 7VM          | Ventral Motor Nucleus of the Facial Nerve | 18 |                 |                 |
| 8n           | Statoacoustic Nerve | 17, 18, 26, 27 | 32, 33, 34, 35 |             |
| 10DN         | Dorsal Motor Nucleus of the Vagus Nerve | 19, 20 |             |                 |
| 10N          | Motor Nucleus of the Vagus Nerve | 20 |                 |                 |
| 12n          | Hypoglossal Nerve | 19, 20 |                 |                 |
| 12N          | Nucleus of the Hypoglossal Nerve | 19, 20 | 32 |
| a            | Alveus | 5, 6, 7, 8, 9, 10, 27, 28, 29, 30, 32, 33, 34, 35 |             |                 |
| A8           | Catecholaminergic Cell Group A8 | 15 | 26 |
| ac           | Anterior Commissure | 8, 9 | 26 | 31, 32 |
| AC           | Nucleus of the Anterior Commissure | 9 |                 |                 |
| Acb          | Accumbens Nucleus | 5, 6, 7, 25, 26, | 31, 32 |
| ADVR         | Anterior Dorsal Ventricular Ridge | 5, 6, 7, 8, 27, 28, 29, 32, 33, 34, 35 |             |                 |
| AHA          | Anterior or Alar Hypothalamic Area | 10 |                 |                 |
| AngC         | Angular Cochlear Nucleus | 17 | 27 |
| AO           | Anterior Olfactory Nucleus | 4 | 26 | 32, 33 |
| AOT          | Nucleus of the Accessory Olfactory Tract | 8 |                 |                 |
| apc          | Anterior Pallial Commissure | 8, 9 | 26, 27 | 31 |
| Arc          | Arcuate Nucleus | 11, 12, 13, 22 | 31, 32 |
| AS           | Anterior Septal Nucleus | 7, 8, 9 | 28 | 31 |
| Au           | Auricle | 16 | 27 |                 |
| BAC          | Bed Nucleus of the Anterior Commissure | 9 | 25 | 31 |
| bc           | Brachium Conjunctivum | 16 | 26 | 34 |
| bop          | Basal Optic Tract | 11, 12, 13, 14, 23 |             |                 |
| BOp          | Nucleus of the Basal Optic Tract | 12, 13, 14, 25 | 34 |
| CAg          | Cerebral Aqueduct | 13, 14, 15, 26 | 31 |
| CC           | Central Canal of the Spinal Cord | 21 |                 |                 |
| Ce           | Cerebellum | 13, 14, 15, 16, 17, 27, 28, 29, 30, 31, 32, 33, 34 |             |                 |
| CeL          | Cerebellar Nucleus, lateral part | 16 | 26 |                 |
| CeM          | Cerebellar Nucleus, medial | 16 | 33 |
| CG           | Central Grey | 12, 13, 14, 15, 16, 25, 26, 27, 31, 32 |             |                 |
| Abbreviation | Brain Region                                      | Coronal Figures | Horizontal Figures | Sagittal Figures |
|--------------|--------------------------------------------------|----------------|-------------------|-----------------|
| chp          | Choroid Plexus                                   | 10             |                   | 32              |
| CPDMCx       | Cell Plate of the Dorsomedial Cortex              | 10             |                   |                 |
| DB           | Nucleus of the Diagonal Band                      | 6, 7           | 24                | 32, 33          |
| dc           | Dorsal Coclear Tract                             | 17, 18, 19     | 27                | 32              |
| DCx          | Dorsal Cortex                                    | 5, 6, 7, 8, 9, 10 | 28, 29, 30        | 33, 34, 35      |
| dcoll        | Dorsal Column Tract                              | 20, 21         |                   | 31, 32          |
| DColl        | Nucleus of the Dorsal Column, lateral part        | 19, 20         |                   | 32              |
| DCollM       | Nucleus of the Dorsal Column, medial part         | 19, 20         |                   |                 |
| DH           | Dorsal Horn of the Spinal Cord                    | 21             |                   |                 |
| DLA          | Dorsolateral Amygdala                            | 9              | 28                | 35              |
| DLH          | Dorsolateral Hypothalamic Nucleus                 | 10, 11         | 24                | 32              |
| DLPR         | Dorsolateral Pallium, Rostral Part                | 4              |                   |                 |
| DLT          | Dorsolateral Thalamic Nucleus                     | 10             | 25, 26            |                 |
| DLTv         | Dorsolateral Vestibular Nucleus                  | 17, 18         | 27                | 33, 34          |
| DIRTF        | Dorsal Nucleus of the Inferior Reticular Formation| 19, 20         | 25, 26, 27        | 32, 33          |
| DMCx         | Dorsomedial Cortex                               | 5, 6, 7, 8, 9, 10 | 30                | 32, 33, 34, 35  |
| DMH          | Dorsomedial Hypothalamic Nucleus                  | 11             |                   | 31              |
| DMS          | Dorsal Median Sulcus of the Cerebellum           | 13, 14, 15, 16 |                   |                 |
| DMT          | Dorsomedial Thalamic Nucleus                      | 10, 11         | 25, 26            | 31              |
| DPR          | Dorsal Pallium, Rostral Part                      | 4              |                   |                 |
| DPT          | Dorsal Pretectal Nucleus                          | 12             | 25, 26            | 33, 34          |
| DS           | Dorsal Septal Nucleus                             | 8              | 27                | 31              |
| DSC          | Dorsal Septal Nucleus, central part               | 9              |                   |                 |
| DSD          | Dorsal Septal Nucleus, dorsal part                | 9              | 27                | 31              |
| DST          | Dorsal Striatum                                  | 5, 6, 7, 8     | 26                | 33, 34          |
| DTg          | Dorsal Tegmental Nucleus                          | 16             |                   | 32              |
| Ep           | Epiphysis                                        | 11, 12         | 28, 29, 30        | 31              |
| EPA          | Entopeduncular Nucleus, anterior part             | 10             |                   |                 |
| EPP          | Entopeduncular Nucleus, posterior part            | 13             | 24                |                 |
| EPT          | External Pretectal Nucleus                        | 11, 12         |                   | 34              |
| EW           | Edinger-Westphal Nucleus                          | 14             |                   |                 |
| f            | Fornix                                           |                |                   | 25              |
| fi           | Fimbria                                          | 10             |                   |                 |
| fr           | Fasciculus Retroflexus                           | 11, 12         | 25, 26            | 32              |
| GL           | Glomerular Layer of the Cerebellum               | 15             |                   | 32              |
| GP           | Globus Pallidus                                  | 7              |                   |                 |
| Hb           | Habenula                                         | 11             | 27                |                 |
| hbc          | Habenular Commissure                             | 11             |                   | 31              |
| iarc         | Internal Arcuate Fibres                          | 18             |                   |                 |
| ic           | Infima Commissure                                | 19, 20         |                   |                 |
| IC           | Nucleus of the Infima Commissure                 | 20             |                   |                 |
| ICc          | Intercollicular Nucleus                           | 14, 15         | 27                | 33, 34          |
| IMLF         | Interstitial Nucleus of the Medial Longitudinal Fasciculus | 13 | 25 |
| iot          | Intermediate Olfactory Tract                     | 7              |                   |                 |
| IPD          | Interpeduncular Nucleus, dorsal part              | 14, 15         | 24                | 31              |
| IPV          | Interpeduncular Nucleus, ventral part             | 14, 15         | 23, 24            | 31              |
| IR           | Inferior Raphe Nucleus                           | 17, 18, 19, 20 | 24, 25, 26, 27    | 31              |
| IS           | Inferior Septal Nucleus                          | 8, 9           |                   | 31              |
| lsD          | Isthmic Nucleus, Diffuse part                    | 15             |                   | 34              |
| Abbreviation | Brain Region | Coronal Figures | Horizontal Figures | Sagittal Figures |
|--------------|--------------|-----------------|------------------|-----------------|
| IsM          | Isthmic Nucleus, Magnocellular part (pre-Isthmic or mesencephalic) | 15, 16 | 26, 27 | 34 |
| IsP          | Isthmic Nucleus, Parvocellular part | 16 | 25, 26 | |
| LA           | Lateral Amygdala | 9 | 26, 27 | 36 |
| LCx          | Lateral Cortex | 5, 6, 7, 8, 9 | 28, 29 | 34, 35, 36 |
| Ifb          | Lateral Forebrain Bundle | 5, 6, 7, 8 | 24, 25, 26, 27 | 32, 33, 34 |
| Ifbd         | Lateral Forebrain Bundle, dorsal peduncle | 10, 11, 12, 13 | 25 | |
| Ifbv         | Lateral Forebrain Bundle, ventral peduncle | 10, 11, 12, 14 | 24, 25 | 34 |
| LGD          | Lateral Geniculate Nucleus, dorsal part | 10 | 26 | |
| LGV          | Lateral Geniculate Nucleus, ventral part | 10, 11 | 24, 25 | 33, 34 |
| LHA          | Lateral Hypothalamic Area | 11, 12, 13 | 23 | 32, 33 |
| LHB          | Lateral Habenula | 10 | | |
| LJC          | Lateral Juxtacommissural Nucleus | 12 | | |
| II           | Lateral Lemniscus | 15, 16, 17 | 25 | 34 |
| LL           | Nucleus of the Lateral Lemniscus | 15, 16, 17 | 24, 25 | 34 |
| LLD          | Nucleus of the Lateral Lemniscus, dorsal part | 25 | 26 | 34 |
| LLV          | Nucleus of the Lateral Lemniscus, ventral part | | | 34 |
| LoC          | Locus Coeruleus | 16 | 25, 26 | 33, 34 |
| lot          | Lateral Olfactory Tract | 4, 5 | 26, 27 | 34, 35 |
| LOT          | Nucleus of the Lateral Olfactory Tract | 5, 6, 7 | 26, 27 | 34, 35 |
| LPO          | Lateral Preoptic Area | 8 | | 32 |
| LPR          | Lateral Pallium, Rostral Part | 4 | | |
| LS           | Lateral Septal Nucleus | 7, 8, 9 | 27 | 32 |
| LTu          | Lateral Tuberal Nucleus | 12 | 22 | |
| LV           | Lateral Ventricle | 4, 5, 6, 7, 8, 9, 10 | 26, 27, 28, 29 | 32, 33, 34, 35 |
| lvesp        | Lateral Vestibulospinal Tract | 18, 20 | 25, 26 | 34 |
| M            | Mammillary Nuclei | 13 | | 31 |
| m5n          | Motor Root of the Trigeminal Nerve | 17 | 25 | |
| m7n          | Motor Root of the Facial Nerve | | 25 | |
| MA           | Medial Amygdala | 9 | 25, 26 | 34 |
| MC           | Magnocellular Cochlear Nucleus | 18 | | 32 |
| MCx          | Medial Cortex | 5, 6, 7, 8, 9, 10, 11 | 28, 29, 30 | 31, 32, 33, 34, 35 |
| mfb          | Medial Forebrain Bundle | 7, 8, 9, 10, 11, 12, 13 | 24, 25, 26 | 32 |
| MHB          | Medial Habenula | 10 | | 31 |
| MJC          | Medial Juxtacommissural Nucleus | 12 | 26 | 31 |
| ml           | Medial Lemniscus | 15, 16, 17, 18, 19, 20 | 23, 24, 25 | 31 |
| ML           | Molecular Layer of the Cerebellum | 15 | | 32 |
| mlf          | Medial Longitudinal Fasciculus | 13, 14, 15, 16, 17, 18, 19, 20 | 24, 25, 26, 27 | 31, 32 |
| MPC          | Medial Parvocellular Nucleus | 19 | | 27 |
| MPO          | Medial Preoptic Area | 8, 9 | 25 | 31 |
| MPR          | Medial Pallium, Rostral Part | | 27 | 32, 33 |
| MRtf         | Middle Reticular Formation | 17, 18 | 23, 24, 25 | 32, 33 |
| MS           | Medial Septal Nucleus | 9 | 27 | |
| MT           | Medial Thalamic Nucleus | 11 | 25 | 31 |
| O            | Oval Nucleus | | 26 | 32 |
| oc           | Optic Chiasm | 8, 9, 10 | 22, 23 | 31, 32 |
| OP           | Olfactory Peduncle | | | 31 |
| ot           | Optic Tract | 9, 10, 11, 12, 13 | 22, 23, 24, 25, 26, 27 | 32, 33, 34 |
| OT           | Optic Tectum | 11, 12, 13, 14, 15 | 25, 26, 27, 28, 29, 30 | 31, 32, 33, 34, 35, 36 |
| p1Tg         | p1 Tegmental Area (former Pretectal Reticular Formation, PrR) | 13, | 25 | 32, 33, 34 |
| p3Tg         | p3 Tegmental Area | 12 | 24 | 32 |
| Abbreviation | Brain Region                                      | Coronal Figures | Horizontal Figures | Sagittal Figures |
|--------------|--------------------------------------------------|-----------------|--------------------|------------------|
| p8n          | Posterior Root of the Statoacoustic Nerve        | 18              | 26, 27             |
| Pa           | Paraventricular Nucleus                          | 9               | 24                 |
| PaO          | Paraventricular Organ                            | 12              | 23                 | 31               |
| PaON         | Paraventricular Organ Nucleus (formerly Periventricular Hypothalamic Nucleus) | 11, 12          | 23, 24             |
| PB           | Parabrachial Nucleus                             | 15              |                    |
| pc           | Posterior Comissure                             | 12              | 26, 27             | 31, 32           |
| PC           | Nucleus of the Posterior Commissure             | 11              | 26                 | 32               |
| PCN          | Posterior Cochlear Nucleus                       | 18              |                    |
| Pct          | Posteroentral Nucleus                            | 11, 12          | 27                 | 33               |
| PDN          | Posterodorsal Nucleus                            | 12              | 27                 | 32               |
| pdt          | Predorsal Tract                                  | 14, 15, 16, 17, 18, 19, 20 | 24, 25 | 31               |
| PDVR         | Posterior Dorsal Ventricular Ridge               | 9, 10           | 9, 10              | 28, 32           |
| PH           | Posterior or Basal Hypothalamus                  | 12              | 24                 |                  |
| PL           | Purkinje Layer of the Cerebellum                 | 15              |                    | 32               |
| PM           | Profound Mesencephalic Area                      | 13, 14          | 26                 | 34               |
| PMN          | Posteromedial Nucleus                            | 11, 12          | 27                 | 31, 32           |
| ppc          | Posterior Pallial Commissure                     | 10              | 27                 | 31, 32           |
| PrPC         | Principal Precommissural Nucleus                 | 11, 12          | 25                 | 32               |
| PT           | Pallial Thickening                               | 5               |                    |
| PTE          | Prethalamic Eminence                             | 25              |                    | 32               |
| PTG          | Pretectal Geniculate Nucleus                     | 11, 12          | 25, 26             | 33, 34           |
| PVSC         | Posterior Nucleus of the Ventral Supraoptic Commissure | 12              | 24                 | 34               |
| R            | Red Nucleus                                      | 14              |                    | 32               |
| r1Tg         | r1 Tegmental Area (Reticular Isthmal Nucleus)    | 15              | 25                 | 33               |
| RM           | Retromammillary Nucleus                          | 13              | 23                 | 31               |
| rmc          | Retromammillary Commissure                       | 13              | 23                 | 31               |
| Rot          | Rotund Nucleus                                   | 11              | 25                 | 31, 32           |
| s            | Septum                                           | 5, 6            |                    |
| s5n          | Sensory Root of the Trigeminal Nerve             | 17              |                    | 34               |
| s7n          | Sensory Root of the Facial Nerve                 | 17              | 26                 |                  |
| SAT          | Striatoamygdaloid Transition Area                | 8, 9            | 25, 26, 27         | 33, 34           |
| SCb          | Suprachiasmatic Nucleus                          | 9               | 24                 | 31               |
| SCO          | Subcommissural Organ                             | 12              | 26                 | 31               |
| SD           | Nucleus of the Supraoptic Decussation            | 11              | 23                 | 33               |
| sh           | Septohypothalamic Tract                          | 9, 10, 11       | 24, 25             | 32               |
| sm           | Stria Medullaris                                 | 9, 10           | 25                 | 32               |
| SN           | Substantia Nigra                                 | 25              |                    | 33, 34           |
| SO           | Superior Olivary Nucleus                         | 17              | 24                 | 33               |
| Sol          | Nucleus of the Solitary Tract                    | 19, 20          |                    |
| sol          | Solitary Tract                                   | 17, 18          | 26, 27             |
| SON          | Supraoptic Nucleus                               | 8, 9            | 24                 | 32               |
| sox          | Supraoptic decussation                           | 23              |                    |
| spc          | Spinocerebellar Tract                            | 16, 17, 18      | 26, 27             | 34               |
| Sph          | Spherical Nucleus                                | 9, 10           | 25, 26             | 35               |
| spl          | Spinal Lemniscus                                 | 15, 16, 17, 18, 19, 20 | 24, 25, 26, 27 | 32, 33, 34 |
| SR           | Superior Rapha Nucleus                           | 15, 16          | 23, 24             | 31               |
| SRtF         | Superior Reticular Formation                     | 15, 16          | 23, 24, 25         | 32, 33           |
| SRtL         | Superior Reticular Area, lateral part            | 16              |                    |
| SRtM         | Superior Reticular Area, medial part             | 16              |                    | 32               |
| STL          | Bed Nucleus of the Stria Terminalis, lateral part| 9               |                    |
the constructed model, using the MINC toolkit (Vincent et al., 2016), and manual corrections. The linear rotational component of the automatic registration was used to measure the angle of alignment of the brain in the skull with respect to our model.

No description exists for the tawny dragon brain or for any agamid brain. A variety of neuroanatomical references available for other lizards (including lacertids, iguanids, and varanids) were used to identify brain areas, including neuroanatomical references for the entire lizard brain (Del Corral, Miralles, Nicolau, Planas, & Rial, 1990; ten Donkelaar, 1998; Medina, Marti, Artero, Fasolo, & Puelles, 1992), the telencephalon (Greenberg, 1982; Northcutt, 1967; Peterson, 1981; Smeets, Hoogland, & Lohman, 1986), the diencephalon (Butler & Northcutt, 1973; Cruce, 1974), the hindbrain (Cruce & Newman, 1981; ten Donkelaar, Bangma, Barbás-Henry, Huizen, & Wolters, 2012; Schwab, 1979; Wolters, ten Donkelaar, & Verhofstad, 1984; Wolters, ten Donkelaar, Steinbusch, & Verhofstad, 1985), and the neuroeric domains (Díaz, Yanes, Trujillo, & Puelles, 2000; Medina, Smeets, Hoogland, & Puelles, 1993; Medina, Puelles, & Smeets, 1994). Another important reference is the turtle brain atlas (Powers & Reiner, 1980).

### RESULTS

The tawny dragon brain model described here can be viewed online and downloaded in NIFTI format from wiley.biolucida.net. The model represents the spatial positioning and intensity of each neural structure based on the nonlinear averaging of thirteen tawny dragon brains. Using the intrinsic three-axis nature of MRI-atlases (Ullmann, Cowin, Kurniawan, & Collin, 2010b), we established a coordinate system with $x$-coordinates running medio-laterally, $y$-coordinates running rostro-caudally, and $z$-coordinates running ventro-dorsally, as per convention (Figure 3). The midline of the brain, which divides the two hemispheres, has been designated as the plane $x = 0$. The center of the epiphysis (defined as the $y$ plane in which the diameter of the epiphysis reaches its maximum) has been designated as the point $(x, y) = (0, 0)$, following...
studies which use the parietal eye as the point \((x,y) = (0,0)\) (Greenberg, 1982). The plane \(z = 0\) is located centrally as defined by the dimensions of the image: there are an equal number of \(z\)-planes above and below \(z = 0\). By convention, \(y\)-values increase caudally and \(z\)-values increase dorsally. The model is bilaterally symmetric about the midline, therefore the positive and negative directions in the \(x\)-plane are arbitrary.

Our model can be matched to novel MRIs, in vivo or ex vivo, and of different preservation and scanning parameters. This process, called model-based segmentation, is commonly used in medical MRI research (e.g., see Friedel, van Eede, Pipitone, Chakravarty, & Lerch, 2014) and could be easily implemented in evolutionary neuroscience to, for example, digitally "extract" lizard brains from the surrounding tissue in an MRI. We registered our model to a representative MRI scan of an intact lizard head, scanned ex-vivo, to demonstrate the position of the brain (Figure 2a,b). During the registration process, we observed that our atlas is not in the natural orientation of the lizard brain; it is rotated by 28° in the \(x\)-plane (Figure 2c). The lizard head MRI is also available for viewing and download from wiley.biolucida.net.

From our atlas, we were able to identify over 200 structures including areas, nuclei, fibre tracts and ventricles (Table 1). Whenever possible, the terminology of ten Donkelaar (1998) was used. Abbreviations follow the standard nomenclature rules as described in the brain atlases co-authored by George Paxinos (e.g., Paxinos & Franklin, 2013; Paxinos & Watson, 2013). Figures 4–20 show our atlas in sequential coronal sections, and we also include a coronal section of the anterior spinal cord (Figure 21). Figures 22–30 show our atlas in sequential horizontal sections, and Figures 31–36 in sequential sagittal sections. Figures 4–36 are presented at the end of this article, after the references, as is traditional for atlases and for ease of use. Higher resolution versions of the atlas figures are freely available from wiley.biolucida.net. The morphology of the lizard brain in our MRI model closely matches coronal nuclear-stained histological sections (Figures 37–45) and therefore our atlas is also relevant for work using traditional neuroanatomical methods.

We have identified the major anatomical divisions of our atlas according to the columnar (Table 2) and neuromeric (Table 3) models. The boundaries between neuromeres are often seen as transverse, dark strips separating grisea. They sometimes run parallel to major fiber tracts, such as the fasciculus retroflexus (adjacent to the boundary between prosomeres 1 and 2), facilitating their visualization. In our model, the three prosomeres of the diencephalon are clearly visible in both coronal and sagittal sections (Figure 46). The divisions between the commonly used columnar regions are not easily distinguished, likely because these regions are artificial; however, we have outlined them for comparative purposes (Figure 46).

In typical MRI images of biological tissue, the signal intensity mainly reflects water content. Since we used a T2*-weighted gradient echo, regions with higher water content appear hyperintense, or close to white in shade. Regions that have low water content appear hypointense, closer to black. The brain regions that have the highest water content are generally regions with high concentrations of cell bodies and/or neuropil and these therefore appear lightest. Fiber tracts tend to be the darkest due to extensive hydrophobic myelination. Nonetheless, in all tissue types signal intensity can vary extensively due to differences in cell size, extent of myelination, and neurochemistry. Signal intensity can even show a gradient within a single region, for example input from the lateral forebrain bundle creates an intensity gradient within the anterior dorsal ventricular ridge (Figure 7). Therefore, different nuclei and fiber tracts are differentiated based not only on differences in signal intensity but also by careful comparison with histological preparations and published literature.

The precise localization of the ventricles is important for identifying surrounding tissue regions, however these structures are particularly difficult to delineate in our atlas. Some ventricles, such as the rostral part of the lateral and tectal ventricles, are filled with aqueous liquid and appear white (e.g., Figures 4, 12–13). Ventricles which have collapsed during perfusion and fixation, and so do not contain any liquid, appear as thin lines of slightly lighter intensity. The majority of the lateral ventricle appears this way (e.g., Figure 7). The third and
fourth ventricles appear black as they have filled with Fomblin, the oil used to immerse the brain during imaging (e.g., Figure 16). Because of this variation, we have outlined ventricles with white dashed lines.

The laminar morphology of some brain regions is readily distinguishable in our atlas, particularly in the cerebral cortex, optic tectum and cerebellum. These layers are not as apparent in the individual MRIs used to make the model, as the differences in intensity are too weak. Only by generating the minimum deformation model from 13 MRIs is the noise reduced, the contrast enhanced, and the layers easily observed. The cerebral cortex generally contains three layers, a main or central cell layer flanked by two plexiform layers. The outer and inner plexiform layers appear relatively light, while the central cell layer appears either lighter or darker than the plexiform layers, depending on cortical area (Figure 47a; also see next paragraph). The darkest layer is the alveus that runs deep within the inner plexiform layer and is continuous with the anterior and posterior pallial commissures. A cell layer, the periventricular layer, exists along the surface of the lateral ventricle, but is not distinguishable from the alveus in our model.

The cerebral cortex is divisible into four main areas, which are distinguishable based on their relative positions and the morphology of the central cell layer. The medial cortex lies above the septum and is characterized by a cell layer that is distinctly darker than the surrounding plexiform layers. In the dorso-medial cortex, the cell layer widens, becomes lighter in intensity and appears slightly convex. An additional cell layer, the cell plate of the inner plexiform layer of the dorso-medial cortex, is visible in the inner plexiform layer. The dorsal cortex shows the distinct three-layered structure with a thin, prominent cell layer anteriorly that becomes less distinct as the inner plexiform layer decreases in intensity posteriorly. The lateral cortex is the most indistinct because its cell layer is diffuse and provides little contrast to the plexiform layers.

The reptilian optic tectum has a marked laminar organization consisting of cell layers separated by fiber layers; in some reptiles a total...
of fourteen layers have been described (Ramón, 1891). These have been grouped in six main layers or strata (ten Donkelaar, 1998), which are readily distinguishable in our model (Figure 47b). The optical layer is only slightly darker than the adjacent superficial grey and fibrous layer, but the two can be distinguished by the dark border between them. The central white layer is the darkest layer, while the central grey layer is of intermediate intensity between the central white and superficial layers. The periventricular grey layer is darker than the superficial layers but lighter than the central white layer, and finally the periventricular white layer is as dark as the central white layer, but much thinner (Figure 47b).

It is also possible identify the three layers of the cerebellum: the outer molecular layer, the central Purkinje layer, and the inner granular layer (Figure 47c). In lizards, including the tawny dragon, the cerebellum is everted (ten Donkelaar & Bangma, 1992). The Purkinje layer is the darkest in our MRI, likely owing to the fact that this layer contains not only the big somas of the Purkinje cells, but also a band of primarily afferent fibers. The granular layer is the lightest in intensity.

Not all structures visible in our MRI model are made up of neural tissue. In the anterior dorsal ventricular ridge, some arteries can clearly be seen as a series of dark spots (Figure 8; indicated by an asterisk). The meninges can be seen as thin, light structures around the edge of the brain, particularly in images of the brain stem (e.g., Figure 17). Droplets of the aqueous storage solution can get trapped around the brain when transferring them to Fomblin for imaging. These appear as bright areas in some images, for example the spaces between the optic tectum and the epiphysis (Figure 12) and between the optic tectum and the cerebellum (Figure 14).
**DISCUSSION**

### 4.1 MRI as a method for studying comparative neuroanatomy

To create an atlas with the best possible resolution, we have used a non-linear image averaging strategy to create an 'idealized' model of a tawny dragon brain (Janke & Ullmann, 2015). The model represents a significant improvement in resolution over the MRIs of individual brains (Figure 1), and this technique is now a standard component of the image registration process for modern structural MRI analysis (Johnson, Calabrese, Badea, Paxinos, & Watson, 2012; Maldjian, Dau-nais, Friedman, & Whitlow, 2014; Ullmann, Watson, Janke, Kurnia-wan, & Reutens, 2013a).

Unlike histology, in MRI brain size impacts the level of discernable detail. For example, an MRI atlas of a monkey brain is able to delineate 720 structures in an image with a 0.5 mm$^3$ voxel size (Maldjian, Dau-nais, Friedman, & Whitlow, 2014), whereas an MRI atlas of a cichlid brain is able to delineate only 54 structures in an image with a 50 μm$^3$ voxel size (Simões, Teles, Oliveira, Van der Linden, & Verhoye, 2012). Though the absolute voxel size in the cichlid atlas is much smaller than the voxel size in the monkey atlas, voxel size relative to brain size is much smaller in the monkey atlas. This provides a two-fold benefit to the monkey atlas: the larger absolute voxel size provides greater signal intensity, while smaller relative voxel size provides greater spatial resolution. Together, these factors allow for much more precise structural delineation in larger brains. This is an important consideration for comparative neuroscience, where comparisons are often made.
between brains that differ in size by orders of magnitude. Using multiple MRIs to create a non-linear average brain model can help offset these issues in species with small brains.

4.2 | The columnar and neuromeric models of brain organization

The study of brain structure requires a model of brain organization that sets easily recognized landmarks that help identify neural structures along pre-established axes (Puelles, 2009). In these models, the relative topological positions of the brain divisions should be invariant, independent of differences in size and shape arising through development or evolution (Nieuwenhuys & Puelles, 2015; Nieuwenhuys, ten Donkelaar, & Nicholson, 1998). Two models are currently used to interpret brain morphology, the columnar and neuromeric/prosomeric models.

The columnar model of neural divisions has been the predominant model of the second half of the twentieth century. It was based on the discovery of distinct functional columns in the spinal cord, the alar plate or dorsal horn and the basal plate or ventral horn. The model was then applied to the brain (Herrick, 1910; reviewed by Puelles, 2009). Thus, the diencephalon was described as containing several dorsoventral columns, including epithalamus, dorsal thalamus, ventral thalamus and hypothalamus. This description is still used by many neuroscientists and is found in the majority of textbooks. However, the columnar model is increasingly being recognized as unnatural.
because it does not consider the curvature of the longitudinal brain axis and the true morphogenetic divisions specified during development (reviewed by Puelles, 2009).

The neuromeric model (called the prosomeric model when discussing the forebrain) was employed by neuroembryologists during late nineteenth and early twentieth centuries, and was based on the periodic transversal bulges (called neumeres) in the neural tube wall during embryonic development (Kupffer, 1906; Orr, 1896; Puelles, 2009; Puelles et al., 2013). This model has recently experienced a resurgence due to its suitability for explaining the expression patterns of developmental regulatory genes and their mutant phenotypes, the results of experimental studies such as transplants and fate mappings, and the trajectories of major fiber tracts (Díaz & Glover, 2002; Marín & Puelles, 1995; Martínez, Marín, Nieto, & Puelles, 1995; Puelles, 2009; Puelles et al., 2013; Puelles & Rubenstein, 1993; Shimamura, Hartigan, Martínez, Puelles, & Rubenstein, 1995). The model is already applied in widely used brain atlases, such as the last edition of the rat brain atlas (Paxinos & Watson, 2013), the Allen Developing Mouse Brain Atlas (http://developingmouse.brain-map.org/), and the chicken brain atlas (Puelles, Martínez-de-la-Torre, Paxinos, Watson, & Martínez, 2007). It is starting to be incorporated into MRI atlases (Watson et al., 2017).

The neuromeric model is powerful for comparative purposes since the same developmental units are found in all vertebrates (Medina, 2006; Puelles et al., 2007; Puelles & Medina, 2002). For these reasons, in this study we used the neuromeric model as our preferred paradigm to interpret MRI data, with the hope that this will be more useful for future functional and evolutionary studies using our atlas. The boundaries between neumeres were identified as dark transversal strips (i.e., thin, cell poor areas) between grisea (which appear lighter). Fiber tracts, easily followed in our 3D atlas, are also useful for understanding the neuromeric organization of the tawny dragon brain, as their main trajectories are often either longitudinal (i.e., parallel to the alar-basal boundary) or transverse (i.e., parallel to the divisions between neumeres). Though this model is based on the natural divisions of the brain and therefore is more desirable than the columnar model, the columnar model remains dominant in everyday use. Therefore, we provide Table 2, Table 3, and Figure 46 comparing the major brain divisions and subdivisions according to each model. The major differences occur in the forebrain, due to the different interpretation of the longitudinal (rostro-caudal) axis and, consequently, opposite view of the transverse (dorso-ventral) divisions.

4.3 | Comparison with other squamates

Although all lizards share a basic pattern of brain organization, there are divergences in morphology that are related to the widespread morphological, ecological, and behavioral differences between species. For instance, the optic tectum is larger in diurnal lizards than in nocturnal ones, and the size of the cerebellum is related to the type of locomotion, being smaller and simpler in limbless than quadrupedal lizards (Dacey & Sereno, 1992; ten Donkelaar, 1998; ten Donkelaar & Bangma, 1992; Platel, 1976). Both the optic tectum and the cerebellum of the tawny dragon are well developed, as predicted for a
quadrupedal diurnal lizard (Gibbons, 1979; Osborne, Umbers, & Keogh, 2013; Osborne, Umbers, Backwell, & Keogh, 2012).

In the tawny dragon, we have identified the four classical cortical areas of the lizard brain: the medial, dorsomedial, dorsal, and lateral cortices (Striedter, 1997). In the dorsomedial cortex there is a cell plate visible in the inner plexiform layer (the CPDMCx in Figure 10), close to the ventricle and associated with a small but distinct ventricular ridge and a thickening of the overlying dorsomedial cortex. A similar organization is also observed in Agama agama (Figure 1B of Wouterlood, 1981). In other lizards, this inner cell plate is not as evident, although some cell clusters can be observed in a similar position (Martinez-Guijarro, Desfilis, & Lopez-Garcia, 1990; Medina et al., 1992; Smeets et al., 1986). In gekkonids, lacertids, and iguanids, the cell clusters are more numerous in the inner plexiform layer of the dorsal cortex instead of the dorsomedial cortex. Some of these form a plate referred as the cell plate of Unger (Lacerta: Medina et al., 1992; Gecko: Smeets et al., 1986) or the supraventricular layer (Iguana: Northcutt, 1967). In the green anole a cell plate is visible in the medial and dorsomedial cortices; Greenberg (1982) labelled it the dorsomedial interposition.

The identification of the dorsal pallium in reptiles has been controversial, for example see Butler (2011) versus Puelles (2006). Based on genoarchitecture during development, Desfilis et al. (2018) proposed that it is located in a very rostral and medial position, resembling that of the avian dorsal pallium, or Wulst. This area shows a different cytoarchitecture compared to medial, dorsomedial and dorsal cortices, which appear more caudally (Desfilis et al., 2018). In our MRI atlas, we identified this dorsal pallial area at very rostral telencephalic levels and accordingly named it rostrodorsal pallium (DPR; Figures 4, 37a). At these very rostral levels we have also identified other pallial divisions: the dorsolateral pallium (DLPR), the lateral pallium (LPR), medial pallium (LPM), and the ventral pallium (including the anterior olfactory nucleus, AO).

The dorsal ventricular ridge, a structure unique to sauropsids, is likely derived from two pallial divisions: most of it belongs to the ventral pallium, while its caudolateral pole belongs to the ventrocaudal

FIGURE 47 Coronal sections through an MRI model (grey scale) and a fluorescent DNA-stained brain (green) compare the appearance of (a) the medial, dorsomedial and dorsal cortices, (b) the optic tectum, and (c) the cerebellum. a = alveus, CGL = central grey layer, CL = cell layer, CWL = central white layer, DCx = dorsal cortex, DMCx = dorsomedial cortex, EZ = ependymal zone, GL = glomerular layer, IPL = inner plexiform layer, LV = lateral ventricle, MCx = medial cortex, ML = molecular layer, OL = optic layer, OPL = outer plexiform layer, PGL = periventricular grey layer, PL = Purkinje layer, PWL = periventricular white layer, SGFL = superficial grey and fibrous layer, TV = tectal ventricle [Color figure can be viewed at wileyonlinelibrary.com]
TABLE 2  The principal rostrocaudal and dorsoventral subdivisions of the central nervous system according to the columnar model of neural divisions. The dorsoventral subdivisions of roof and floor, which are universally present, are omitted

| Primary rostrocaudal subdivisions | Secondary rostrocaudal subdivisions | Primary dorsoventral divisions |
|----------------------------------|------------------------------------|-------------------------------|
| Prosencephalon or forebrain      | Telencephalon                       | Pallium                       |
| Diencephalon                     | Epithalamus                         | Dorsal thalamus               |
| Mesencephalon or midbrain        | Mesencephalon or midbrain           | Ventral thalamus              |
| Rhombencephalon or hindbrain     | Metencephalon                       | Hypothalamus                  |
| Mesencephalon or midbrain        |                                    | Midbrain tegmentum            |
| Myelencephalon                   |                                    | Cerebellum                    |
| Spinal Cord                      | Spinal segments                     | Pontine tegmentum             |
|                                  |                                    | Medullar tegmentum            |
|                                  |                                    | Alar plate/dorsal horn         |
|                                  |                                    | Basal plate/ventral horn       |

pallium (Desfilis et al., 2018). These two sectors of the dorsal ventricular ridge are evident at the caudal telencephalic levels of our atlas (e.g., Figure 10), since they appear as two light areas separated by a dark (i.e., cell poor) strip of tissue. In Iguana these two areas are also separated by a cell-poor lamina (Northcutt, 1967). In birds, the corresponding regions are the nidopallium and arcopallium, which are again separated by a cell-poor lamina (Desfilis et al., 2018).

In our model, the most prominent component of the ventrocaudal pallium is the nucleus sphericus, a structure that exhibits substantial variation in size and complexity between species. This nucleus is involved in vomerolfaction and receives massive afferents from the accessory olfactory bulb (Lanuza & Halpern, 1997; Lohman & Smeets, 1993; Martínez-García, Olucha, Teruel, Lorente, & Schwerdtfeger, 1991). The degree of development of this nucleus likely relates to its chemosensory function. In species that use the vomeronasal system extensively, such as snakes and lizards with forked tongues, the spherical nucleus occupies a large proportion of the dorsal ventricular ridge (Cooper, 1995; Halpern, 1980; Schwenk, 1993). In species with a reduced vomeronasal organ, this nucleus may be practically nonexistent, as is the case in Anolis (Greenberg, 1982). In the tawny dragon, the spherical nucleus appears to be of intermediate size, similar to Iguana (Northcutt, 1967). Both of these species, like Anolis, do not have forked tongues and are not thought to be heavily reliant on vomeronasal input. However, unlike Anolis, Ctenophorus and Iguana have femoral pores which produce a waxy substance (Gray, 1827) that is likely used for chemosensory signaling through the vomeronasal system (Baeckens, Edwards, Huyghe, & Van Damme, 2015; Martin & Lopez, 2000).

4.4  Relevance of the tawny dragon for studies on the neurobiological basis of behavior

Among squamate reptiles, agamids (dragon lizards, Hamilton, May, & Waters, 2015), with more than 300 species, form an enormously diverse group with extensive morphological, ecological, and behavioral differences between species. Agamids are considered a good model for the study of evolutionary biology (Chen, Stuart-Fox, Hugall, & Symonds, 2012; Melville, Ritchie, Chapple, Glor, & Schulte, 2011; Stuart-Fox & Owens, 2003). In particular, the genus Ctenophorus has been the object of numerous ecological and behavioral comparative studies (Osborne, 2005a; Stuart-Fox, Moussalli, Johnston, & Owens, 2004; Umbers, Osborne, & Keogh, 2012).

Some tawny dragon populations are color-polymorphic and each morph exhibits different social and reproductive strategies (McLean, Stuart-Fox, & Moussalli, 2014; Teasdale, Stevens, & Stuart-Fox, 2013; Yewers, Pryke, & Stuart-Fox, 2016). Recently, there has been intense interest in studying color polymorphic lizards as models of the origin and maintenance of intraspecific phenotypic and genetic diversity (Corl, Davis, Kuchta, & Sinervo, 2010; McLean & Stuart-Fox, 2014; Vercken, Massot, Sinervo, & Clobert, 2007; Zamundio & Sinervo, 2003). One common finding is variation in reproductive strategy between color morphs (McLean & Stuart-Fox, 2014; Osborne, 2005b, 2005a; Osborne et al., 2012; Teasdale et al., 2013; Wellenreuther, Svensson, & Hansson, 2014; Yewers et al., 2015; Zamundio & Sinervo, 2003). However, little attention has been paid to neural differences between morphs, despite their obvious potential role in driving behavioral variation, including reproductive strategies (but see LaDage et al., 2009,2013). Another Ctenophorus species, the painted dragon (C. pictus), is also color polymorphic (Healey, 2008; Olsson, Schwartz, Uller, & Healey, 2009; Tobler, Healey, & Olsson, 2011), but most remaining Ctenophorus species are monomorphic, making this genus an ideal system for studying the evolution of color polymorphism and its relationship to behavioral and neural variation. Further work using color polymorphic lizard species holds great potential in elucidating the neural underpinnings of different reproductive strategies.

5  CONCLUSIONS

This is the first time, to our knowledge, that an MRI atlas of a lizard brain has been produced. MRI is an innovative technique used frequently in the medical sciences. Here, we have added the first reptile
to the growing list of MRI atlases available for non-traditional study organisms. The resolution obtained in this atlas is significantly higher than that of other atlases for animals with similarly-sized brains. We hope this atlas provides inspiration to further the study of the reptile brain, the correlation between brain structure and function, and the study of brain evolution, particularly using comparative methods. Only by advancing research in all these fields can we understand the general principles of vertebrate brain organization and identify selective pressures and mechanisms behind variation in the functional organization of the brain. We aspire to develop a range of MRI atlases representing, as much as possible, the diversity of vertebrates. Our goal is to make these universally available through a virtual museum, similar to those provided by brain collections in traditional brick-and-mortar museums (Iwaniuk, 2010,2011), and more recently by the on-line brain collections such as the Comparative Mammalian Brain Collection (http://neurosciencelibrary.org) and BrainMaps.org (http://brainmaps.org/).

### 6 | DATA ACCESSIBILITY

The MRI model of a tawny dragon brain (Ctenophorus decresii), the dragon brain atlas, and the MRI of a tawny dragon head are freely available for download from the Wiley Biolucida Server at wiley.biolucida.net.

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| Primary rostrocaudal subdivisions | Secondary rostrocaudal subdivisions (protosegments) | Tertiary rostrocaudal subdivisions (segments) | Primary dorsoventral subdivisions |
|-----------------------------------|---------------------------------------------------|---------------------------------|----------------------------------|
| Prosencephalon or forebrain       | Secondary prosencephalon                           | Terminal or rostral hypothalamic prosomere (hp2) | Alar Preoptic area Alar terminal hypothalamus |
|                                   |                                                   | Peduncular or caudal hypothalamic prosomere (hp1) | Basal Basal terminal hypothalamus |
| Diencephalon                      | Prosomere 3 (p3)                                  | Alar Prethalamic eminence Prethalamus | Basal Basal peduncular hypothalamus |
| Mesencephalon or midbrain         | Mesencephalon or midbrain                          | Mesomerese 1 and 2                | Alar Tectum |
| Rhombencephalon or hindbrain      | Prepontine (Istmo-cerebellar) division             | Rhombomeres 0, 1, 2 (r0, r1, r2)  | Basal Tegmentum (III motor nuclei) |
|                                   |                                                   | Rhombomeres 3, 4 (r3, r4)         | Alar Istmic nuclei Locus coeruleus Rostral vestibular nuclei Main trigeminal nucleus Cerebellum |
|                                   |                                                   | Rhombomeres 5, 6 (r5, r6)         | Alar Parts of vestibular nuclei Part of descending trigeminal nucleus |
|                                   |                                                   | Rhombomeres 7-11 (r7-r11)        | Alar Parts of vestibular nuclei Part of descending trigeminal nucleus |
| Spinal Cord                       | Cervical, Thoracic, Lumbar, Sacral, Coccygeal regions | Myelomeres                      | Basal IX, X & XI motor nuclei & nerve exits |

TABLE 3. The principal rostrocaudal and dorsoventral subdivisions of the central nervous system according to the neuromeric model of neural subdivisions. The dorsoventral subdivisions of roof and floor, which are universally present, are omitted.
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FIGURES 4–20 Coronal sections through the MRI model of the tawny dragon (Ctenophorus decresii) brain. Figures are in rostro-caudal order and each section is 25 voxels or 500 μm caudal to the previous section except for Figure 5, which is 250 μm caudal to Figure 4 and 250 μm anterior to Figure 6. The plane of each section according to our coordinate system is indicated in the upper left corner. The bar in the lower right corner = 1 mm. A list of abbreviations is found in Table 1.
FIGURE 21  A coronal section through the MRI model of the anterior spinal cord of the tawny dragon (*Ctenophorus decresii*). The plane, according to our coordinate system, is indicated in the upper left corner. The bar in the lower right corner = 1 mm. cc = central canal, dc = dorsal column tract, DH = dorsal horn, VH = ventral horn

FIGURES 22–30  Horizontal sections through the MRI model of the tawny dragon (*Ctenophorus decresii*) brain. Figures are in ventro-dorsal order and each section is 25 voxels or 500 μm dorsal to the previous section. The plane of each section according to our coordinate system is indicated in the upper left corner. The bar in the lower right corner = 1 mm. A list of abbreviations is found in Table 1
FIGURES 31–36 Sagittal sections through the MRI model of the tawny dragon (Ctenophorus decresii) brain. Figures are in medio-lateral order. Each section is 25 voxels or 500 μm lateral to the previous section except for Figure 32, which is 20 voxels (400 μm) lateral to Figure 31. This is because Figure 32 is offset from the midline by 100 μm. The plane of each section according to our coordinate system is indicated in the upper left corner. The bar in the lower right corner = 1 mm. A list of abbreviations is found in Table 1.
