



# A three-dimensional digital atlas of the Nile crocodile (*Crocodylus niloticus*) forebrain

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## Abstract

The phylogenetic position of crocodylians in relation to birds and mammals makes them an interesting animal model for investigating the evolution of the nervous system in amniote vertebrates. A few neuroanatomical atlases are available for reptiles, but with a growing interest in these animals within the comparative neurosciences, a need for these anatomical reference templates is becoming apparent. With the advent of MRI being used more frequently in comparative neuroscience, the aim of this study was to create a three-dimensional MRI-based atlas of the Nile crocodile (*Crocodylus niloticus*) brain to provide a common reference template for the interpretation of the crocodylian, and more broadly reptilian, brain. Ex vivo MRI acquisitions in combination with histological data were used to delineate crocodylian brain areas at telencephalic, dien-cephalic, mesencephalic, and rhombencephalic levels. A total of 50 anatomical structures were successfully identified and outlined to create a 3-D model of the Nile crocodile brain. The majority of structures were more readily discerned within the forebrain of the crocodile with the methods used to produce this atlas. The anatomy outlined herein corresponds with both classical and recent crocodylian anatomical analyses, barring a few areas of contention predominantly related to a lack of functional data and conflicting nomenclature.

**Keywords** MRI · Reptile · Neuroanatomy · 3D atlas

## Abbreviations

3 V	3rd ventricle
AS	Anterior septum
CNIII	Cranial nerve 3 (occulomotor nerve)
CNIV	Cranial nerve 4 (trochlear nerve)

CO	Chiasma opticum
CP	Posterior commissure
D	Nucleus diagonalis
dADVR	Dorsal anterior dorsal ventricular ridge
DBB	Diagonal band of brocca
DC	Dorsal cortex
DMA/DLA	Dorsal thalamic nuclei (medial and lateral)
dStr	Dorsal striatum
E	Entopallium
Field L	Field L
Fim	Fimbria
GLDd	Dorsal lateral geniculate nucleus
GLv	Ventral lateral geniculate nucleus
Hb	Habenula
HC	Hippocampal commissure
Hp	Hippocampus
Hyp	Hypothalamus
I	Isthmic nuclei
LC	Lateral cortex
LFB	Lateral forebrain bundle
LoC	Locus coeruleus
LS	Lateral septum

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LV	Lateral ventricle
MC	Medialis complex nuclei
MFB	Medial forebrain bundle
ML	Mesencephalic lentiform nucleus
MS	Medial septum
nBOR	Basal optic root nucleus
nDCP	Dorsal posterior commissure nucleus
nVd	Nucleus et tractus descendens nervi trigemini
OT	Optic tract
ov	Nucleus ovalis
PAG	Periaqueductal gray
PDVR	Posterior dorsal ventricular ridge
Prim.Hp	Primordial hippocampus
Re	Nucleus reuniens
RF	Posterior reticular formation
Rt	Nucleus rotundus
SM	Stria medullaris
TeO	Optic tectum
TOL	Lateral olfactory tract nucleus
TS	Torus semicircularis
TU	Olfactory tuberculum
vADVR	Ventral anterior dorsal ventricular ridge
vStr	Ventral striatum

## Introduction

Research on the brain and behaviour of reptiles<sup>1</sup> has experienced a recent growth in interest, with studies examining aspects of brain evolution (Striedter 2016; Desfilis et al. 2018; Tosches et al. 2018), learning and cognition (Northcutt 2013; Noble et al. 2014; Krochmal et al. 2015; Siviter et al. 2017; Matsubara et al. 2017), neural structure and function (Ngwenya et al. 2016; Pritz 2016; Fournier et al. 2018), and post-hatching neurogenesis (Powers 2016; Ngwenya et al. 2018). Historically relatively little research effort has been focused on reptiles (Bonnet et al. 2002; Manger et al. 2008), despite the diversity of this class, their occupation of multiple environmental niches, and their broad behavioural repertoires (Butler and Hodos 2005; Burghardt 2013; Nomura et al. 2013). For example, turtles and crocodiles show object play (Burghardt 2015), lizards succeed in problem-solving tasks, and show behavioural flexibility (Powell and Leal 2012; Vasconcelos et al. 2012), and tortoises can learn by observing conspecifics (Wilkinson et al. 2010). Importantly, reptiles represent the third major group of the amniotes, the other two groups being mammals and birds, and thus, studies of their brains and behaviour may

reveal important insights in the evolution of brain structure and function found across amniotes.

Although considered to have a simple organization (MacLean 1990; Aboitiz 1995; Butler et al. 2011; Patton 2015), the reptilian forebrain has been shown to possess many similarities with mammalian and avian forebrains (Jarvis 2009; Striedter 2016; Briscoe and Ragsdale 2018). Sensory processing networks (Reiner and Powers 1980; Berson and Hartline 1988; Reiner and Northcutt 2000; Manger et al. 2002; Vergne et al. 2009; Belekova et al. 2010; Belekova and Kenigfest 2018; Behroozi et al. 2018a), amygdaloid functions (Striedter 1997; Fernandez et al. 1998; Lanuza 1998; Puelles and Kuwana 2000), motor control (Medina and Smeets 1991; Reiner et al. 1998, 2005), and the resultant behaviours (Peterson 1980; Powell and Leal 2012; Nomura et al. 2013) question the idea of a simply organized reptilian telencephalon. The structure and development of the reptile forebrain is an area of interest with particular reference to understanding avian and mammalian homologies (Aboitiz 1995, 1999; Puelles 2001; Butler and Molnár 2002; Martínez-García et al. 2002; Medina 2010; Butler et al. 2011; Dugas-Ford and Ragsdale 2015; Montiel et al. 2016). Crocodylians, which represent the closest extant relative of birds (Green et al. 2014; Güntürkün et al. 2017a), and share a stem amniote ancestor with mammals (Jarvis 2009), are an excellent model for understanding the structure, function, and evolution of amniotes brains.

The growing interest in reptilian neurobiology underscores the need for precise anatomical maps and brain atlases to allow more sophisticated experiments, such as electrophysiological studies, lesion experiments, or non-invasive imaging like functional MRI or PET (Behroozi et al. 2018a). Currently, despite specific aspects of the crocodylian forebrain having been examined (Pritz 1974a, b, 1975, 1995; Brauth and Kitt 1980; Pritz and Stritzel 1992; Derobert et al. 1999; Briscoe et al. 2018; Briscoe and Ragsdale 2018), no atlas of the crocodylian brain established with modern scanning techniques is available, and our global understanding of the crocodylian brain is heavily dependent on early studies using classical neuroanatomical methods (Crosby 1917; Huber and Crosby 1926; Riss et al. 1969). Thus, we have created a three-dimensional atlas of the Nile crocodile brain based on MRI data that describes the telencephalon, diencephalon, mesencephalon, and rhombencephalon.

## Materials and methods

### Acquisition of the crocodile specimen

For this study, one young Nile crocodile (*Crocodylus niloticus*) with a body mass of 2.8 kg and snout to tail length of 95.5 cm was used. This crocodile was sourced from the

<sup>1</sup> \*Reptile refers to non-avian reptiles.

Thaba Kwena Crocodile Farm based in Bela Bela, Limpopo Province, South Africa. The size of the selected animal was restricted by the size of the scanner tube of the MRI machine based in Germany, which allowed a maximum head width of 100 mm. The crocodile was treated and used according to the guidelines of the University of the Witwatersrand Animal Ethics Committee, which parallel those of the NIH (National Institutes of Health) for the care and use of animals in scientific experimentation. The ethical clearance number for the project: 2015/06/25/A, the ordinary permit number: 029,562, and the CITES export clearance number: 152,535 were certified and issued by the relevant governmental authorities in South Africa. The German import clearance number: E-04349/15 was issued by the Bundesamt für Naturschutz (German Federal Agency for Nature Conservation). The animal was sacrificed (i.p. Euthapent, 2 ml/kg, containing 100 mg/ml sodium pentobarbital) and then transcardially perfused with a phosphate-buffered saline solution (0.9% NaCl, 0.12 M phosphate buffer, PB), followed by a mixture of 4% paraformaldehyde and 1% Dotarem (Gadoteric acid: a paramagnetic MR contrast agent) in 0.1 M PB. The head was separated from the remainder of the body and stored in the paraformaldehyde/Dotarem solution at 4 °C and imaged within a week of preparation.

### Data acquisition

3D MRI data sets of the crocodile brain were acquired using a horizontal bore small animal scanner (Bruker BioSpec, 70/30 USR, Germany) using an 80 mm transmit quadrature birdcage resonator and a planar single-loop 20 mm receiver coil from the same vendor. The imaging and shim unit was a BGA 12 s model with 444 mT/m maximal strength.

T2-weighted 3D MR images were created using a Rapid Acquisition with Relaxation Enhancement (RARE) sequence of Bruker with the following parameters: RARE factor = 1; spectral bandwidth = 120 kHz; number of average = 3; repetition time = 600 ms, effective echo time = 10, 20, 30, 40, 50 ms; field of view = 32 × 32 × 25 mm<sup>3</sup>; matrix size = 400 × 400 × 256; spatial resolution = 80 × 80 × 98 μm<sup>3</sup>. The total acquisition time was 51 h 12 min.

### Histological protocol

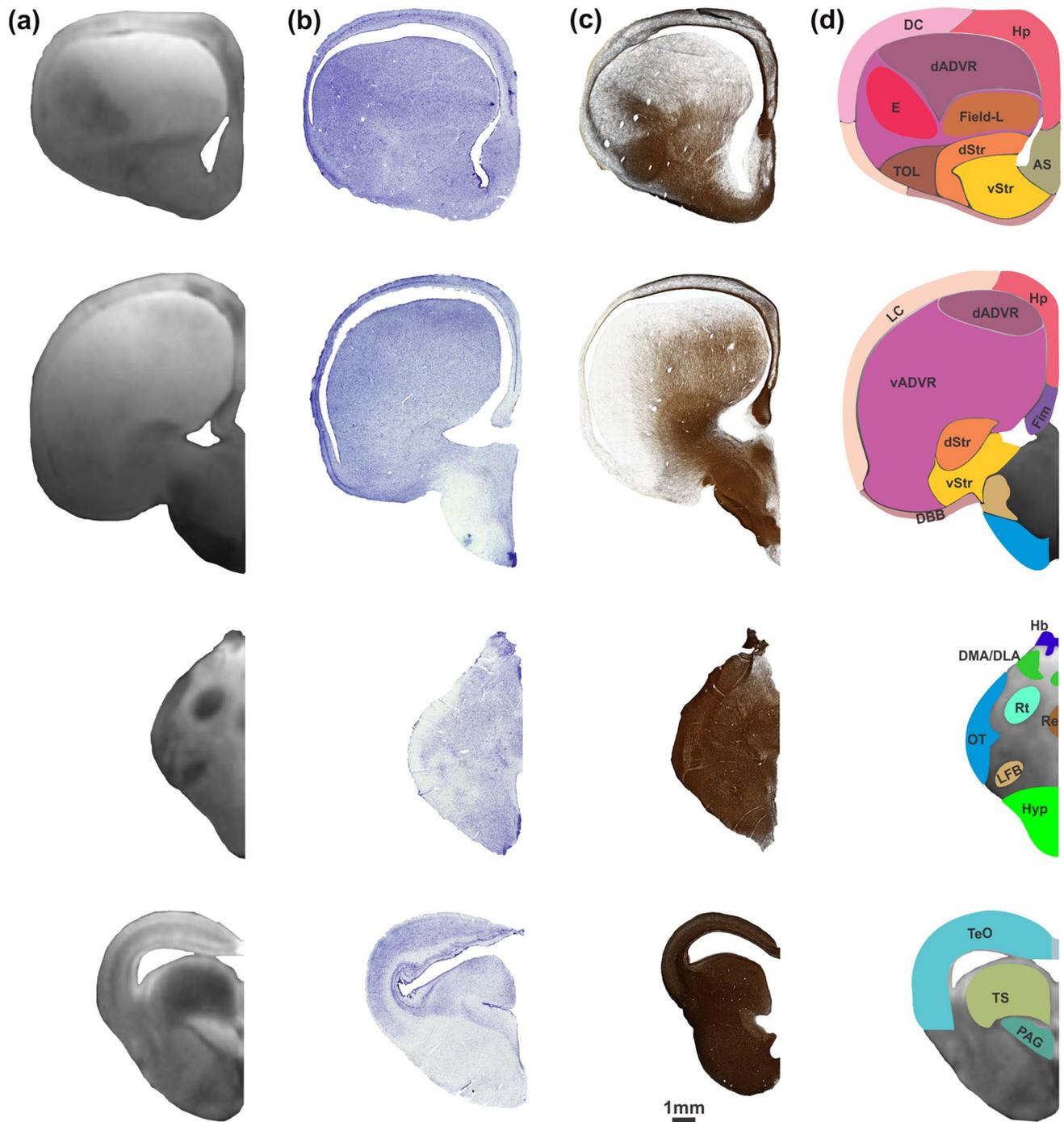
The scanned brain was extracted from the skull and post-fixed in 4% paraformaldehyde and 30% sucrose in 0.1 M PB for 2 h at 4 °C, and then cryoprotected in 30% sucrose in 0.1 M PB at 4 °C for 48 h. The brain was sectioned in the coronal plane from rostral to caudal at 40 μm thickness on a freezing microtome. Sections were collected in two parallel sequences and subsequently stored in 0.12 M PBS containing 0.1% sodium azide at 4 °C before being mounted on 0.5% gelatine-coated glass slides. The two series of sections

were then stained for either cresyl violet or myelin. In brief, for cresyl violet staining, the sections were first rinsed in 96% ethanol and then placed in a 1% Cresyl Violet (Sigma-Aldrich, USA) solution for 15 min. The stained sections were then differentiated in 0.2% acetic acid, dehydrated in graded series of alcohol, cleared in xylene, and cover slipped.

Myelin sections were stored in 5% formalin for a period of 2 weeks and were then mounted on 1.5% gelatine-coated glass slides and subsequently stained with a modified silver staining protocol (Gallyas 1979).

### Brain area delineation and 3-D construction

The scanned images in conjunction with the histological sections were used to create a 3D atlas of the crocodile telencephalon, including some areas in the diencephalon and a few areas in the mesencephalon and anterior rhombencephalon. Due to the limited field of view of the utilized receiver coil, delineation of the olfactory bulb and areas posterior to the anterior rhombencephalon were not analysed, as the resolution and/or contrast of the images were unsuitable for reliable identification of structures in these regions. Delineation of all regions of interest (ROIs) were performed in the frontal plane of one hemisphere using the software FSL view [FSL, Oxford, UK, version 5.0.9 (Smith et al. 2004)] and labelled with itk-snap (Version 3.4.0). 3D reconstructions were carried out with the software 3D slicer (Fedorov et al. 2012). A comprehensive consolidated histological atlas of the crocodilian brain is not available, and thus, anatomical areas were matched to multiple studies that focussed on different aspects of the crocodilian brain (Crosby 1917; Huber and Crosby 1926; Riss et al. 1969; Brauth and Kitt 1980; Brauth et al. 1983, 1988; Derobert et al. 1999; Pritz 2015). Anatomical regions were identified based on the contrast gradient of the images. For example, T2-weighted images present with a lighter contrast for regions depicting cell bodies and neuropil and a darker contrast gradient for myelinated fibre tracts. In addition, structures were also identified according to their shape and relative location (e.g., nucleus reuniens has a distinctive circular shape and is positioned centrally within the diencephalon when compared to nucleus rotundus, which is bilaterally placed). The general topographical arrangement of the structures outlined aided in the delineation of borders. Furthermore, anatomical borders and transitional regions that were unclear were addressed by matching the MRI image to the appropriate Nissl and myelin stained section taken from the same brain (e.g., Fig. 1). As a general approach when specific structures within a region of the brain were difficult to identify with the techniques used, for example, the hypothalamic nuclei, these structures were grouped under the rubric of



**Fig. 1** Comparison between coronal sections of T2-weighted MRI images (a), Nissl stained sections (b), myelin stained sections (c), and the overlay of the delineated structures based on corresponding MRI images (d). Delineations of areas in the atlas are based on frontal

T2-weighted MRI images and any identified area was confirmed by analysis of Nissl and myelin sections. Refer to Table 1 for the corresponding colour codes and anatomical region

larger structural entities, for example the hypothalamus. The nomenclature used in the current study was adopted

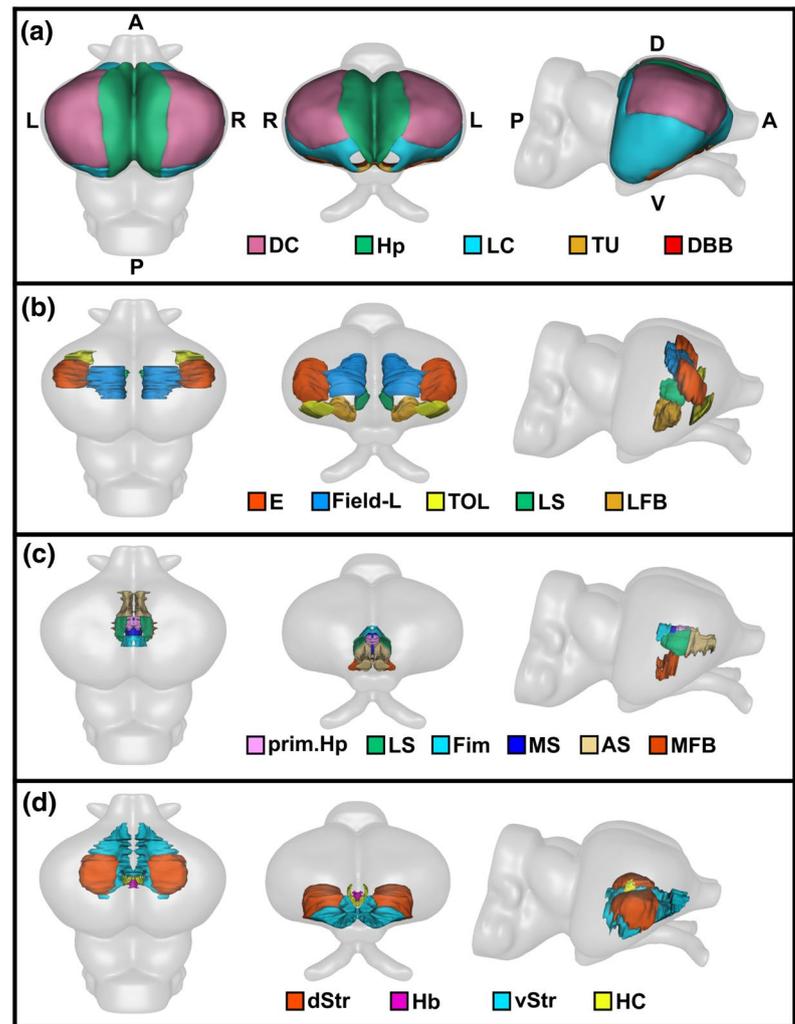
from multiple sources (e.g., Crosby 1917; Huber and Crosby 1926; Brauth and Kitt 1980; Pritz 2016; Table 1).

**Table 1** Nomenclature of delineated subdivisions, nuclei, and fibre tracts in Nile crocodile brain

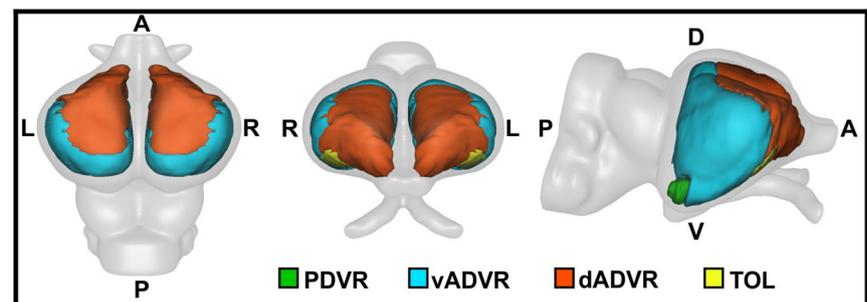
		Abbreviation	Structure		
Telencephalon	Pallium	Cortical pallium	DC	Dorsal Cortex	
			Hp	Hippocampus	
			Fim	Fimbria	
			LC	Lateral Cortex	
	Nuclear pallium (DVR)	dADVR	dorsal Anterior Dorsal Ventricular Ridge		
		vADVR	ventral Anterior Dorsal Ventricular Ridge		
		Field L	Field L		
		E	Entopallium		
		TOL	Lateral Olfactory Tract Nucleus		
		PDVR	Posterior Dorsal Ventricular Ridge		
	Sub-Pallium	Septal complex	LS	Lateral Septum	
			AS	Anterior Septum	
			MS	Medial Septum	
			DBB	Diagonal Band of Broca	
			Prim.Hp	Primordial Hippocampus	
			Pallidostriatal complex	dStr	dorsal Striatum
	vStr	ventral Striatum			
	TU	Olfactory Tuberculum			
	Diencephalon	Hypothalamus		Hyp	Hypothalamus
			Epithalamus	Hb	Habenula
		SM		Stria medullaris	
		Dorsal thalamus	DMA/DLA	Dorsal Thalamic nuclei (medial and lateral)	
			GLDd	dorsal Lateral Geniculate Nucleus	
			D	Nucleus Diagonalis	
			MC	Medialis complex nuclei	
			Re	Nucleus Reuniens	
			Rt	Nucleus Rotundus	
Ventral thalamus		GLv	ventral Lateral Geniculate Nucleus		
		ov	Nucleus ovalis		
Pretectum		ML	Mesencephalic lentiform nucleus		
		nDCP	dorsal posterior commissure nucleus		
Mid and Hindbrain		Mesencephalon	nBOR	Basal optic root nucleus	
			TeO	Optic Tectum	
			TS	Torus semicircularis	
			PAG	Periaqueductal gray	
		Cranial nerves	CN III	Cranial nerve 3 (oculomotor nerve)	
	CN IV		Cranial nerve 4 (trochlear nerve)		
	Rhombencephalon	I	Isthmic nuclei		
		LoC	Locus coeruleus		
		nVd	nucleus et tractus descendens nervi trigemini		
		RF	posterior reticular formation		
	Tracts & Ventricles	Fiber tracts	LFB	Lateral Forebrain Bundle	
			MFB	Medial Forebrain Bundle	
OT			Optic Tract		
Commissural Fibers		HC	Hippocampal commissure		
		CO	Chiasma Opticum		
		CP	Posterior Commissure		
Ventricle structures		3V	3rd ventricle		
		LV	Lateral ventricle		



**Fig. 3** 3-D rendering of the Nile crocodile brain with several identifiable telencephalic structures delineated. **a** Cortical mantle and associated subdivisions as well as the basal forebrain structures. **b** Nuclear structures within the ADVR such as the lateral olfactory tract nucleus (TOL) and the sensory functional areas for vision (E, entopallium) and audition (Field L). In addition, the lateral forebrain bundle (LFB) and lateral septum (LS) are shown. **c** Subpallial structures localised within the medial aspect of the telencephalon. **d** Pallidostriatal structures with the inclusion of the habenular nuclei (Hb) and the hippocampal commissure (HC). For further abbreviations, see Table 1 and main text

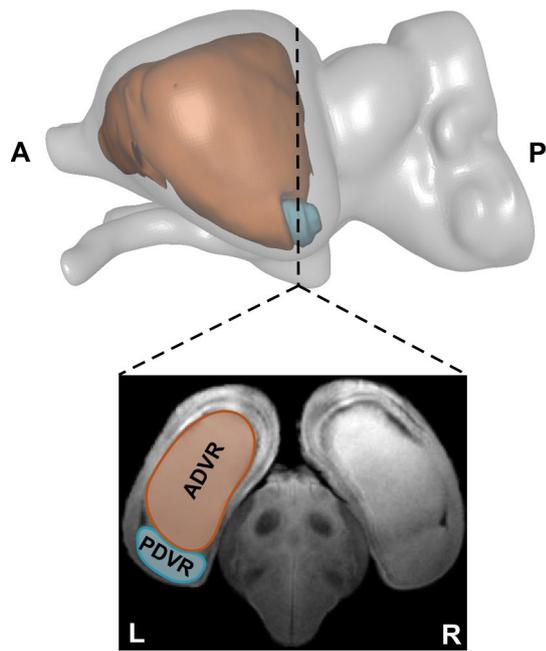


**Fig. 4** 3-D rendering of the Nile crocodile brain and structures identified within and associated with the dorsal ventricular ridge (DVR) delineated. Depicted are a dorsal (left), anterior (middle), and lateral (right) views of the brain. For further abbreviations, see Table 1 and main text



the cortical mantle and the associated three major cortices typically described in crocodylians, including the hippocampus (Hp), the dorsal cortex (DC), and the lateral cortex (LC) (Figs. 1, row 1 and 2, 2, 3a). The hippocampus in this outline is defined according to Crosby's (1917) interpretation and includes the hippocampus pars dorsalis and pars dorso-medialis. The Hp extends the entire rostro-caudal length of the pallium along the medial aspect and is directly bordered by the lateral cortex at the rostral and caudal poles (Fig. 3a).

The hippocampal formation also includes the fimbria (Fim) located immediately caudal to the hippocampal commissure as outlined within the current template (Figs. 1, row 2, 3c). The lateral cortex, positioned along the lateral border of the pallium, also extends throughout the rostro-caudal extent of the pallium. The dorsal cortex is positioned between the Hp and LC, and is absent in the extreme rostral and caudal poles (Figs. 1, row 1 and 2, 3a). Although subcomponents of these areas were partly visible in our MRI images (e.g.,



**Fig. 5** The anterior (ADVR, red) and posterior (PDVR, blue) subdivisions of the dorsal ventricular ridge could be readily demarcated using the MRI images obtained in the current study

hippocampal subdivisions and cortical layers), we refrained from delineating them due to uncertainties regarding the exact borders.

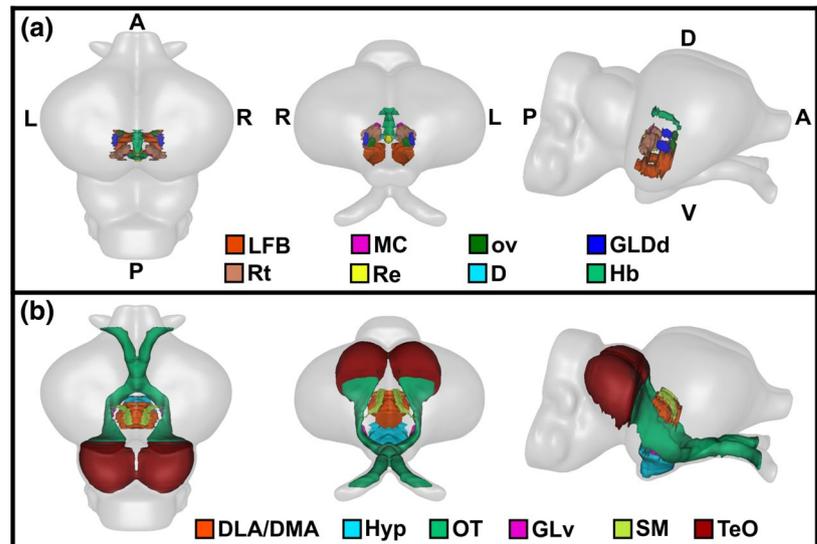
**Nuclear pallium:** The nuclear pallium, the largest anatomical structure within the crocodylian telencephalon, consists of the dorsal ventricular ridge (DVR). Based on our MRI images and on previous delineations, we subdivided the DVR into anterior (ADVR) and posterior (PDVR) subdivisions (Ulinski 1983) (Figs. 4, 5). Using the contrast of the MRI images in combination with the matching Nissl stained sections, we could further subdivide the ADVR into two separate divisions, one ventral and one dorsal along the rostro-caudal axis (Figs. 1, row 1 and 2, 2b–i, 4). In addition, we were able to identify and outline the nucleus of the lateral olfactory tract (TOL), and confirm the observation of the sensory regions for vision (E) and audition (Field L) within the ADVR of crocodylians as per a previous study (Figs. 1, row 1, 2b–i, 3b, 4) (Behroozi et al. 2018a). The PDVR was readily separated from the ADVR, in both coronal and sagittal planes (Fig. 5). The PDVR was separated from the ADVR with the use of cytoarchitectural criteria, such as a sparse cell zone similar to the zona limitans described below and the more homogenous cellular organisation of the PDVR when compared to the heterogeneous ADVR. Subdivisions within the PDVR were, however, not evident.

**Subpallium:** Subpallial structures were located within the ventral telencephalon by a boundary that presented as a cell free zone (i.e., zona limitans) separating the striatum from

the ADVR (Fig. 1, row 1), the medial cortex from the primordial hippocampus, and the basal forebrain nuclei from the lateral cortex. As such, we were able to delineate the striatum, the septal nuclei, the primordial hippocampus, as well as several basal forebrain nuclei. The striatum, located directly ventral to the ADVR, could be further separated into dorsal (dStr) and ventral (vStr) subdivisions (Figs. 1, row 1 and 2, 3d). The striatum in part matches the classical VLA (i.e., ventrolateral area as described by Crosby 1917). Specifically, our observations match those described by Brauth and Kitt (1980), where the dorsal striatum corresponds to their large cell VLA (VLA lc), and the ventral striatum to their small cell VLA (VLA sc). It should be noted that the border separating the striatum and the nucleus accumbens was difficult to determine with accuracy, and hence, we have grouped these structures in the rostral aspect of the atlas, labelled as vStr (Fig. 1 row 1, 3d). In addition, it was equally difficult to determine the borders separating two specialised regions defined as the ventro-caudal region of the VLA lc, and the dorso-medial region of the VLA lc, and hence, these structures were also grouped into the broader outline of the VLA lc, labelled as the dorsal striatum. Along the medial aspect of the pallium, ventral to the primordial hippocampus (Prim.Hp), the septal nuclei were identified. The rostral pole of the septum was homogenous and named the anterior septum (AS) (Figs. 1, row 1, 3c). Caudally, the grey matter mass of the septal nuclear complex was bisected by the medial forebrain bundle (MFB, see below) into medial (MS) and lateral (LS) septal subdivisions (Fig. 3c). We could readily identify and delineate the basal forebrain nuclei, olfactory tuberculum (TU), and the diagonal band of Broca (DBB), located at the most ventral aspect of the subpallium, with TU transitioning into DBB (Figs. 1, row 1 and 2, 2, 3a). The ventro-medial nucleus, located at the intersection of the TOL, striatum, ADVR, TU and/or DBB, was not readily identifiable and has been co-opted into the dorsal striatum. Dorsal-to-the septal nuclei, the Prim. Hp was identified, but this structure was difficult to separate from the ventral aspect of the hippocampus (medial) rostrally, although the caudal portion of this cortical structure was readily identified (Fig. 3c). The primordial hippocampus is thought to form part of the septal nuclei (Riss et al. 1969), but here was separated and outlined in accordance with the description provided by Crosby (1917).

**Commissural fibres and fibre bundles:** The two major fibre tracts of the reptilian telencephalon, the lateral (LFB) and medial forebrain bundles (MFB), were readily discernible (Fig. 3b, c). The lateral forebrain bundle became apparent in a central position, between the dorsal and ventral striatum, and continued medio-ventrally to merge with the MFB. The MFB originated immediately caudal to the termination of the anterior septum, between the LS and MS, and continued latero-ventrally to merge with the LFB ventrally. The largest

**Fig. 6** 3-D rendering of the Nile crocodile diencephalon and mesencephalon. **a** Epithalamic and dorsal thalamic nuclei within the diencephalon. **b** Dorsal thalamic anterior nuclei (DMA\DLA) and ventral diencephalic structures. Additionally, the complete trajectory of the optic nerve, chiasm, and tract to the optic tectum is outlined (OT). For further abbreviations, see Table 1 and main text



commissure within the telencephalon, the hippocampal commissure (HC), was readily observed and outlined (Fig. 3d). The anterior commissure, however, was not included in the current atlas, as it was not visible in the images generated in the current study. Both the anterior commissure nucleus and the hippocampal commissure nucleus (Crosby 1917) could not be identified.

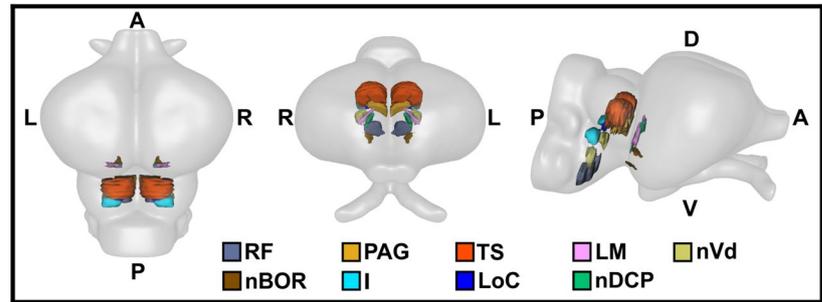
## Diencephalon

Due to the limited field of view of the receiver coil and the centering of the coil over the telencephalon, the ventral and caudal aspects of the diencephalon, mesencephalon, and rhombencephalon suffered from low signal contrast, which restricted us from delineating many nuclei in these regions. Nevertheless, we were able to delineate numerous structures in the dorsal and rostral diencephalon, including thalamic sensory nuclei, the habenular nucleus, the hypothalamus (though without subdivisions), and the major diencephalic fibre tracts.

In the dorsal aspect of the diencephalon, we identified the habenular complex (Hb) (Figs. 1, row 1, 2a, 3d, 6a). The Hb was accompanied by the stria medullaris (SM), a fibre tract connecting the Hb with other diencephalic structures as well as with the telencephalic septum (Fig. 6b). The specific morphological features that assisted with the identification of the Hb were based on the association to the fibre tract SM, the unique triangular shape of the nuclei, and its dorsal position within the diencephalon. The SM was clearly defined on MRI images as darker in colour, which is a common feature of the major fibre tracts, and its dorsolateral position which is closely associated with the Hb complex. The anterior dorsal thalamic nuclei, the most rostral of the ventral thalamic nuclei, positioned ventral to the habenular

nucleus, were difficult to subdivide into medial and lateral components; therefore, we grouped these nuclei as DLA/DMA (Fig. 6b). The thalamic sensory nuclei described for crocodylians were clearly visible, including nucleus reuniens (Re, auditory), nucleus ovalis (ov), nucleus rotundus (Rt), nucleus geniculatus lateralis pars dorsalis (GLDd) and pars ventralis (GLv, all five visual), medialis complex (MC, somatosensory), and nucleus diagonalis (D, specific function not defined as yet, possibly somatic if considered the counterpart of the turtle nucleus ventralis) (Pritz 2016) (Figs. 1 row 3, 6a, b). The sensory thalamic nuclei were also identified using morphological features based on shape, position, symmetry, and association of structures. Nucleus ovalis, as the name suggests, was oval in shape and positioned rostrally in close approximation to the ventral aspect of SM. Nucleus rotundus was bilaterally positioned at the transition of the caudal aspects of the DLA/DMA, at which point Rt changed in shape from round to oval from its rostral to caudal representation. Nucleus reuniens was centrally placed within the diencephalon, round in shape, and bordered dorsolaterally by the bilateral Rt nuclei. The medialis complex was positioned intimately around the medio-dorsal aspect of the Rt, whilst the D was positioned more closely along the ventral border of the Re. Both the GLDd and GLv were identified on the extreme lateral border of the diencephalon; however, the GLDd was positioned more dorsally and the GLv more ventrally, both of which were in close proximity to the laterally placed optic tract as it encapsulates the diencephalon on its outer margin (Fig. 6a, b). Thalamic nuclei that were a challenge to confidently identify, and were thus not included, were the posterocentralis and the area ventrolateralis as described by Pritz (2014). Ventral to the thalamus, the only region that we could reliably identify was the hypothalamus (Hyp) as a single structure (Figs. 1, row 3, 2a–c, 6b), since

**Fig. 7** 3-D rendering of the Nile crocodile mesencephalon and rhombencephalon (dorsal, anterior, and lateral views). This figure shows the nuclei that could be readily identified in these regions with the MR images obtained in the current study. For further abbreviations, see Table 1 and main text



the faint signal contrast in this region limited delineation of subdivisions with accuracy. Finally, regarding the major fibre tracts, the extension of the lateral and medial forebrain bundles within the telencephalon were clearly visible, but the separation of these fibre bundles within the diencephalon was not clear and was thus collectively referred to as the LFB within the diencephalon (Figs. 1, row3, 6a). The optic nerves and optic chiasm (CO) were readily identified at the ventral aspect of the telencephalon. At the rostral border of the hypothalamus (Hyp), the optic chiasm (CO) was observed to decussate into bilateral optic tracts (OT), which traversed the caudo-lateral extremes of the diencephalon, from ventral to dorsal (Fig. 6b). The optic tract could be traced along the caudo-lateral aspects of the diencephalon until its insertion into the pronounced optic tectum (TeO) of the mesencephalon (Figs. 1, row 3 and 4, 6b).

### Mesencephalon and rhombencephalon

Similar to the diencephalon, reduced signal quality restricted nuclear identification to a few structures mostly within the rostral and dorsal aspects of the mesencephalon and rhombencephalon.

The pretectum originated at the caudal most aspect of the diencephalon and transitions into the rostral mesencephalic structures. Within the pretectum, structures that could be identified included the posterior commissure (CP), the nucleus lentiformis mesencephali (LM), and the nucleus dorsalis commissura posterioris (nDCP) (Fig. 7). As mentioned, the pretectum transitioned into the rostral mesencephalon with the emergence of the optic tectum (TeO) (Figs. 1, row 4, 6b), followed caudally by the ventrally placed torus semicircularis (TS). Ventral to the torus semicircularis, the periaqueductal gray matter (PAG) was identified (Figs. 1, row 4, 7). In the ventral aspect of the rostral midbrain, only the basal optic root nucleus (nBOR) was confidently identified, based on its shape and neuro-anatomical position (Fig. 7). The optic tectum and the torus semicircularis terminated at the caudal most extreme of the mesencephalon (Fig. 7).

At the level of the rhombencephalon, specifically the pons, the locus coeruleus (LoC) was observed to lie medial

to the isthmic nuclei (I) (the combination of nucleus isthmi pars magnocellularis and parvocellularis), which was closely bordered by the fourth cranial nerve (NIV) inferior to the isthmic nuclei. In the lateral aspect of this brainstem region, the nucleus descendens nervi trigemini (nVd) (Fig. 7) was identified, while the most ventrolateral aspect housed the large posterior reticular formation (RF) (Fig. 7).

### Discussion

Brain atlases form the foundation for the understanding of the topography, typology, cytology, and ontogeny of the brain of any given species. The revealed neuroanatomy plays an integral role in the interpretation of developmental, behavioural, physiological, genetic, and behavioural studies to address broader concepts of comparative brain evolution (Pollen and Hofmann 2008). Modern neuroanatomical methods, such as the various scanning modalities, have provided several digital brain atlases. MR imaging, which was originally adopted as a non-invasive imaging technique designed for humans (Haxby et al. 2001; Behroozi and Daliri 2014, 2015), now allows for detailed and rapid analysis of a variety of different species that were previously overlooked for detailed presentations of brain structure (Behroozi et al. 2017, 2018a,b). This is reflected in both the number of three-dimensional digital atlases now available and the diversity of species that have been examined (Güntürkün et al. 2013; De Groof et al. 2016; Ella et al. 2017; Majka et al. 2017; Liu et al. 2018; Hoops et al. 2018).

There are multiple benefits to using MR imaging to develop digital brain atlases; however, most significant is that this technique allows the rendering of 3D visualisations of the brain and the structures within. 2D-based histological brain atlases are generally biased toward the researcher's specific interests and methodological preferences in terms of slice thickness, delineation, and orientation (De Groof et al. 2016; Hamaide et al. 2017). This holds true for the studies of crocodilian brains, many of which focus on specific regions of interest (Crosby 1917; Huber and Crosby 1926; Pritz and Northcutt 1980; Derobert et al. 1999). MR imaging digital atlases can improve on

certain histological limitations and are most often based upon the anatomy of the brain, while it is still within the skull, producing minimal deformation and reducing the effects of shrinkage and possible mechanical damage to the brain. Ideally, MRI imaging of the whole brain in situ should be a routine procedure in the future preparations of atlases, and if possible, accompanied by associated high quality histological atlases based on serial sections and high-resolution images.

MR imaging digital brain atlases are also useful when using techniques that require localization of focal target areas (e.g., anatomical tract tracer injections or electrode placement for electrophysiological recordings) (De Groof et al. 2016; Karten et al. 2013; Vellema et al. 2011; Pritz and Northcutt, 1980; Pritz 1975, 2016), and functional MR imaging methods (Van Meir et al. 2005; Berns et al. 2012; Van Ruijssevelt et al. 2013) such as manganese-enhanced MR imaging (Inoue et al. 2011) and diffusion tensor imaging (De Groof et al. 2008). These imaging applications are becoming more prevalent with reference to reptiles. For example, a recent fMRI study that was conducted on the Nile crocodile forebrain provided the means to decipher the functional anatomy of the visual and auditory system of the reptilian forebrain (Behroozi et al. 2018a). Consequently, the appropriately adapted fMRI technique for reptiles now allows for the possibility of conducting repeated scans and longitudinal analyses, which can be applied to a variety of studies from cognitive-based learning to developmental changes with reference to the crocodilian brain. These atlases also contribute to high-resolution whole brain imaging data banks (Ullmann et al. 2015).

There are only a handful of Reptilian stereotaxic brain atlases (e.g., Greenberg 1982), with only one recent 3D atlas of the lizard brain (Hoops et al. 2018). While the crocodilian brain has been intensively studied with classical techniques using varied nomenclature that has no clear consensus, a few studies have provided a holistic account of crocodilian neuroanatomy. As mentioned, the atlas presented here follows the general anatomical pattern outlined in previous studies but also has the inclusion of a recent study (Briscoe and Ragsdale 2018). The study of Briscoe and Ragsdale (2018) is of particular interest, as the researchers applied an avian-based interpretation and nomenclature to the alligator forebrain. Phylogenetically, crocodilians and birds are the only extant archosaurs (Green et al. 2014), raising the question of whether the crocodilian brain is more a “reptilian” or “avian”-type brain, or perhaps exhibiting features of both. In the current study, we have maintained the more “classical” nomenclature and interpretation used for reptilian brains in our analysis of the Nile crocodile, but where relevant we note the potential for the application of avian-based nomenclature and analysis. In addition, we provide a consolidated account of the avian and crocodilian neuroanatomy

and associated nomenclature based on possible homologies identified from previous studies (Tables 2, 3, 4, 5).

## Telencephalon

*Cortical mantle:* The specific borders and number of cortical regions differ amongst reptiles, depending on the species examined and nomenclature used. The cortical mantle of the crocodilian forebrain includes the hippocampus (or medial cortex), dorsal cortex, and lateral cortex (or piriform cortex) (Ulinski 1990; Nieuwenhuys et al. 1998). These cortical divisions have been shown to share cortical functions and fibre projections with mammals and birds. For example, the reptilian medial cortex (hippocampus) is considered homologous to the mammalian and avian hippocampus (Striedter 2016). Similarly, the reptilian dorsal cortex (Striedter 1997), the avian wulst (Medina and Reiner 2000), and the mammalian neocortex (Jarvis et al. 2013) are also considered homologues. The “olfactory” lateral cortex is considered homologous to the mammalian and avian piriform cortex (Bruce 2007). An interesting consideration with reference to the avian wulst includes the recent description of a “reptilian wulst” identified in squamates at the extreme rostral pole of the telencephalon (Desfilis et al. 2018; Hoops et al. 2018). This reptilian wulst has been posited to reside within the crocodilian telencephalon as well, based on a bulge like feature depicted from Crosby’s (1917) neuroarchitectural account of the alligator forebrain (Desfilis et al. 2018). This bulge like feature was not readily identifiable in the current atlas, and hence, the presence of a crocodilian wulst like area could not be confirmed within this study. Additionally, a large component of the reptilian dorsal cortex has been suggested to align more closely to the avian dorsolateral corticoid area (CDL) which has been implicated in the avian limbic (Atoji and Wild, 2005) and/or hippocampal complex (Behroozi et al. 2017; Medina et al. 2017; Desfilis et al. 2018). However, Briscoe and Ragsdale (2018) also provide a compelling proposal relating cell groups of the alligator dorsal cortex and the avian wulst, exemplified by the striking similarity of DACH expression across the alligator dorsal cortex and within the interstitial nucleus of the hyperpallium of the wulst. See Table 2 for a consolidated account of possible homologies specific to the telencephalon between birds and crocodilians.

The cortical regions delineated here predominantly match earlier descriptions (Crosby 1917). In addition, despite differences in the onset and exact location of the lateral, hippocampal, and dorsal cortices in the very rostral parts of the telencephalon, our data are also congruent with Riss (Riss et al. 1969) (*Caiman sclerops*) and Briscoe and Ragsdale (Briscoe and Ragsdale 2018) (*Alligator mississippiensis*), with the aforementioned differences possibly attributable to species or age differences. A feature associated with

**Table 2** Nomenclature of shared neuroanatomical structures between crocodylians and birds (Telencephalon)

	Abbreviation	Structure	Corresponds in birds to	Homology	References
Telencephalon					
Pallium					
Cortical Pallium	DC	Dorsal Cortex	Dorsolateral corticoid area (CDL)/ Wulst	Homology suggested	Medina et al. (2017); Desfilis et al. (2018); Briscoe and Ragsdale (2018)
	Hp	Hippocampus	Hippocampus (HP)	Homology likely	Medina, (2010); Striedter (2016); Herold et al. (2018)
	Fim	Fimbria	Hippocampal fimbria (Fim)	Homology likely	Puelles et al. (2017); Güntürkün et al. (2017b)
	LC	Lateral Cortex	Pyriiform cortex [Cpi]	Homology likely	Puelles et al. (2000); Butler and Hodos (2005); Medina (2010)
Nuclear pallium (DVR)	dADVR	Dorsal Anterior Dorsal Ventricular Ridge	Mesopallium (M)	Homology suggested	Briscoe and Ragsdale (2018)
	vADVR	Ventral Anterior Dorsal Ventricular Ridge	Nidopallium (N)	Homology suggested	Briscoe and Ragsdale (2018)
	Field L	Field L	Field L	Homology likely	Briscoe and Ragsdale (2018); Behroozi et al. (2018a)
	E	Entopallium	Entopallium (E)	Homology likely	Briscoe and Ragsdale (2018); Behroozi et al. (2018a)
	TOL	Lateral Olfactory Tract Nucleus	Nucleus basorostralis (Bas)	Homology suggested	Clarke and Ulinski (1984); Briscoe and Ragsdale (2018)
	PDVR	Posterior Dorsal Ventricular Ridge	Caudal aspect of the Nidopallium (N)/Arcopallium	?	Desfilis et al. (2018); Briscoe and Ragsdale (2018)
Sub-pallium					
Septal complex	LS	Lateral Septum	Lateral septal nucleus (SL)	Homologue (as septal complex)	Font et al. (1995); Goodson (2004); Butler and Hodos (2005); Sánchez-Camacho et al. (2006)
	AS	Anterior Septum	?		
	MS	Medial Septum	Medial septal nucleus (MS)	Homology likely	Semba (2004)
	DBB	Diagonal Band of Brocca	Nucleus of the diagonal band (NDB)	Homology likely	
	Prim.Hp	Primordial Hippocampus	?	?	
<i>Pallidostratial complex</i>	dStr	Dorsal Striatum	Globus pallidus (GP) and striatum	Homologue likely	Briscoe and Ragsdale (2018)
	vStr	Ventral Striatum	Medial and Lateral striatum (MS/LS)	Homologue (as pallidostratial complex)	Brauth et al. (1983, 1985); Butler and Hodos (2005); Reiner et al. (1998, 2005); Semba (2004)
	TU	Olfactory Tuberculum	Olfactory Tubercle (OTu)		

List of brain areas of the Nile crocodile identified in this atlas and their relation to corresponding areas in the bird brain based on information from the literature. Areas were considered "homologue" to their avian counterpart when there was a general consensus in the literature with respect to their homology. In three cases (pallidostratial complex, septal complex, and habenular complex) sufficient evidence for homology was only available for a whole nuclear complex but not for single nuclei within the complex. We thus labeled only the complex as homologue between the two clades. For some areas, data strongly indicate a homology to the corresponding avian structure, but a final conclusion has not been reached yet. We thus labeled these areas with "homology likely". In a few cases, homology has been suggested by only a few studies and data are currently not sufficient to draw a conclusion. We labelled these cases as "homology suggested". In the remaining cases, labelled with a question mark, there are currently no data at all to draw any conclusion

**Table 3** Nomenclature of shared neuroanatomical structures between crocodylians and birds (Diencephalon)

	Abbreviation	Structure	Corresponds in birds to	Homology	References
Diencephalon					
Hypothalamus	Hyp	Hypothalamus	Hypothalamus (Hyp)	Homologue (as a whole structure)	Moreno and González (2005); Puelles and Rubenstein (2015)
Epithalamus	Hb	Habenula	Habenular complex (HB)	Homologue (as habenular complex)	Sutherland (1982); Amo et al. (2010); Moreno et al. (2017); Jesuthasan (2018); Bianco and Wilson (2009)
Dorsal thalamus	SM	Stria medullaris	Stria medullaris (Sme)	Homologue	Butler and Hodos (2005)
	DMA/DLA	Dorsal thalamic nuclei (medial and lateral)	Dorsal thalamic nuclei (DLM/L DMA)	Homology likely	Pritz (2014); Butler and Hodos (2005)
	GLDd	Dorsal lateral geniculate nucleus	Dorsal lateral geniculate nucleus/Nucleus opticus principalis thalami (GLDd/OPT)	Homology likely	Butler and Hodos (2005)
	D	Nucleus diagonalis	?	?	
Ventral Thalamus	MC	Medialis complex nuclei	Nucleus dorsolateralis posterior thalami (DLP)	Homology likely	Korzeniewska and Güntürkün (1990)
	Re	Nucleus Reuniens	Nucleus ovoidalis (Ov)	Homologue	Butler and Hodos (2005); Güntürkün et al. (2017b)
	Rt	Nucleus Rotundus	Nucleus rotundus (IRT)	Homologue	Pritz (2014); Moreno et al. (2017)
Pretectum	GLv	ventral lateral geniculate nucleus	Ventral lateral geniculate nucleus (GLv)	Homologue	Butler and Hodos (2005)
	ov	Nucleus ovalis	? (possibly GLDv)	?	Butler and Hodos (2005)
Pretectum	ML	Mesencephalic lentiform nucleus	Mesencephalic lentiform nucleus (NLM)	Homologue	Ebbeson and Karten (1981); Butler and Hodos (2005); )
	nDCP	Dorsal posterior commissure nucleus	Nucleus spiriformis lateralis (Spl)	Homology likely	Reiner et al. (1998); Butler and Hodos (2005); Güntürkün et al. (2017b)

List of brain areas of the Nile crocodile identified in this atlas and their relation to corresponding areas in the bird brain based on information from the literature. Areas were considered "homologue" to their avian counterpart when there was a general consensus in the literature with respect to their homology. In three cases (pallidostriatal complex, septal complex, and habenular complex) sufficient evidence for homology was only available for a whole nuclear complex but not for single nuclei within the complex. We thus labeled only the complex as homologue between the two clades. For some areas, data strongly indicate a homology to the corresponding avian structure, but a final conclusion has not been reached yet. We thus labeled these areas with "homology likely". In a few cases, homology has been suggested by only a few studies and data are currently not sufficient to draw a conclusion. We labelled these cases as "homology suggested". In the remaining cases, labelled with a question mark, there are currently no data at all to draw any conclusion

the dorsal cortex, termed the primordial general cortex by Crosby (1917), or the mesopallial bridge by Briscoe and Ragsdale (2018), was not identified in the current analysis. This could be due to the resolution limitations of the MR images or may be a feature that is not present in the Nile crocodile. It has been suggested that the mesopallial bridge (i.e., primordial general cortex) could be the equivalent of the reptilian pallial thickening (Pritz 2014), a feature considered to be absent in crocodylians. Furthermore, the specific medial and lateral subdivisions of the dorsal cortex identified by Briscoe and Ragsdale (2018) in the alligator were not visible in the MR images obtained in the current study.

**Dorsal Ventricular Ridge (DVR):** The DVR is the largest structure in the crocodylian telencephalon and appears relatively larger than the homologous structure in other reptiles (e.g., turtles). This telencephalic region has been extensively discussed regarding specific homologies with structures within bird and mammal brains (Karten 1991, 2015; Butler 1994; Striedter 1997; Puelles and Kuwana 2000; Jarvis 2009; Butler et al. 2011; Jarvis et al. 2013; Fouragnan et al. 2015; Belekova and Kenigfest 2018; Desfilis et al. 2018). We parcellated the DVR into anterior (ADVR) and posterior (PDVR) divisions, with the ADVR further subdivided into a ventral (vADVR) and dorsal (dADVR) division. These

**Table 4** Nomenclature of shared neuroanatomical structures between crocodylians and birds (Mesencephalon and Rhombencephalon)

	Abbreviation	Structure	Corresponds in birds to	Homology	References
Mid and Hindbrain					
Mesencephalon	nBOR	Basal optic root nucleus	Basal optic root nucleus (nBOR)	Homologue	Butler and Hodos (2005)
	TeO	Optic tectum	Optic tectum (Teo)	Homologue	Butler and Hodos (2005)
	TS	Torus semicircularis	Mesencephalicus lateralis pars dorsalis (MLd)	Homologue	Puelles et al. (1994); Logerot et al. (2011)
	PAG	Periaqueductal gray	Periaqueductal gray (PAG)	Homologue	Linman et al. (2012); Goodson and Kingsbury (2013)
Cranial nerves	CNIII	Cranial nerve 3 (oculomotor nerve)	Cranial nerve 3 (CNIII)	Homologue	Butler and Hodos (2005)
	CNIV	Cranial nerve 4 (trochlear nerve)	Cranial nerve 4 (CNIV)	Homologue	Butler and Hodos (2005)
Rhombencephalon	I	Isthmic nuclei	Isthmic nuclei (1)	Homologue	Künzle and Schnyder (1984); Butler and Hodos (2005); Güntürkün et al. (2017b)
	LoC	Locus coeruleus	Locus coeruleus (LoC)	Homologue	Brauth (1988); Smeets and González (2000)
	nVd	Nucleus et tractus descendens nervi trigemini	Nucleus et tractus descendens nervi trigemini (TDV)	Homologue	Richard et al. (2004); Butler and Hodos (2005)
	RF	Posterior reticular formation	Posterior reticular formation (RF)	Homologue	Ebbesson and Goodman (1981); Butler and Hodos (2005)

List of brain areas of the Nile crocodile identified in this atlas and their relation to corresponding areas in the bird brain based on information from the literature. Areas were considered "homologue" to their avian counterpart when there was a general consensus in the literature with respect to their homology. In three cases (pallidostratial complex, septal complex, and habenular complex), sufficient evidence for homology was only available for a whole nuclear complex but not for single nuclei within the complex. We thus labeled only the complex as homologue between the two clades. For some areas, data strongly indicate a homology to the corresponding avian structure, but a final conclusion has not been reached yet. We thus labeled these areas with "homology likely". In few cases, homology has been suggested by only a few studies and data are currently not sufficient to draw a conclusion. We labelled these cases as "homology suggested". In the remaining cases, labelled with a question mark, there are currently no data at all to draw any conclusion

**Table 5** Nomenclature of shared neuroanatomical structures (fibre bundles/tracts and ventricles) between crocodylians and birds

	Abbreviation	Structure	Corresponds in birds to	Homology	References
Fibre bundles, tracts and ventricles					
Fibre tracts	LFB	Lateral Forebrain Bundle	Lateral Forebrain Bundle (LFB)	Homologue	Butler and Hodos (2005)
	MFB	Medial Forebrain Bundle	Medial Forebrain Bundle (MFB)	Homologue	Butler and Hodos (2005)
	OT	Optic Tract	Optic tract (OT)	Homologue	Butler and Hodos (2005)
Commissural Fibers	HC	Hippocampal commissure	Hippocampal commissure (HC)	Homologue	Suárez (2017)
	CO	Chiasma Opticum	Chiasma opticum (CO)	Homologue	Butler and Hodos (2005)
	CP	Posterior Commissure	Posterior commissure (PC)	Homologue	Suárez (2017)
Ventricles	3 V	3rd ventricle	3rd ventricle (3 V)	Homologue	Butler and Hodos (2005)
	LV	Lateral ventricle	Lateral ventricle (LV)	Homologue	Butler and Hodos (2005)

List of brain areas of the Nile crocodile identified in this atlas and their relation to corresponding areas in the bird brain based on information from the literature. Areas were considered "homologue" to their avian counterpart when there was a general consensus in the literature with respect to their homology. In three cases (pallidostratial complex, septal complex, and habenular complex), sufficient evidence for homology was only available for a whole nuclear complex but not for single nuclei within the complex. We thus labeled only the complex as homologue between the two clades. For some areas, data strongly indicate a homology to the corresponding avian structure, but a final conclusion has not been reached yet. We thus labeled these areas with "homology likely". In few cases, homology has been suggested by only a few studies and data are currently not sufficient to draw a conclusion. We labelled these cases as "homology suggested". In the remaining cases, labelled with a question mark, there are currently no data at all to draw any conclusion

divisions match recent genetic expression data provided for the alligator telencephalon (Briscoe and Ragsdale 2018). Briscoe and Ragsdale (2018) applied avian neuroanatomical nomenclature to the alligator dorsal telencephalon, where the mesopallium is equivalent to the dADVR in our study, and the nidopallium equivalent to the vADVR in our study. The dorsal and ventral ADVR divisions outlined here in the Nile crocodile also appear to be equivalent to the ADVR divisions proposed by Riss et al. (1969) for the caiman telencephalon. Specifically, the anatomical subdivision zone 5 within the caiman histological atlas appears to be equivalent to the dADVR identified herein, while zone 8 of Riss et al. (1969) appears equivalent to the vADVR identified herein. A similar region or sector to the dADVR was also identified based on the genoarchitecture described for the lizard (included as part of the lateral pallium; Desfilis et al. 2018) and agamid lizard (included as part of the rostral lateral pallium; Hoops et al. 2018). Within the vADVR, we also identified primary visual and auditory functional areas (labelled E and Field L) which were recently identified using functional MR imaging (Behroozi et al. 2018a). These functional regions match the areas described as the entopallium (visual) and Field L (auditory) by Briscoe and Ragsdale (2018). Behroozi et al. (2018a, b) also identified two secondary auditory areas, one of which was specific to the processing of complex sounds suggestive of hierarchical auditory processing within the telencephalon of the Nile crocodile forebrain. These structures were readily discernible with the use of functional MRI, but not evident from the use of structural MRI only and were thus not included in the current atlas.

A feature of the ADVR defined with the MR images is the nucleus of the lateral olfactory tract (TOL) (Crosby 1917). Although Riss et al. (1969) also identified this anatomically distinct region in the caiman, their zone 7, it was found to lack projections from the olfactory system (Scalia et al. 1969). In birds, the nucleus basorostralis is found in a very similar location within the telencephalon as the crocodilian TOL (Clark and Ulinski 1984; Güntürkün et al. 2017b). The avian nucleus basorostralis has been implicated in somatosensory, auditory, and vestibular functions (Wild et al. 2010; Briscoe and Ragsdale 2018). Briscoe and Ragsdale (2018) labelled the TOL as the putative alligator basorostralis nucleus; however, functional and hodological data are needed to clarify the role of this structure for crocodilians and the appropriate nomenclature to apply. Given the lack of clarity on the function of this anatomical region, we have applied the nomenclature of Crosby (1917), but are aware that this may need to be amended.

The posterior DVR, as described by Ulinski (1983), is present in all reptiles and was identified for the Nile crocodile. However, the crocodilian PDVR differs from other reptiles (e.g., squamates), due to the reported lack of the

nucleus sphericus, a large, clearly visible nucleus embedded within the PDVR. We could not identify a distinct nucleus sphericus in the Nile crocodile with the acquired MR images, although a recent study suggests the possibility of a nucleus sphericus in the Nile crocodile (Ngwenya et al. 2018). Functionally, the PDVR is considered to be part of the amygdaloid complex (Striedter 1997; Ulinski 1983), while others have described this region as a multimodal sensory integration centre within reptiles (Lanuza 1998). In birds, some have understood the reptilian posterior DVR to be the homologue of the arcopallium, but this view has been considered too simplistic (Jarvis 2009; Jarvis et al. 2013). Briscoe and Ragsdale (2018) label the PDVR in their alligator study of the arcopallium and also provide specific subdivisions of this region, but they do not propose specific homology of the arcopallium (including associated subdivisions) between alligators and birds. Alternatively, a recent genoarchitectural study on lizards suggests that the reptile PDVR is rather more comparable to the caudal pole of the nidopallium in birds (Desfilis et al. 2018); however, others have defined a clear border between the junction of the nidopallium and arcopallium in birds with the genetic marker DACH2. The same applies for the alligator suggesting that the PDVR (i.e., arcopallium) is a separate structure and not a part of the nidopallium (vADVR) (Briscoe and Ragsdale 2018). A similar border between the ADVR and PDVR with the use of the DACH2 marker was also defined for the non-avian reptiles (e.g., turtles and lizards) (Tosches et al. 2018). As such, proposing equivalence of the reptilian PDVR with the avian caudal nidopallium (e.g., Desfilis et al. 2018) is difficult to justify based on molecular evidence at present and requires further investigation. The placement of the PDVR in the current atlas of the Nile crocodile brain is concurrent with the alligator arcopallium described by Briscoe and Ragsdale (2018), but due to the resolution limitations of the MR images, specific subdivisions within the PDVR could not be established with certainty.

*Subpallial structures:* In terms of the subpallial structures identified with MR imaging, the majority of what was observed is in agreement with previous descriptions of this region in crocodilians (Crosby 1917; Riss et al. 1969), although a few differences should be noted. Within reptiles, the subpallium can be subdivided into a septal nuclear complex and a ventral and dorsal striatopallidal complex. It has been shown that the ventral and dorsal striatopallidal complexes are highly conserved across vertebrates (Medina and Reiner 1995; Reiner et al. 1998). In mammals, the dorsal striatopallidal complex is further subdivided into striatum (caudate and putamen) and globus pallidus (internal and external/entopenduncular nucleus), while the ventral striatopallidal complex includes the nucleus accumbens, olfactory tubercle, and ventral pallidum (Butler and Hodos 2005). The MR

images obtained in the current study did not allow similar detailed parcellation of these regions. Thus, we have only delineated the ventral striatum (including nucleus accumbens in the rostral aspect of the vStr) and the dorsal striatum (including nucleus ventro-medialis, which is not part of the striatopallidal complex). The ventral striatum delineated herein is in part congruent with the VLA described by (Crosby 1917), but more specifically, the VLA sc (small cell area: equivalent of the caudate and the putamen) and the dorsal striatum, VLA lc (large cell, specifically the ventro-caudal division: equivalent of the globus pallidus) (Brauth and Kitt 1980; Brauth et al. 1983). Briscoe and Ragsdale (2018) delineated the striatum in their study based on the expression of PPP1R1B (DARPP32), and found an absence of expression in the ventral aspect of the striatum, which was considered to be the likely homologue of the mammalian and avian globus pallidus. However, as mentioned, the reason for simply delineating the striatopallidal complex in our atlas as the ventral and dorsal striatum is due to the lack of resolution of these structures using MR imaging. More specifically, the dorsal striatum in this study is composed of both the striatum (i.e., VLA lc: specifically the dorso-medial region) and pallidum (i.e., VLA lc: specifically the ventro-caudal region) with specific reference to Brauth et al. (1983). Similarly, structures within the ventral striatum (e.g., VLA sc) could not be readily dissociated from the nucleus accumbens (area c in Riss et al. 1969) and has been combined and labelled the vStr (i.e., our ventral striatum includes both ventral and dorsal striatum if defined according to the mammalian striatopallidal complex).

It should be noted that while many aspects of the striatopallidal complex are shared amongst reptiles, birds, and mammals, distinct localised striatal anatomical regions do differ in position and subdivisions. Additionally, more details pertaining to these complexes are known for birds than reptiles (Butler and Hodos 2005). Pritz (2016) again highlights this point and further suggests that the striatum in crocodylians might possess many more subdivisions than previously described; therefore, further studies are required to understand the various subfields that comprise this region. Another striatal structure, the olfactory tuberculum (TU) was clearly outlined in the rostral aspects of the telencephalon, which caudally transitioned into the DBB along the basal aspect of the pallia. With reference to the septal nuclear complex, an anterior septum was identified rostrally and separated caudally into the medial and lateral subdivisions with the presence of the MFB as described for crocodylians. Further septal subdivisions described for other reptiles (Font et al. 1998) were not identified or included in the current atlas and require further investigation.

## Diencephalon

The diencephalic structures of the crocodile brain outlined herein with MR imaging are in broad agreement with classical histological atlases (Crosby 1917; Huber and Crosby 1926) and more recent studies (Derobert et al. 1999; Pritz 2015). Vertebrates in general have four major divisions of the diencephalon, which include the epithalamus, dorsal and ventral thalamus, and the hypothalamus (although the hypothalamus is now considered to be a derivative of the prosencephalon, which also gives rise to the telencephalon, and thus, its designation as part of the diencephalon may need to be revised in line with this recent developmental data, Puelles et al. 2013). The epithalamus in the current atlas includes the Hb and the associated afferent fibre tract the SM as described for crocodylians. The dorsal thalamic nuclei were readily outlined with the MR images obtained (e.g., DLA/DMA, Re, Rt, MC, D, and GLDd) and are in agreement with previous studies in crocodylians (Pritz 2014, 2015) and other reptiles (Ulinski 1986; Butler and Hodos 2005). The ventral thalamic nuclei identified was the GLv and ov, both of which are involved in visual processing (Butler and Hodos 2005). In terms of the Hyp, specific subdivisions were difficult to accurately delineate with MR imaging as depicted by (Subheddar et al. 1989) for the gharial crocodile (*Gavialis gangeticus*), and thus, the hypothalamus is only represented as a single structure here. Comparative studies on the Hyp have demonstrated a highly conserved topographical organisation across vertebrates, with only subtle differences in the expression of molecular markers and connectivity between birds and reptiles likely representing adaptations to specific environmental niches across these taxonomic groupings (Moreno and Gonzalez 2005; Domínguez et al. 2015; Puelles and Rubenstein 2015; Kuenzel 2018). The connectivity pattern between the amygdaloid complex, vomeronasal system, and the hypothalamus, which is considered homologous between reptiles and mammals, is an example of such an adaptation driven difference (Moreno and Gonzalez 2005). Since birds do not possess a vomeronasal system, the organisation of their amygdaloid complex does differ from reptiles, and thus also the associated connectivity of the hypothalamus (Moreno and Gonzalez 2005). Hence, the bird Hyp shows some homology at the level of topographical organisation with the crocodile as a whole structure, but it has also been shown that there is significant divergence in its associated connectivity with other forebrain structures, demonstrating the complexity of defining homology when structures are shared but connectivity is divergent. The major fibre bundles connecting the diencephalon to the telencephalon (MFB and LFB) were combined and labelled the LFB due to the difficulty in discriminating between these fibre bundles in the MR images obtained. We were unable to identify the rostral reticular nucleus (associated to the LFB

peduncle) (Pritz 2016). See Tables 3 and 5 for a consolidated account of possible homologies specific to the diencephalon and fibre bundles between crocodylians and birds.

## Mesencephalon and rhombencephalon

The pretectal, mesencephalic, and hindbrain structures outlined on the MR images were guided by previous crocodylian studies (Huber and Crosby 1926; Carl Huber and Crosby 1933; Brauth and Kitt 1980; Derobert et al. 1999). More structures were visible in the MR images in the dorsal component of the pretectum when compared to the ventral tegmentum. Several histochemical, hodological, and functional studies have been made to elucidate the structure and function of the mesencephalic and rhombencephalic components of crocodylians (Brauth and Kitt 1980; Brauth et al. 1988; Ferguson et al. 1978; Heric and Kruger 1965; Manley 1971; Médina et al. 2004). While we could only identify a limited number of structures in the crocodile mesencephalon with the MR images obtained, the major sensory structures including the TeO (equivalent of the superficial division of the mammalian superior colliculus) and the TS (equivalent of the mammalian inferior colliculus) were readily identified. Caudally, within the hindbrain the I, LoC, and RF were identified, as well as specific cranial nerves and nuclei. Table 4 depicts a consolidated account of possible homologies specific to the mesencephalon and rhombencephalon of crocodylians and birds.

## Conclusion

The atlas of the Nile crocodile brain developed here provides a 3D reference template that will complement the application of other research techniques to the study of the crocodylian brain. While the structural outline provided herein for the Nile crocodile brain is in general agreement with previous studies, there are a few incongruencies with previously published histological data. For example, Briscoe and Ragsdale (2018) describe a number of novel subdivisions of the hippocampus and the dorsal cortex, but these could not be confirmed with the MR images obtained. While these authors deserve acknowledgement for attempting to implement a strictly avian nomenclature to the crocodylian forebrain, caution should be exercised with specific reference to the functional properties and associated implications with the use of the designated anatomical terms. Given the phylogenetic proximity of the only living archosaurs (birds and crocodylians), a change in the nomenclature applied to the crocodile brain does warrant consideration. Despite this, it is extremely challenging to create an anatomical atlas using the same nomenclature for crocodiles and birds, due to the variance in gross and histological structure between these

two groups of Archosaurs, especially when the anatomy of other reptilian brains is considered. The identification of a putative crocodylian nucleus sphericus and the possibility of a crocodylian pallial thickening are two examples that highlight this point. A consolidated neuroanatomical nomenclature framework applicable to all reptiles, including crocodylians and definitively proven homologues with regions of the avian brain (e.g., Reiner 2005) is required, but this requires a great deal more research to be undertaken. The neuroanatomical nomenclature used for reptiles has been an area riddled with conflict (Nieuwenhuys et al. 1998). In addition, as novel techniques and the variety of reptilian species studied increases, the anatomical nomenclature becomes more confusing. Disagreements pertaining to amniote neuroanatomical homologies further exacerbate these nomenclatorial difficulties. The crocodylian brain is particularly challenging for the derivation of a parsimonious nomenclature due to its unique phylogenetic position amongst amniotes, and, fundamentally, we are still questioning if the crocodylian brain is more similar to reptiles or birds. Additionally, the newly co-opted phylogenetic inclusion of the turtles into the archelosaurians (the sister taxa to lepidosaurians) (Crawford et al. 2015) may provide a different interpretation and approach to the understanding of the anatomy and associated nomenclature (Striedter 2016). Despite these potential pitfalls, we have attempted to identify possible homologues, or the lack thereof, to highlight the similarities and differences in the nomenclature used for birds and crocodiles, based on the anatomical areas defined within this atlas. This exercise is important to consolidate and define the nomenclature used to describe the crocodylian brain, and with the advent of new techniques, newly recognised homologies, and new phylogenetic interpretations, a resolution to the nomenclature problem may ensue.

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## Compliance with ethical standards

**Conflict of interest** All authors declare that they have no conflicts of interests.

**Human and animal rights statement** The crocodile used in this study was treated and used according to the guidelines of the University of the Witwatersrand Animal Ethics Committee, which parallel those of the NIH (National Institutes of Health) for the care and use of animals in scientific experimentation. The ethical clearance number for the project: 2015/06/25/A, the ordinary permit number: 029562, and the CITES export clearance number: 152535 were certified and issued by the relevant governmental authorities in South Africa. The German import clearance number: E-04349/15 was issued by the Bundesamt für Naturschutz (German Federal Agency for Nature Conservation).

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