Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Chemical Neuroanatomy 70 (2015) 42–57

Contents lists available at ScienceDirect

Journal of Chemical Neuroanatomy

journal homepage: www.elsevier.com/locate/jchemneu

Organization of cholinergic, catecholaminergic, serotonergic and orexinergic nuclei in three strepsirrhine primates: *Galago demidoff*, *Perodicticus potto* and *Lemur catta*

Tanya Calvey [a], Nina Patzke [a], Consolate Kaswera-Kyamakya [b], Emmanuel Gilissen [c,d,e], Mads F. Bertelsen [f], John D. Pettigrew [g], Paul R. Manger [a,*]

[a] School of Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2193, Johannesburg, South Africa
[b] Faculté des Sciences, University of Kisangani, B.P 1232 Kisangani, Democratic Republic of Congo
[c] Department of African Zoology, Royal Museum for Central Africa, Leuvensesteenweg 13, B-3080 Tervuren, Belgium
[d] Laboratory of Histology and Neuropathology, Université Libre de Bruxelles, 1070 Brussels, Belgium
[e] Department of Anthropology, University of Arkansas, Fayetteville, AR 72701, USA
[f] Centre for Zoo and Wild Animal Health, Copenhagen Zoo, Frederiksberg, Denmark
[g] Queensland Brain Institute, University of Queensland, St. Lucia 4072, Australia

ARTICLE INFO

Article history:
Received 30 September 2015
Received in revised form 21 October 2015
Accepted 21 October 2015
Available online 10 November 2015

Keywords:
Choline acetyltransferase
Tyrosine hydroxylase
Serotonin

ABSTRACT

The nuclear organization of the cholinergic, catecholaminergic, serotonergic and orexinergic systems in the brains of three species of strepsirrhine primates is presented. We aimed to investigate the nuclear complement of these neural systems in comparison to those of simian primates, megachiropterans and other mammalian species. The brains were coronally sectioned and immunohistochemically stained with antibodies against choline acetyltransferase, tyrosine hydroxylase, serotonin and orexin-A. The nuclei identified were identical among the strepsirrhine species investigated and identical to previous reports in simian primates. Moreover, a general similarity to other mammals was found, but specific differences in the nuclear complement highlighted potential phylogenetic interrelationships. The central feature of interest was the structure of the locus coeruleus complex in the primates, where a central compactly

Abbreviations: III, oculomotor nucleus; IV, trochlear nucleus; Vmot, motor division of trigeminal nerve nucleus; Vsens, sensory division of trigeminal nerve nucleus; VI, abducens nucleus; VIId, dorsal division of facial nerve nucleus; VIIv, ventral division of facial nerve nucleus; X, dorsal motor vagus nucleus; XII, hypoglossal nucleus; 5n, trigeminal nerve; 3V, third ventricle; 4V, fourth ventricle; A1, caudal ventrolateral medullary tegmental nucleus; A2, caudal dorsomedial medullary nucleus; A4, dorsolateral division of locus coeruleus; A5, fifth arcuate nucleus; A6c, compact portion of locus coeruleus; A6d, diffuse portion of locus coeruleus; A7d, nucleus subcoeruleus, diffuse portion; A7sc, nucleus subcoeruleus, compact portion; A8, retrorubral nucleus; A9l, substantia nigra, lateral; A9m, substantia nigra, medial; A9pc, substantia nigra, pars compacta; A9v, substantia nigra, ventral, pars reticulata; A10, ventral tegmental area; A10c, ventral tegmental area, central; A10d, ventral tegmental area, dorsal; A10dc, ventral tegmental area, dorsal caudal; A11, caudal diencephalic group; A12, tuberal cell group; A13, zona incerta cell group; A14, rostral periventricular nucleus; A15d, anterior hypothalamic group, dorsal division; A15v, anterior hypothalamic group, ventral division; A16, catecholaminergic neurons of the olfactory bulb; ac, anterior commissure; Amyg, amygdaloid body; AON, anterior olfactory nucleus; AP, area postrema; B9, supralemniscal serotonergic nucleus; C, caudate nucleus; C1, rostral ventrolateral medullary tegmental group; C2, rostral dorsomedial medullary nucleus; ca, cerebral aqueduct; Cb, cerebellum; cc, corpus callosum; Cl, claustrum; CLi, caudal linear nucleus; CO, cochlear nuclear complex; CVL, caudal ventrolateral serotonergic group; DCN, deep cerebellar nuclei; dfu, dorsal funiculus; Diag.B, diagonal band of Broca; DRc, dorsal raphe, caudal division; DRd, dorsal raphe, dorsal division; DRif, dorsal raphe, interfascicular division; DRl, dorsal raphe, lateral division; DRp, dorsal raphe, peripheral division; DRv, dorsal raphe, ventral division; DT, dorsal thalamus; EW, Edinger–Westphal nucleus; f, fornix; fr, fasciculus retroflexus; GC, central gray matter; GP, globus pallidus; Hbl, lateral habenular nucleus; Hbm, medial habenular nucleus; Hip, hippocampus; Hyp, hypothalamus; Hyp.d, dorsal hypothalamic cholinergic nucleus; Hyp.l, lateral hypothalamic cholinergic nucleus; Hyp.v, ventral hypothalamic cholinergic nucleus; IC, inferior colliculus; ic, internal capsule; icp, inferior cerebellar peduncle; io, inferior olivary nuclear complex; IP, interpeduncular nucleus; Is.Call/TOL, islands of Calleja/olfactory tubercle; LDT, laterodorsal tegmental nucleus; lfp, longitudinal fasciculus of the pons; LGn, lateral geniculate nucleus; lot, lateral olfactory tract; LV, lateral ventricle; Mc, main cluster of orexinergic neurons; mcp, middle cerebellar peduncle; mlf, medial longitudinal fasciculus; MnR, median raphe nucleus; N.Acc, nucleus accumbens; N.Amb, nucleus ambiguus; N.Bas, nucleus basalis; NEO, neocortex; OB, olfactory bulb; ON, optic nerve; OT, optic tract; Otc, optic tract cluster of orexinergic neurons; P, putamen nucleus; pVII, preganglionic motor neurons of the superior salivatory nucleus or facial nerve; pIX, preganglionic motor neurons of the inferior salivatory nucleus; PBg, parabigeminal nucleus; PC, cerebral peduncle; pg, pineal gland; PIR, piriform cortex; PPT, pedunculopontine tegmental nucleus; py, pyramidal tract; pyx, decussation of the pyramidal tract; R, thalamic reticular nucleus; Rmc, red nucleus, magnocellular division; RMg, raphe magnus nucleus; ROb, raphe obscurus nucleus; RPa, raphe pallidus nucleus; RtTg, reticulotegmental nucleus of the pons; RVL, rostral ventrolateral serotonergic group; S, septal nuclear complex; SC, superior colliculus; scp, superior cerebellar peduncle; Sep.m, medial septal nucleus; Sp5, spinal trigeminal tract; Stn, subthalamic nucleus; vfu, ventral funiculus; vh, ventral horn of spinal cord; VPO, ventral pontine nucleus; xscp, decussation of the superior cerebellar peduncle, zizona incerta; Zic, zona incerta cluster of orexinergic neurons.

* Corresponding author. Tel.: +27 11 717 2497; fax: +27 11 717 2422.
E-mail address: Paul.Manger@wits.ac.za (P.R. Manger).

http://dx.doi.org/10.1016/j.jchemneu.2015.10.002

Orexin
Mammalian evolution
Chiroptera
Neural systems.

packed core (A6c) of tyrosine hydroxylase immunopositive neurons was surrounded by a shell of less densely packed (A6d) tyrosine hydroxylase immunopositive neurons. This combination of compact and diffuse divisions of the locus coeruleus complex is only found in primates and megachiropterans of all the mammalian species studied to date. This neural character, along with variances in a range of other neural characters, supports the phylogenetic grouping of primates with megachiropterans as a sister group.

1. Introduction

Extant Primates are currently split into Strepsirrhini (Lemuriformes) and Haplorhini (Anthropoidea and *Tarsius*), with Strepsirrhini being the earliest distinct branch of the extant primates (Kay et al., 1997a,b; Bloch et al., 1997; Ni et al., 2013). Genomic data suggest that the estimated time of the most recent common ancestor of Strepsirrhinin and Haplorhini ranged between 58.9 and 68.6 million years (Jameson et al., 2011). Strepsirrhines and, among the haplorhines, the Tarsiidae whose unique extant representative are the tarsiers, appear to have retained several plesiomorphic features of stem primates (Matsui et al., 2009; Rosa et al., 1996). This includes the Dermoptera (colugos or flying lemurs), which have recently been identified as a primate sister taxon that diverged very early at ~86 million years ago (e.g. Arnason et al., 2002). For this reason the organization of the various neural systems within the strepsirrhine brain is of interest in understanding the phylogenetic trajectory of primates as a group. To date, no comprehensive mapping of the nuclei of the cholinergic, catecholaminergic, serotonergic or orexinergic systems has been undertaken for any strepsirrhine primate, but data for anthropoid primates such as the pygmy marmoset and the common marmosets, the squirrel monkey, the macaque monkey, the baboon and the human are available (summarized in Maseko et al., 2007; Dell et al., 2010). In this sense, there is a gap in our knowledge of the evolution of the nuclear organization of these systems between primates and other mammals.

In addition to this knowledge gap, studies of the nuclear organization of the cholinergic, catecholaminergic, serotonergic and orexinergic systems in a range of mammalian species, which are relatively conservative in their evolution (Manger, 2005; Dell et al., 2010), are of interest in providing data of relevance to the "Flying Primate" hypothesis. This hypothesis proposes that megachiropterans and microchiropterans are two evolutionarily distinct mammalian groups not related to each other in the same monophyletic order. Instead, the megachiropterans are proposed to have evolved from the dermopteran lineage of gliders (also known as the colugos or "flying lemurs"), which are the acknowledged sister group to primates (Pettigrew, 1986; Pettigrew et al., 1989; Meredith et al., 2011). The "Flying Primate" hypothesis is contentious because of DNA sequence data that appears to refute it, but which may reflect convergent DNA evolution under the selection pressure of high temperature associated with powered flight (Pettigrew and Kirsch, 1995, 1998; Kirsh and Pettigrew, 1998; Hutcheon et al., 1998; Jabbari and Bernadri, 2004). Nevertheless, there is substantial data coming from brain studies that supports this hypothesis (Pettigrew, 1986; Pettigrew et al., 1989, 2008; Manger et al., 2001; Maseko and Manger, 2007; Maseko et al., 2007; Kruger et al., 2010a,b; Dell et al., 2010, 2013).

Changes in the complexity of neural system structure, in terms of the number and complement of distinct subdivisions, are thought to occur only during the evolutionary events leading to the establishment of a new mammalian order (Manger, 2005). All progeny of the newly established order will then likely retain the same complement of distinct subdivisions of the various systems irrespective of the subsequently evolution of brain size, phenotype or life history parameters (Manger, 2005). While there are some cases that do not adhere directly to this hypothesis, for example the organization of the locus coeruleus in murid rodents is different from all other rodent groups (Kruger et al., 2012), this framework for understanding changes in mammalian brain evolution at the systems level has been supported by a number of studies (see summaries in Dell et al., 2010; Calvey et al., 2013). Given this background, the current study aimed to analyse the nuclear organization of the cholinergic, catecholaminergic, serotonergic and orexinergic systems in three previously unstudied strepsirrhine primate species. This was done for two reasons: (1) to determine whether the strepsirrhine and haplorhine primates share an identical complement of nuclei for these systems, providing a primate specific nuclear complement of these systems (Manger, 2005); and (2) to determine whether this potentially primate specific complement of nuclei shares enough similarities with the nuclear complement of the same systems found in megachiropterans (Maseko et al., 2007; Dell et al., 2010) to provide a further test of the "Flying Primate" hypothesis using neuroanatomical data.

2. Methods and materials

Brains from two *Galago demidoff* (brain masses of 3.27 and 3.45 g), two *Perodicticus potto* (brain masses of 12.79 and 14.12 g) and two *Lemur catta* (brain masses of 24.11 and 26.72 g) were used in the present study. Permits were obtained from the relevant wildlife authority in the Democratic Republic of Congo for *G. demidoff* and *P. potto*, as well as from the Copenhagen Zoo for *L. catta*. All animals were handled according to the guidelines of the University of the Witwatersrand Animal Ethics Committee. Each animal was weighed, anaesthetized and subsequently euthanized with appropriate doses of sodium pentobarbital (200 mg sodium pentobarbital/kg). Upon cessation of respiration the animals were perfused intracardially with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB), approximately 1 l/kg of each solution, both solutions having a temperature of approximately 4 °C. The brains were then carefully removed from the skulls and post-fixed overnight in 4% paraformaldehyde in 0.1 M PB, followed by equilibration in 30% sucrose in 0.1 M PB at 4 °C. Each brain was then frozen in crushed dry ice and sectioned into 50 μm thick serial coronal sections on a freezing microtome. A one in six series of sections, cut at 50 μm thickness in the coronal plane, was made for Nissl substance, myelin, choline acetyltransferase (ChAT), tyrosine hydroxylase (TH), serotonin (5HT) and orexin-A (hypocretin/OxA) staining. Sections used for the Nissl series were mounted on 0.5% gelatine-coated glass slides, cleared in a solution of 1:1 chloroform and absolute alcohol, then stained in 1% cresyl violet to reveal cell bodies. Myelin sections were first stored in 5% formalin for two weeks at 4 °C then mounted on 1.5%

gelatine-coated slides and subsequently stained with silver solution to reveal myelin sheaths (Gallyas, 1979).

For the immunohistochemical staining, each section was treated with endogenous peroxidase inhibitor (49.2% methanol:49.2% 0.1 M PB: 1.6% of 30% H_2O_2) for 30 min and subsequently subjected to three 10 min 0.1 M PB rinses. Sections were then preincubated for 2 h, at room temperature, in blocking buffer (containing 3% normal goat serum for the TH, 5-HT and OxA sections or 3% normal rabbit serum for the ChAT sections, plus 2% bovine serum albumin and 0.25% Triton-X in 0.1 M PB). This was followed by three 10 min rinses in 0.1 M PB. The sections were then placed in the primary antibody solution that contained the appropriate diluted primary antibody in blocking buffer for 48 h at 4 °C under gentle agitation. Anti-choline acetyltransferase (AB144P, Millipore, raised in goat) at a dilution of 1:3000 was used to reveal cholinergic neurons. Anti-tyrosine hydroxylase (AB151, Millipore, raised in rabbit) at a dilution of 1:7500 revealed the catecholaminergic neurons. Serotonergic neurons were revealed using anti-serotonin (AB938, Millipore, raised in rabbit) at a dilution of 1:7500. Orexinergic neurons were revealed using anti-Orexin A (AB3704, Millipore, raised in rabbit) at a dilution of 1:3000. This incubation was followed by three 10 min rinses in 0.1 M PB and the sections were then incubated in a secondary antibody solution (1:1000 dilution of biotinylated anti-rabbit IgG, BA-1000, Vector Labs, for TH, 5-HT and OxA sections, or a 1:1000 dilution of biotinylated anti-goat IgG, BA-5000, Vector Labs, for ChAT sections, in a blocking buffer containing 3% NGS/NRS and 2% BSA in 0.1 M PB) for 2 h at room temperature. This was followed by three 10 min rinses in 0.1 M PB, after which sections were incubated for 1 h in avidin-biotin solution (at a dilution of 1:125, Vector Labs), followed by three 10 min rinses in 0.1 M PB. Sections were then placed in a solution of 0.05% diaminobenzidine (DAB) in 0.1 M PB for 5 min, followed by the addition of 3 μl of 3% hydrogen peroxide to each 1 ml of solution in which each section was immersed. Chromatic precipitation was visually monitored and verified under a low power stereomicroscope. Staining was continued until such time as the background stain was at a level that would assist architectural reconstruction without obscuring the immunopositive neurons. Development was arrested by placing sections in 0.1 M PB, followed by two more rinses in this solution. Sections were then mounted on 0.5% gelatine coated glass slides, dried overnight, dehydrated in a graded series of alcohols, cleared in xylene and coverslipped with Depex. To test for non-specific staining, the primary antibody or the secondary antibody was omitted in selected section, which resulted in no staining of the tissue.

Sections were examined under a low power stereomicroscope and using a camera lucida, the architectonic borders of the sections were traced following the Nissl and myelin stained sections. Sections containing the immunopositive neurons were matched to the drawings and the neurons were marked. All drawings were then scanned and redrawn using the Canvas 8 drawing programme (Fig. 1). The nomenclature used for the cholinergic nuclei was adopted from Woolf (1991), Limacher et al. (2008), Bhagwandin et al. (2008), Gravett et al. (2009), Pieters et al. (2010), and Calvey et al. (2013), the catecholaminergic nuclei from Hökfelt et al. (1984), Smeets and González (2000), Limacher et al. (2008), Bhagwandin et al. (2008), Gravett et al. (2009), Pieters et al. (2010) and Calvey et al. (2013), the serotonergic nuclei from Törk (1990), Limacher et al. (2008), Bhagwandin et al. (2008), Gravett et al. (2009), Pieters et al. (2010) and Calvey et al. (2013), and the orexinergic nuclei from Kruger et al. (2010b), Bhagwandin et al. (2011), Gravett et al. (2011) and Calvey et al. (2013).

3. Results

The current study describes and defines the nuclear organization of the cholinergic, catecholaminergic, serotonergic and orexinergic neural systems in three species of strepsirrhine primates (Demidoff's dwarf bushbaby—*G. demidoff*, the potto—*P. potto* and the ring-tailed lemur—*L. catta*). For the most part, the systems investigated exhibited an organization that may be thought of as generally mammalian, and typically primate-like; however, the structure of the locus coeruleus in the primates appears to be quite different to that observed in most other mammals, although it is very similar to that seen in megachiropteran bats (Maseko et al., 2007; Dell et al., 2010). As all species investigated showed a near identical pattern of nuclear organization, the following description applies to all three species unless otherwise noted. Despite this it should be noted that hile the nuclear subdivisions of these systems are the same across the species, the exact morphology of the brains of the different species does differ to some extent, and these minor differences lead to slightly different placement of the nuclei of the systems investigated in relation to the broader neuroanatomy.

3.1. Cholinergic nuclei

The cholinergic system is generally subdivided into six main regions containing a cluster of distinct nuclei: cortical cholinergic neurons, striatal, basal forebrain, diencephalic, pontomesencephalic and cranial motor nerve nuclei (Woolf, 1991). Choline acetyltransferase immunoreactive neurons (ChAT+) were identified in all these subdivisions except the cerebral cortex (Fig. 1), where no cholinergic interneurons were identified, as observed in some other mammals (e.g. Bhagwandin et al., 2006; Calvey et al., 2013).

3.1.1. Striatal cholinergic interneurons

ChAT+ neurons were found in the caudate/putamen complex, the globus pallidus, the nucleus accumbens, the Islands of Calleja and the olfactory tubercle in all three species (Fig. 1D–L). A moderate to high density of ChAT+ interneurons were found throughout the caudate/putamen complex, although the caudate and the putamen nuclei were clearly separated by the internal capsule in all three species. The nucleus accumbens exhibited a low to moderate density of ChAT+ neurons surrounding the ventral border of the anterior commissure directly ventral to the caudate/putamen complex in all three species. These neurons were predominantly bipolar and ovoid in shape, but a few neurons were multipolar. The globus pallidus contained a low density of ChAT+ neurons that were predominantly multipolar and mostly located around the margins of this nucleus. The ChAT+ neurons within the olfactory tubercle and Islands of Calleja were found in the most ventral portion of the cerebral hemisphere (Fig. 2C). Throughout the olfactory tubercle a moderate density of ChAT+ neurons were observed, and within the most ventral portion of this region distinct clusters of ChAT+ neurons were observed to constitute the Islands of Calleja.

3.1.2. Cholinergic nuclei of the basal forebrain

Cholinergic nuclei identified within the basal forebrain of the three strepsirrhine species studied included the medial septal nucleus, the diagonal band of Broca and the nucleus basalis (Fig. 1F–H). The ChAT+ neurons representing the medial septal nucleus appeared in the rostral portion of the septum adjacent to the midline. These neurons were bipolar with vertically oriented dendrites. The ChAT+ cells representing the diagonal band of Broca were located ventral to the cells of the medial septal nucleus, in the ventromedial corner of the cerebral hemisphere. No clear dendritic orientation of these bipolar and multipolar neurons was evident. Ventral to the globus pallidus and lateral to the hypothalamus, a moderate to high density of ChAT+ neurons were assigned to the nucleus basalis (Fig. 2). These cells were found to intermingle with

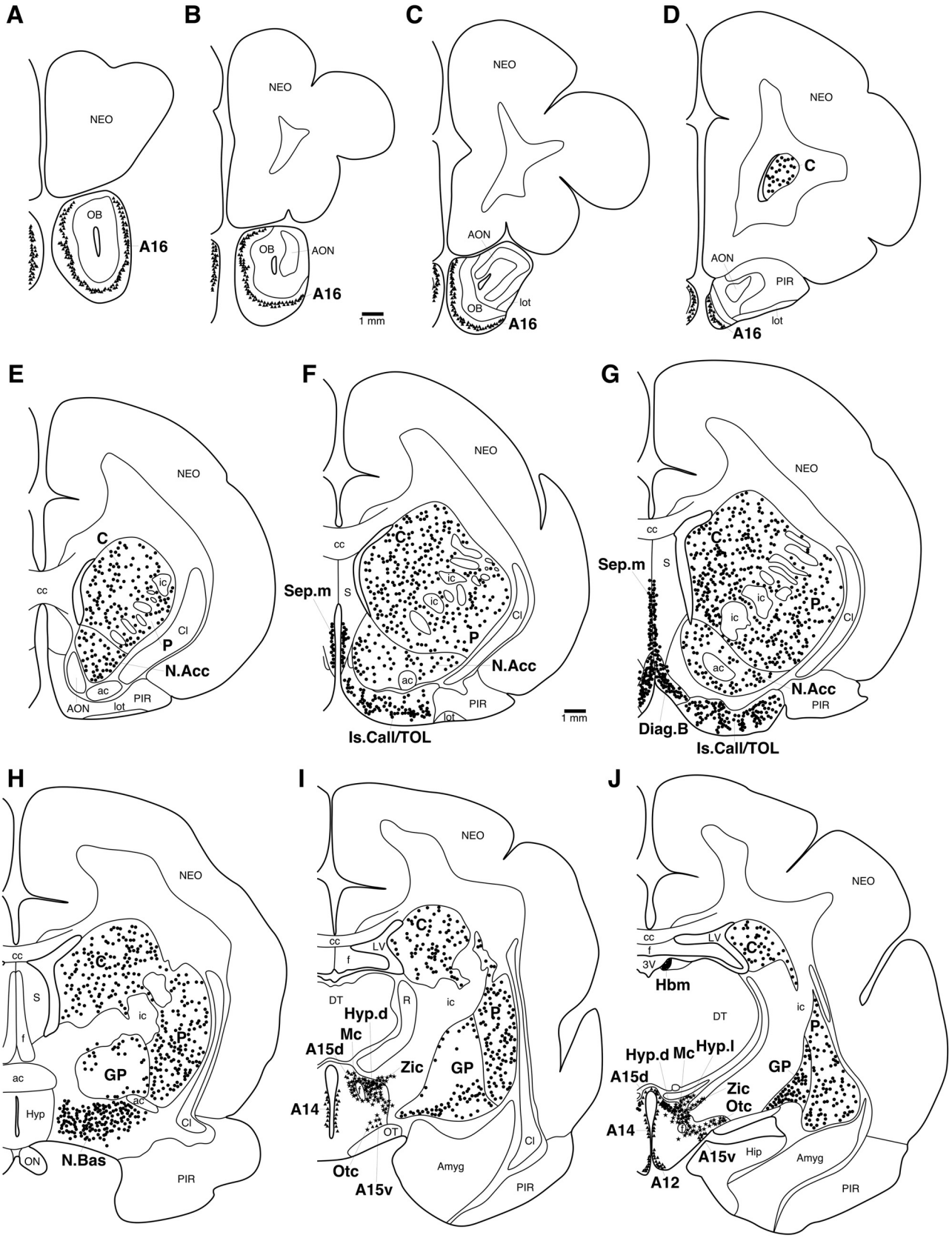

Fig. 1. Serial drawings of coronal sections through one half of the potto (*Perodicticus potto*) brain from the olfactory bulb through to the spinomedullary junction. **A** is the most rostral section, **V** the most caudal. The outlines of the architectonic regions were drawn using Nissl and myelin stains and immunoreactive cells marked on the drawings. Solid black circles depict cholinergic neurons, solid triangles depict catecholaminergic neurons (those immunoreactive for tyrosine hydroxylase), open squares depict serotonergic neurons and closed stars represent orexinergic neurons. Each circle, triangle, square or star represents an individual neuron. The figures are approximately 1500 μm apart. See list for abbreviations.

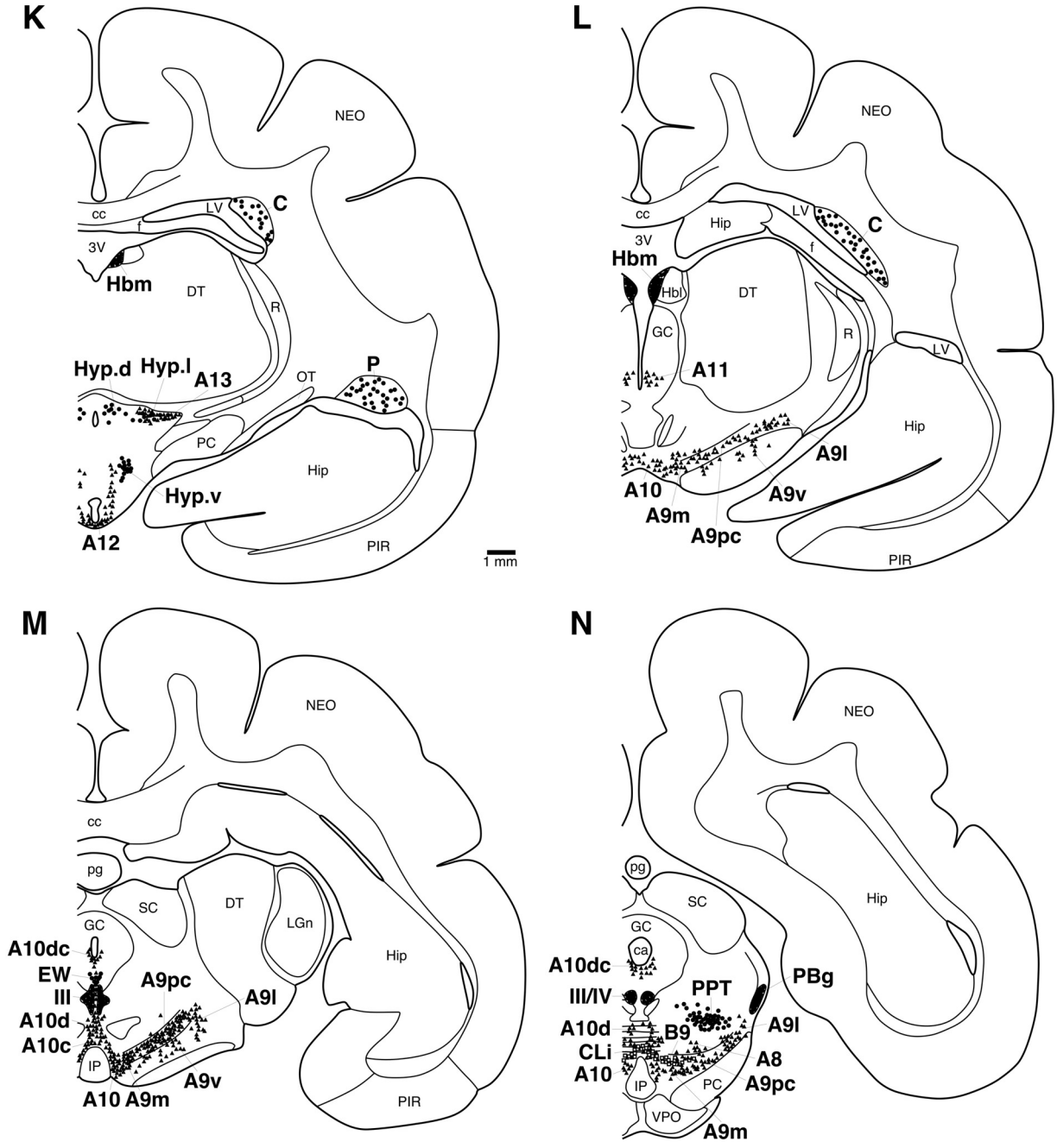

Fig. 1. (*Continued*).

the most ventral cells of the globus pallidus, were ovoid in shape, and showed no clear dendritic orientation.

3.1.3. Diencephalic cholinergic nuclei

Within the strepsirrhine diencephalon, ChAT+ neurons were observed in the medial habenular nucleus, as well as forming three distinct nuclei, dorsal, lateral and ventral, within the hypothalamus (Fig. 1I–L). The ChAT+ neurons of the medial habenular nucleus were small, ovoid in shape and densely packed within this nucleus. The ChAT+ neurons within the hypothalamic nuclei were small in size, sparsely distributed and faintly immunostained, but were readily located. The ChAT+ neurons forming the dorsal hypothalamic cholinergic nucleus were found in the mediodorsal hypothalamus dorsal and medial to the fornix. The lateral hypothalamic cholinergic nucleus appeared to be a lateral extension of the neurons forming the dorsal nucleus, but located lateral to the fornix in the dorsolateral aspect of the hypothalamus. The ventral hypothalamic cholinergic nucleus was found in the ventromedial aspect of the hypothalamus, near, but not within, the arcuate nucleus.

3.1.4. Pontomesencephalic cholinergic nuclei

ChAT+ immunoreactive neurons delineated the parabigeminal nucleus (PBg), the pedunculopontine (PPT) and laterodorsal (LDT) tegmental nuclei in all three strepsirrhine species investigated (Fig. 1N–O). The parabigeminal nucleus was located at the very lateral margin of the pontine tegmentum in a location ventral to the posterior pole of the superior colliculus. The ChAT+ neurons were small and densely packed with no clear dendritic orientation. The PPT was a large nucleus with bipolar and multipolar cells found in a moderate density throughout much of the midbrain and rostral pontine tegmentum (Fig. 3). This nucleus appeared at the level of the oculomotor nuclei and terminated at the level of the

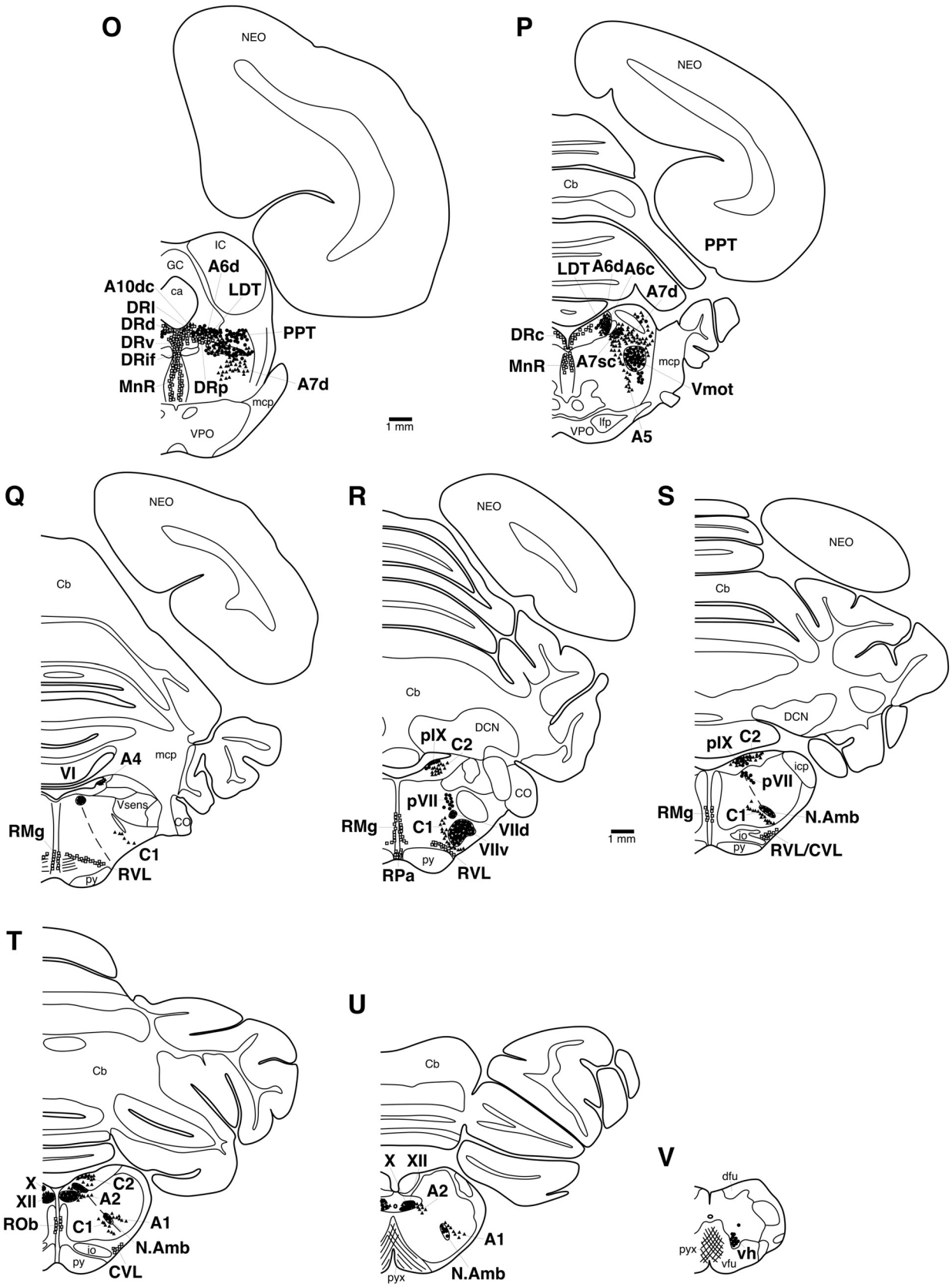

Fig. 1. (*Continued*).

trigeminal motor nucleus. The ChAT+ neurons forming the LDT nucleus were found in the ventrolateral periaqueductal and periventricular grey matter. The most ventrolateral aspect of this nucleus abutted the dorsomedial aspect of the PPT nucleus, with the ventrolateral edge of the gray matter delineating their mutual border (Fig. 3). The ChAT+ immunoreactive neurons of the LDT and PPT showed a very similar morphology, being mostly multipolar and showing no specific dendritic orientation.

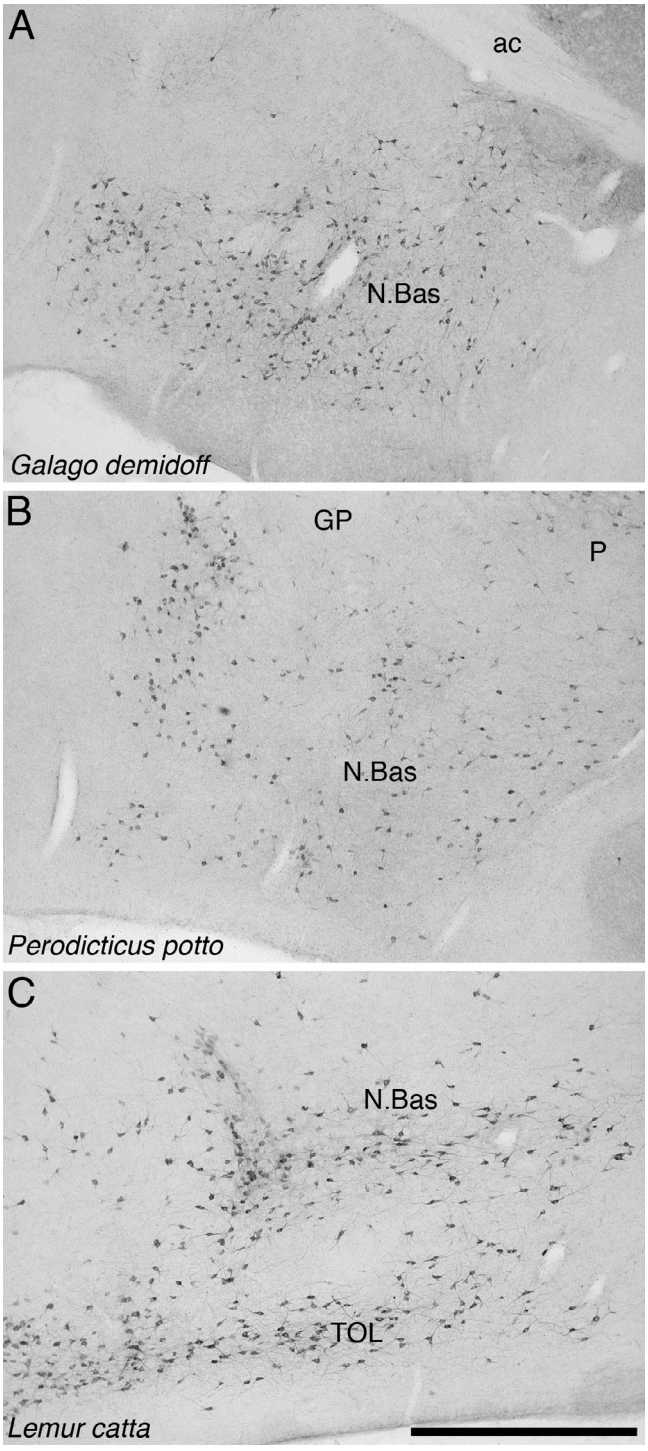

Fig. 2. Photomicrographs showing neurons immunoreactive for choline acetyltransferase in the nucleus basalis (**N.Bas.**) and olfactory tubercle (**TOL**) in the three species studied: (A) Demidoff's dwarf bushbaby (*Galagoides demidoff*); (B) the potto (*Perodicticus potto*); and (C) the ring-tailed lemur (*Lemur catta*). Note the similarity in appearance of this region of the brain in all species. Scale bar in **C** = 1000 μm and applies to all. **ac**—anterior commissure, **P**—putamen. In all images, medial is to the left and dorsal to the top.

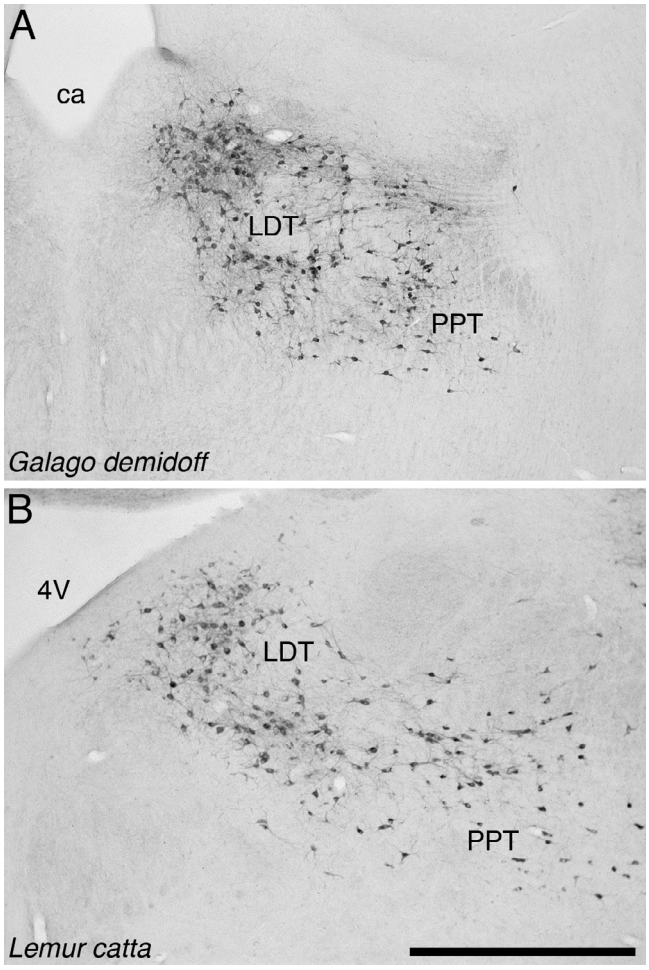

Fig. 3. Photomicrographs showing neurons immunoreactive for choline acetyltransferase in the laterodorsal tegmental (**LDT**) and peduculopontine (**PPT**) nuclei in two of the species studied: (A) Demidoff's dwarf bushbaby (*Galago demidoff*); and (B) the ring-tailed lemur (*Lemur catta*). Note the similarity in appearance of this region of the brain in both species. Scale bar in **B** = 1000 μm and applies to both. **4V**—fourth ventricle; **ca**—cerebral aqueduct. In both images, medial is to the left and dorsal to the top.

3.1.5. Cholinergic cranial nerve nuclei

The ChAT+ immunoreactive neurons forming various cranial nerve nuclei were found in positions typical of all mammals studied to date (Woolf, 1991; Maseko et al., 2007; Dell et al., 2010; Kruger et al., 2010a). The ChAT+ nuclei identified in the three strepsirrhine species studied included the oculomotor(III) and the trochlear(IV) nerve nuclei, the motor division of the trigeminal nerve nucleus (Vmot), the abducens nerve nucleus(VI), the dorsal and the ventral subdivisions (VIId and VIIv) of the facial nerve nucleus, the nucleus ambiguus, the dorsal motor vagus(X), the hypoglossal(XII), and the Edinger–Westphal (EW) nerve nuclei, the preganglionic motor neurons of the superior salivatory (pVII) and inferior salivatory (pIX) nuclei and the ventral horn of the spinal cord (vh) (Fig. 1M–V). ChAT+ immunoreactive neurons that could be classified as the medullary tegmental field (mtf) were absent in all three species. Most nuclei contained large, strongly immunostained ChAT+ motor neurons. The cells in the dorsal motor vagus nerve nucleus, while having a similar morphology were slightly smaller than the neurons observed in the other nerve nuclei, and the neurons within the superior and inferior salivatory nuclei were also smaller and more scattered than in the other nuclei.

3.2. Catecholaminergic nuclei

Tyrosine hydroxylase (TH+) immunoreactivity revealed catecholaminergic neurons in the brains of the three species examined. The nuclei formed by these neurons were arranged in a number of identifiable nuclear complexes that extended from the olfactory bulb through to the spinomedullary junction. These complexes

correspond to that seen in other mammals (e.g. Smeets and González, 2000) and could be divided into the olfactory bulb, diencephalic, midbrain, pontine and medullary nuclear clusters. In the current description the nuclei are referred to using the nomenclature of Dahlström and Fuxe (1964) and Hökfelt et al. (1984), as no catecholaminergic nuclei outside the classically defined nuclei (e.g. Smeets and González, 2000) were observed. The TH+ nuclei found in the three species studied were similar to that seen in many other mammals. The rodent typical C3 nucleus (rostral dorsal midline medullary nucleus) was absent in all three species (e.g. Smeets and González, 2000; Dell et al., 2010; Kruger et al., 2012). The primate and megachiropteran appearance of the locus coeruleus (A6), having a compact and diffuse portion was present in all three species (Dell et al., 2010).

3.2.1. The olfactory bulb (A16)

The TH+ neurons forming the A16 nucleus were observed as dense clusters of cells surrounding the inner and lateral aspects of the olfactory glomeruli in all three species (Fig. 1A–D). These neurons likely represent the periglomerular dopaminergic neurons, were small in size, and exhibited dendrites oriented to the edges of the glomeruli. No TH+ neurons were observed outside of the glomerular layer of the olfactory bulb.

3.2.2. Diencephalic catecholaminergic nuclei (A15–A11)

In the hypothalamus of all three species TH+ neurons formed six distinct nuclei: the dorsal division of the anterior hypothalamic group (A15d), the ventral division of the anterior hypothalamic group (A15v), the rostral periventricular cell group (A14), the zona incerta (A13), the tuberal cell group (A12), and the caudal diencephalic group (A11) (Fig. 1I–K). A15d contained a moderate density of bipolar TH+ neurons lateral to the third ventricle in the dorsal medial portion of the hypothalamus. The TH+ neurons of the A15 v nucleus were found medial to the optic tract in the ventrolateral portion of the hypothalamus. These neurons were also small and bipolar and exhibited no specific dendritic orientation. The TH+ neurons forming the A14 nucleus were observed as two distinct columns of moderately dense neurons adjacent to the lateral borders of the third ventricle in the more rostral portion of the hypothalamus. The dendrites of these bipolar neurons exhibited a dorsoventral orientation. The A13 nucleus consisted of a moderate density of predominantly oval, bipolar cells in the dorsolateral portion of the mid-level of the hypothalamus caudal to the fornix. TH+ neurons representing the A12 nucleus, were found in a moderate to high density lateral and ventral to the ventral portion of the third ventricle in the caudal third of the hypothalamus. At the most caudal level of the hypothalamus, the TH+ neurons forming the A11 nucleus were found caudal to the caudal pole of the third ventricle. In comparison to the other TH+ neurons within the hypothalamus, the A11 neurons were larger and multipolar and exhibited no specific dendritic orientation.

3.2.3. Midbrain nuclei (A10–A8)

Tyrosine hydroxylase immunoreactive neurons in the midbrain were found within the ventral tegmental area (the A10 complex, including the A10, A10 central, A10 dorsal, and A10 dorsocaudal nuclei), the substantia nigra (the A9 complex, including the A9 pars compacta, A9 lateral, A9 ventral, and A9 medial nuclei) and the retrorubral nucleus (A8) within the midbrain tegmentum in all three species studied (Fig. 1L–O). In the ventral border of the tegmentum, rostral to and surrounding the interpeduncular nucleus, TH+ neurons representing the A10 nucleus were located. Caudally, these cells were found dorsal and dorsolateral to the interpeduncular nucleus, between it and the root of the oculomotor nerve. Immediately dorsal to the inteperduncular nucleus, a dense cluster of TH+ neurons representing the A10c nucleus was located. Dorsal to the A10c nucleus, and ventral to the oculomotor nucleus, a moderate density of TH+ neurons, forming a distinctive triangular aggregation, represent the A10d nucleus. Within the periaqueductal grey matter, bipolar and multipolar cells representing the A10dc nucleus were found surrounding the ventral aspect of the cerebral aqueduct. The TH+ neurons in all these nuclei were a mixture of bipolar and multipolar types, with ovoid soma, with dendrites showing no specific orientation.

The substantia nigra nuclear complex was observed in the ventral and lateral portions of the midbrain tegmentum, just dorsal to the cerebral peduncles and ventral to the medial lemniscus. The A9m nucleus appeared as a moderate to high density cluster of bipolar and multipolar cells located lateral to the A10 nucleus and lateral to the root of the oculomotor nerve. The A9pc (pars compacta) appeared to be a lateral extension of the A9m nucleus, but exhibited a slightly higher density of TH+ neurons. The neurons of the A9pc were found as a distinct band lying immediately dorsal to the cerebral peduncle. Throughout the pars reticulata of the substantia nigra, in a position ventral to the A9pc, scattered TH+ neurons were assigned to the A9v (ventral) nucleus. At the lateral edge of A9pc, a loose aggregation of TH+ neurons formed the A9l (lateral) nucleus. The TH+ neurons in all these A9 nuclei were a mixture of bipolar and multipolar types, with ovoid soma and dendrites showing no specific orientation except in the A9pc, where the dendrites were oriented in a roughly mediolateral plane. Dorsal to the substantia nigra complex, extending into the caudal aspect of the midbrain tegmentum, numerous TH+ neurons, found in a low to moderate density, represented the A8 nucleus. The morphology of these neurons was similar to that observed in the A10 and A9 nuclear complexes.

3.2.4. Rostral rhombencephalon—The locus coeruleus complex (A7–A4)

Within the pontine region of all three species a large number of TH+ neurons forming the locus coeruleus complex was readily observed. This complex represents the noradrenergic component of the catecholaminergic system (Smeets and González, 2000) and could be subdivided into six nuclei: the subcoeruleus compact portion (A7sc), the subcoeruleus diffuse portion (A7d), the locus coeruleus compact portion (A6c), the locus coeruleus diffuse portion (A6d), the fifth arcuate nucleus (A5), and the dorsolateral division of locus coeruleus (A4) (Figs. 1O–Q and Fig. 4). Within the dorsal portion of the pontine tegmentum adjacent to the ventrolateral edge of the periaqueductal grey matter, a tightly packed cluster of TH+ neurons represented the A7 compact portion of the subcoeruleus (A7sc) (Fig. 4A). This division is the same as what was previously described as the subcoeruleus by Dahlström and Fuxe (1964) and Olson and Fuxe (1972) in the laboratory rat. Ventral and lateral to the A7sc, a diffuse aggregation of TH+ neurons formed the A7d nuclear complex (Fig. 4C). These neurons were located both medially and laterally around the trigeminal motor nucleus (Vmot) and the superior cerebellar peduncle. Within the lateral portion of the periventricular grey matter a tightly packed, moderate to high density cluster of TH+ neurons were assigned to the A6c nucleus (Fig. 4). This tightly packed cluster of TH+ neurons was surrounded by a band of more loosely packed neurons that were assigned to the diffuse portion of the locus coeruleus (A6d) (Fig. 4). In the ventrolateral pontine tegmentum lateral to the superior olivary nucleus and lateral to Vmot and A7d, a small cluster of TH+ neurons formed the A5 nucleus. These neurons formed a rough mesh-like dendritic network around the ascending fascicles located within the ventrolateral pontine tegmentum. Immediately adjacent to the wall of the fourth ventricle, in the dorsolateral portion of the periventricular grey matter, a dense, but small cluster of TH+ neurons represented the A4 nucleus.

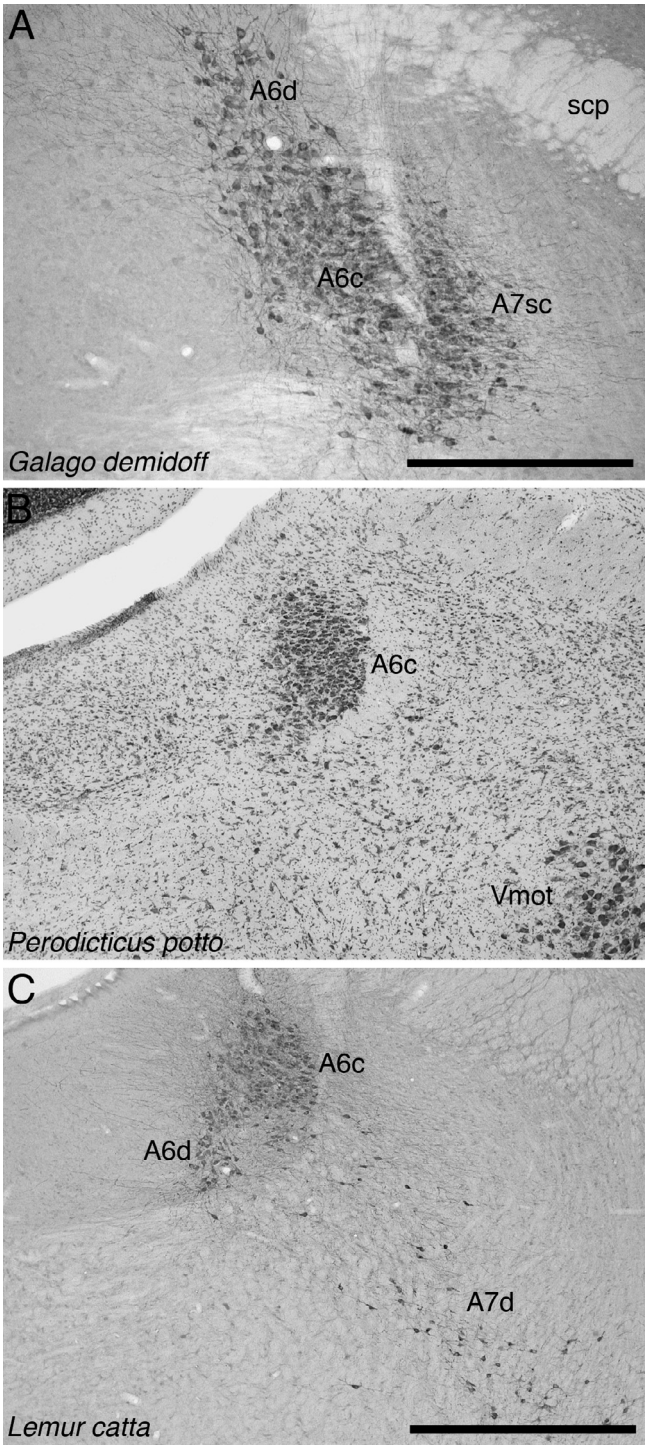

Fig. 4. Photomicrographs showing neurons immunoreactive for tyrosine hydroxylase (A and C) and stained for Nissl substance (B) in the locus coeruleus complex of the three species studied: (A) Demidoff's dwarf bushbaby (*Galago demidoff*); (B) the potto (*Perodicticus potto*); and (C) the ring-tailed lemur (*Lemur catta*). Note the presence of both the compact (**A7sc**) and diffuse (**A7d**) portions of the subcoeruleus, and the compact (**A6c**) and diffuse (**A6d**) portions of the locus coeruleus. The A6c is only present in primates and megachiropterans and is readily visible even in Nissl stained sections (B). Scale bar in **A** = 500 μm, scale bar in **C** = 1000 μm and applies to (B and C). **Vmot**—motor division of trigeminal nucleus; **scp**—superior cerebellar peduncle. In all images, medial is to the left and dorsal to the top.

3.2.5. Medullary nuclei (C1, C2, A1, A2, area postrema)

In the medulla of all three species five catecholaminergic nuclei were observed: the rostral ventrolateral tegmental group (C1), the rostral dorsomedial group (C2), the caudal ventrolateral tegmental group (A1), the caudal dorsomedial group (A2), and the area postrema (AP) (Fig. 1R–U). C1 and C2 represent the adrenergic component of the catecholaminergic system while A1 and A2 are dopaminergic and noradrenergic (Smeets and González, 2000). Medioventral to the facial nerve nucleus and the nucleus ambiguus, on the ventrolateral aspect of the medulla, TH+ neurons representing the C1 nucleus appeared in a moderate to high density. These multipolar neurons had dendrites that formed a mesh-like network in this region around the ascending and descending fascicules of the medulla. The C2 nucleus was represented by ovoid shaped, bipolar TH+ neurons on the dorsal aspect of the medulla adjacent to the ventral border of the fourth ventricle. The nucleus ambiguus separated the TH+ neurons of the C1 nucleus from those of the A1 nucleus. The A1 nucleus was found lateral to the nucleus ambiguus, while the C1 nucleus was medial to the nucleus ambiguus. The neurons forming the A1 nucleus had a similar appearance to those of the C1 nucleus. The TH+ neurons assigned to the A2 nucleus were found in the dorsomedial medulla, between the X and XII cranial nerve nuclei, although some of these neurons were found within the adjacent dorsomedial medullary tegmentum. The A2 neurons were both bipolar and multipolar and were slightly larger than the neurons within the C2 nucleus. Straddling the midline, dorsal to the central canal and the dorsal motor vagus nucleus, and between the most caudal aspect of the bilateral C2 nucleus, was a single large, densely packed cluster of intensely stained TH+ neurons, the area postrema.

3.3. Serotonergic nuclei

The serotonergic nuclei identified in the brains of all three species in this study were found to be the same as in the other eutherian mammals studied to date (Steinbusch, 1981; Maseko et al., 2007; Dell et al., 2010; Kruger et al., 2012; Calvey et al., 2013). These nuclei were all located within the brainstem and can be divided into a rostral and caudal cluster (Törk, 1990). Both of these clusters contained distinct nuclei, and these were found throughout the brainstem from the level of the decussation of the superior cerebellar peduncle through to the spinomedullary junction. All three species examined exhibited the same complement of serotonergic nuclei in both the rostral and caudal clusters.

3.3.1. Rostral cluster

Serotonergic neurons (5HT+) representing the caudal linear nucleus (CLi), the supralemniscal nucleus (B9), the median raphe nucleus (MnR) and the dorsal raphe complex were found in all three species (Fig. 1N–P). The CLi nucleus was the most rostral of the serotonergic nuclei found and the bipolar 5HT+ neurons formed a cluster of moderate density around the midline immediately dorsal to the interpeduncular nucleus in a location just anterior to the decussation of the superior cerebellar peduncle in all three species. The serotonergic neurons forming the B9 nucleus appeared to be a lateral extension of the most ventral portion of CLi (Fig. 5A). The predominantly bipolar 5HT+ B9 neurons were found in a moderate to high density and extended as an arc of neurons into the ventrolateral portion of the midbrain tegmentum with cell density increasing caudally. The median raphe nucleus (MnR) was characterized by two distinct, densely packed 5HT+ neuronal columns on either side of the midline in a para-raphe position, extending from the level of the oculomotor nucleus to the anterior portion of the pons. The dendrites of mostly bipolar MnR neurons were oriented in a dorsoventral plane. In the ring-tailed lemur, the columns of the MnR expanded laterally along the ventral border of the medial longitudinal fasciculus, a feature not observed in the other two species.

Within the dorsal raphe nuclear complex we identified six distinct nuclei in all three species: the dorsal raphe interfascicular

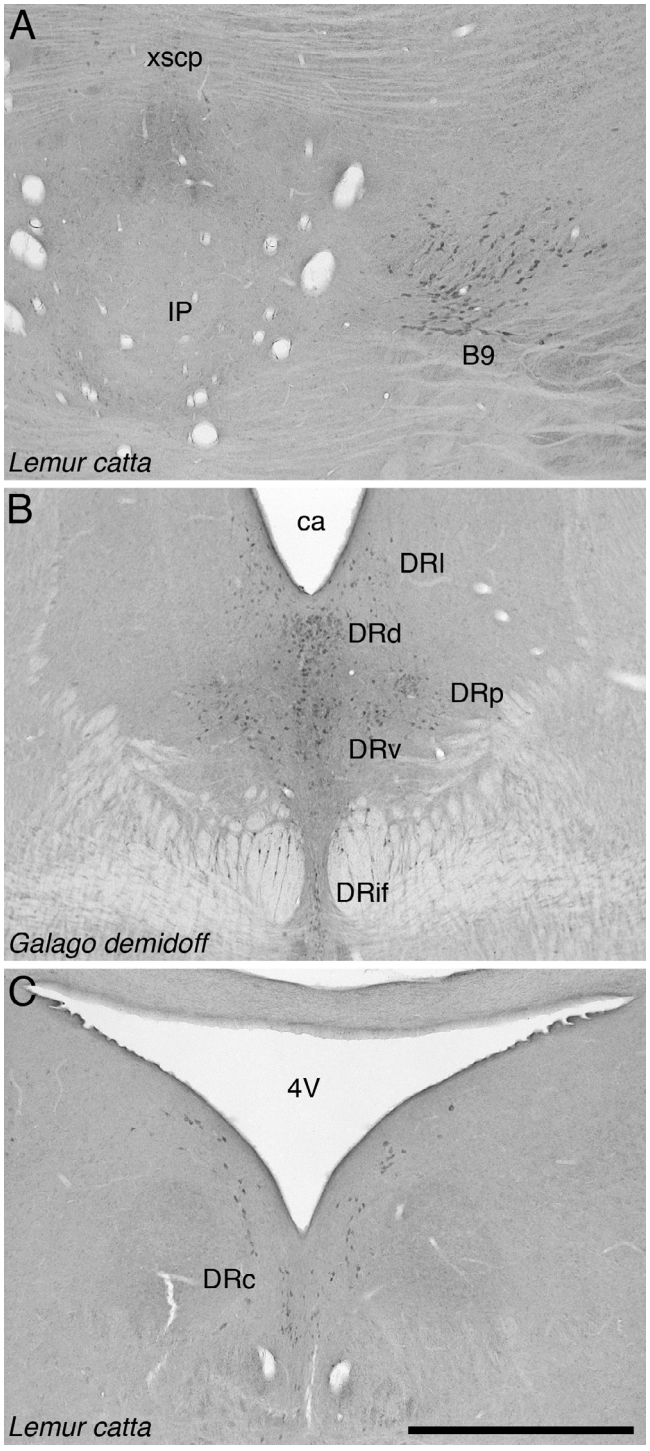

Fig. 5. Photomicrographs showing neurons immunoreactive for serotonin in the rostral serotonergic cluster in two of the species studied: (A and C) the ring-tailed lemur (*Lemur catta*); and (B) Demidoff's dwarf bushbaby (*Galago demidoff*). (A) The supralemniscal serotonergic group (**B9**) in the ventral midbrain tegmentum, located lateral to the interpeduncular nucleus (**IP**) and ventrolateral to the decussation of the superior cerebellar peduncle (**xscp**). (B) The most expanded portion of the dorsal raphe nuclear complex showing the lateral (**DRl**), dorsal (**DRd**), ventral (**DRv**), peripheral (**DRp**) and interfascicular (**DRif**) divisions. (C) The caudal (**DRc**) division of the dorsal raphe nuclear complex. Scale bar in **C** = 1000 μm and applies to all. **4V**—fourth ventricle, **ca**—cerebral aqueduct.

nucleus (DRif), the dorsal raphe ventral nucleus (DRv), the dorsal raphe dorsal nucleus (DRd), the dorsal raphe lateral nucleus (DRl), the dorsal raphe peripheral nucleus (DRp) and the dorsal raphe caudal nucleus (DRc) (Fig. 5B). These six nuclei were found, for the most part, within the periaqueductal and periventricular grey matter from the level of the oculomotor nucleus to the trigeminal motor nucleus and contained bipolar and multipolar neurons. Two pararaphe columns of 5HT+ neurons located between the bilaterally paired medial longitudinal fasciculi represent the DRif nucleus in all three species. The DRv was found immediately dorsal to the DRif, within the periaqueductal grey matter, and was represented by a high density of 5HT+ neurons. Immediately dorsal to DRv, between it and the ventral border of the cerebral aqueduct, a high-density cluster of bipolar 5HT+ neurons was designated as the DRd nucleus. A moderate density of larger, multipolar 5HT+ neurons representing the DRp, was located in the ventrolateral portion of the periaqueductal grey matter. A small number of DRp neurons was found in the adjacent midbrain tegmentum, outside the periaqueductal grey matter, and these were the only neurons of the dorsal raphe complex found external to the central grey matter. The larger, multipolar 5HT+ neurons of the DRl were located dorsolateral to the DRd forming clear aggregations along the edges of the cerebral aqueduct with a low to moderate density. Caudal to DRl, where the cerebral aqueduct opened into the fourth ventricle and the DRd, DRv and DRif disappeared, the neurons of the DRl formed an arc of 5HT+ neurons across the midline of the dorsal portion of the periventricular grey matter, and this represents the DRc nucleus. The neurons of the DRc evinced a similar morphology to those of the DRl and DRp (Fig. 5C).

3.3.2. Caudal cluster

Within the caudal serotonergic cluster we found evidence for the raphe magnus (RMg), rostral and caudal ventrolateral (RVL and CVL), raphe pallidus (RPa) and raphe obscurus (ROb) nuclei (Fig. 1Q–T). The RMg was observed to be two columns of loosely aggregated moderate to large 5HT+ neurons, with dendrites oriented dorsoventrally, on either side of the midline from the level of the trigeminal motor nucleus to the nucleus ambiguus. Appearing at the same level as RMg within the left and right ventrolateral medullary tegmentum, a distinct anteroposterior column of 5HT+ neurons was found, the rostral and caudal ventrolateral serotonergic columns. The RVL began as a ventrolateral continuation of 5HT+ neurons from the lower portion of the RMg extending over the pyramidal tracts and lateral to the inferior olivary complex. The inferior olivary complex topologically distinguishes left and right RVL, and at the approximate level of nucleus ambiguus the RVL becomes the CVL. The CVL continues in the caudal ventrolateral medullary tegmentum until the spinomedullary junction is reached. The neurons of the RVL and CVL exhibited a similar morphology to those of the RMg. The 5HT+ neurons forming the RPa nucleus were found in the ventral midline of the medulla associated with the pyramidal tracts. These bipolar neurons were for the most part located between the two pyramidal tracts in a tightly packed bundle. Two loosely arranged bilateral columns of large, multipolar 5HT+ neurons located on each side of the midline from the level of the nucleus ambiguus to the spinomedullary junction were classified as the ROb.

3.4. Orexinergic (hypocretinergic) nuclei

The vast majority of orexin-A immunopositive neurons (OxA+) identified in the brains of the three strepsirrhine species studied were found within the hypothalamus. These neurons were ovoid in shape and bipolar, exhibiting no clear dendritic orientation (except where noted below). Within the area where orexinergic neurons were located we could readily divide them into three distinct clusters: a main cluster (Mc), a zona incerta cluster (Zic) and an optic tract cluster (Otc) (Figs. 1I–J and Fig. 6). The main cluster (Mc) was identified as a large group of moderately densely packed OxA+ neurons located lateral to the third ventricle in the perifornical

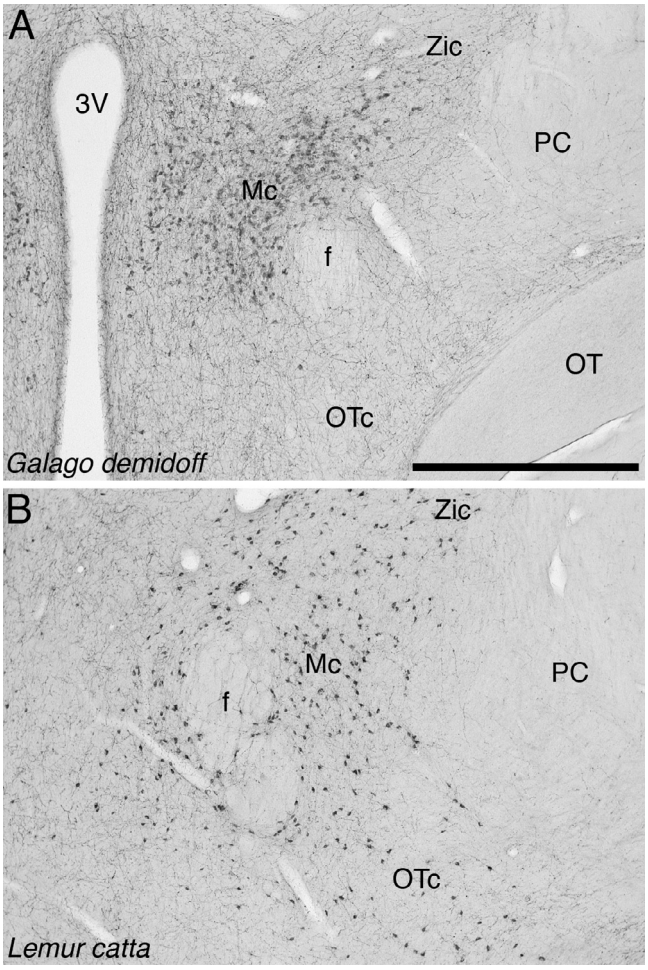

Fig. 6. Photomicrographs showing neurons immunoreactive for orexin-A in the hypothalamus in two of the species studied: (A) Demidoff's dwarf bushbaby (*Galago demidoff*); and (B) the ring-tailed lemur (*Lemur catta*). Note the similarity of the clustering of the orexinergic neurons into the main cluster (**Mc**) near the fornix (**f**), the zona incerta cluster (**Zic**) in the dorsolateral hypothalamus above the cerebral peduncle (**PC**), and the optic tract cluster (**OTc**) in the ventrolateral hypothalamus near the optic tract (**OT**). Scale bar in **A** = 1000 μm and applies to both images. In both images, medial is to the left and dorsal to the top. **3V**—third ventricle.

region of the hypothalamus, with a moderate number of neuronal cell bodies extending medially from this area into the dorsomedial hypothalamus. From the main cluster a group of OxA+ neurons extended laterally into the dorsolateral hypothalamus, with some cells found just outside of the hypothalamus in the region of the zona incerta. This zona incerta cluster (Zic) had a moderate density of OxA+ neurons with dendrites oriented in the mediolateral plane. The third cluster, the optic tract cluster (Otc), extended ventrolaterally from the main cluster to the ventrolateral region of the hypothalamus adjacent to the optic tract. The Otc contained a low to moderate density of OxA+ neurons that showed no specific dendritic orientation.

4. Discussion

The nuclear organization and complement of four immunohistochemically identifiable neural systems within the brains of three previously unstudied species of strepsirrhine primates (Demidoff's dwarf bushbaby—*G. demidoff*, the potto—*P. potto* and the ring-tailed lemur—*L. catta*) were analysed in this study. For the most part, the organization and complement of nuclei of these systems were similar to that observed in many other Eutherian mammals previously described (e.g. Maseko et al., 2007; Dell et al., 2010), but despite this there were specific differences of note (summarized in Table 1) that may be related either to the phylogenetic history or to the current life histories of these species. The locus coeruleus complex, presenting with both a compact (A6c) and a diffuse (A6d) portion was present in all three strepsirrhine species studied, which is the same as previously observed in haplorhine primates and in megachiropterans (Maseko et al., 2007; Dell et al., 2010). In microchiropterans, as in most other mammals, only the A6d portion of the locus coeruleus is present (Maseko and Manger, 2007; Kruger et al., 2010a). All three strepsirrhines had a large and clearly expressed lateral division of the dorsal raphe, a typical primate feature that has been described in megachiropterans (Dell et al., 2010). All three strepsirrhines had the catecholaminergic A4 and A15d nuclei, as well as the optic tract cluster (Otc) of the orexinergic system. These nuclei are present in all previously studied megachiropterans, but are absent in all previously studied microchiropterans (Maseko and Manger, 2007; Kruger et al., 2010a,b). The cholinergic parabigeminal nucleus was present in these strepsirrhines as well as in megachiropterans, whereas this nucleus is absent in four out of the six previously studied microchiropterans (Maseko and Manger, 2007; Kruger et al., 2010a). There was a clear presence of the catecholaminergic ventral division of the substantia nigra nuclear complex in all three strepsirrhine species, but this nucleus appears to be incipient in microchiropterans and is completely absent in one species (Maseko and Manger, 2007; Kruger et al., 2010a). Overall, the results of the current study, along with those from previous studies support chiropteran diphyly and the link between megachiropterans and primates.

4.1. Cholinergic nuclei

The complement of nuclei within the cholinergic system of the three strepsirrhines was identical to that observed in previously studied primates, and very similar to that observed in most other mammals (e.g. Dell et al., 2010). While there can be significant variation in the complement of cholinergic nuclei in the mammalian brain, it can be said that the complement of cholinergic nuclei in strepsirrhine primates likely reflects that of a generalized Eutherian mammal. In the strepsirrhine primates no cholinergic interneurons were observed in the cerebral cortex, as observed in Murid rodents and the hottentot golden mole (e.g. Bhagwandin et al., 2006; Calvey et al., 2013), or in other regions of the brain such as the olfactory bulb, amygdala, hippocampus, superior and inferior colliculi, or cochlear nuclei as seen in some Afrotherians (Pieters et al., 2010; Calvey et al., 2013). As with most other mammals, the parabigeminal nucleus was present in all three strepsirrhines, but as mentioned, this nucleus was not found in four species of microchiropterans (Maseko and Manger, 2007; Kruger et al., 2010a). In addition, the strepsirrhine primates did not exhibit parvocellular cholinergic neurons surrounding the pontine cholinergic nuclei (LDT and PPT) as observed in the rock hyrax (Gravett et al., 2009). Thus, the variance in the complement of cholinergic nuclei does, to an extent, distinguish the primates from several other mammalian lineages, but does not specifically align them with the megachiropterans; however, it does not argue against such an alignment, and the complement of cholinergic nuclei does serve to distinguish the megachiropterans from the microchiropterans.

4.2. Catecholaminergic nuclei

For the most part, the complement of nuclei identified as belonging to the catecholaminergic system in the strepsirrhines studied was similar to that observed in most eutherian mammals

Table 1
Summary of the nuclei delineated in the current study of strepsirrhine primates in comparison to similar studies previously undertaken in microchiropterans, megachiropterans and haplorhine primates (data for these species from Dell et al., 2010). Cells with a dark grey background indicate features distinguishing the microchiroptera from the other species. Cells with a light grey background indicate features that align the megachiropterans with the primates to the exclusion of all other mammals.

	Microchiroptera						Megachiroptera			Strepsirrhines			Haplorhines					
Species	*Miniopterus schreibersii*	*Chaerophon pumilis*	*Hipposideros commersoni*	*Cardioderma cor*	*Coleura afra*	*Triaenops persicus*	*Rousettus aegyptiacus*	*Eidolon helvum*	*Epomophorus wahlbergii*	*Galagoides demidoff*	*Perodicticus potto*	*Lemur catta*	*Cebuella pymaea*	*Callithirix jacchus*	*Saimiri sciureus*	*Macaca sp.*	*Papio papio*	*Homo sapiens*
Common name	Schreiber's long fingered bat	Little free-tailed bat	Commerson's leaf-nosed bat	Heart-nosed bat	African sheath-tailed bat	Persian trident bat	Egyptian Rousette	Straw coloured fruit bat	Wahlberg's epaletted fruit bat	Demidoff's dwarf bushbaby	Potto	Ring-tailed lemur	Pygmy marmoset	Common marmoset	Squirrel monkey	Macaque monkey	Baboon	Human
Cholinergic																		
Islands of Calleja	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Olfactory tubercle	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Nucleus accumbens	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Caudate/Putamen	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Globus pallidus	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Medial septal nucleus	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Diagonal band of Broca	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Nucleus basalis	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Dorsal hypothalamic	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Ventral hypothalamic	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Lateral hypothalamic	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Medial habenular	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Parabigeminal nucleus	–	–	–	+	+	–	+	+	+	+	+	+	?	+	+	+	+	+
Pedunculopontine nucleus	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+	+
Laterodorsal tegmental nucleus	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+	+
Edinger–Westphal nucleus	–	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+	+
Oculomotor nucleus	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+	+
Trochlear nucleus	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Trigeminal motor nucleus	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Abducens nucleus	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Facial nucleus dorsal	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Facial nucleus ventral	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Nucleus ambiguous	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Vagus motor nucleus	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Hypoglossal nucleus	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Ventral horn	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Superior salivatory nucleus	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+

Table 1 (*Continued*)

Species	Microchiroptera						Megachiroptera			Strepsirrhines			Haplorhines					
	Miniopterus schreibersii	*Chaerophon pumilis*	*Hipposideros commersoni*	*Cardioderma cor*	*Coleura afra*	*Triaenops persicus*	*Rousettus aegyptiacus*	*Eidolon helvum*	*Epomophorus wahlbergii*	*Galagoides demidoff*	*Perodicticus potto*	*Lemur catta*	*Cebuella pymaea*	*Callithirix jacchus*	*Saimiri sciureus*	*Macaca sp.*	*Papio papio*	*Homo sapiens*
Common name	Schreiber's long fingered bat	Little free-tailed bat	Commerson's leaf-nosed bat	Heart-nosed bat	African sheath-tailed bat	Persian trident bat	Egyptian Rousette	Straw coloured fruit bat	Wahlberg's epaletted fruit bat	Demidoff's dwarf bushbaby	Potto	Ring-tailed lemur	Pygmy marmoset	Common marmoset	Squirrel monkey	Macaque monkey	Baboon	Human
Inferior salivatory nucleus	+	+	+	+	+	+	+	+	+	+	+	+	?	+	?	+	+	+
Medullary tegmental field	–	–	–	–	–	–	–	–	–	–	–	–	?	–	?	–	–	–
Catecholaminergic																		
A1	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
A2	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
C1	+	+	+	+	+	+	+	+	+	+	+	+	?	?	+	+	+	+
C2	+	+	+	+	+	+	+	+	+	+	+	+	?	?	+	+	?	+
Area postrema	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
A4	–	–	–	–	–	–	+	+	+	+	+	+	+	?	+	+	+	+
A5	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
A6d diffuse	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
A6c compact	–	–	–	–	–	–	+	+	+	+	+	+	+	?	+	+	+	+
A7sc compact	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
A7d diffuse	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
A8	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
A9pc	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
A9m	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
A9v	–	+/–	+/–	+/–	+/–	+/–	+	+	+	+	+	+	+	?	+	+	+	+
A9l	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
A10	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
A10c	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
A10dc	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
A10d	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
A11	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
A12	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
A13	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+
A14	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	?	+
A15d	–	–	–	–	–	–	+	+	+	+	+	+	+	?	+	+	?	+
A15v	-	+	+	–	–	+	+	+	+	+	+	+	+	?	+	+	?	+
A16	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	?	?
Serotonergic																		
CLi	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	?	+
B9	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	?	+
MnR	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	?	+
DRl	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	?	+
DRv	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	?	+
DRd	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	?	+
DRif	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	?	+
DRp	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	?	+
DRc	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	?	+
RMg	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	?	+
RPa	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	?	+
RVL	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	?	+
CVL	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	?	+
ROb	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	?	+
Orexinergic																		
Main cluster	?	+	+	+	+	+	+	+	+	+	+	+	?	?	?	?	?	+
Zona incerta cluster	?	+	+	+	+	+	+	+	+	+	+	+	?	?	?	?	?	+
Optic tract cluster	?	–	–	–	–	–	+	+	+	+	+	+	?	?	?	?	?	+

(Dell et al., 2010; Calvey et al., 2013); however, one feature of the catecholaminergic system, the presence of the locus coeruleus compact division (A6c) specifically aligns the primates with the megachiropterans to the exclusion of all other mammals, including the microchiropterans (Maseko and Manger, 2007; Kruger et al., 2010a). Thus, in primates and megachiropterans, the locus coeruleus has a distinct core (which we term A6c) region of densely packed tyrosine hyodroxylase immunoreactive neurons, surrounded by a shell of less densely packed neurons (which we term A6d). In most other mammals, the core region (A6c) is absent, and the neurons of the locus coeruleus are less densely packed (e.g. Dell et al., 2010; Calvey et al., 2013). The one group of possible exceptions to this distinguishing and aligning feature of primates and megachiropterans are the murid rodents. In murid rodents, the A6 region is seen as a very densely packed core of tyrosine hydroxylase immunoreactive neurons (Dahlström and Fuxe, 1964; Kruger et al., 2012); however, in all other rodents previously studied, including bathyergid mole rats, the greater cane rat, the African porcupine and the Highveld gerbil (Da Silva et al., 2006; Moon et al., 2007; Dwarika et al., 2008; Bhagwandin et al., 2008; Limacher et al., 2008), the A6 region presents as a more loosely packed cluster of tyrosine immunoreactive neurons and has been classified as the diffuse portion of the locus coeruleus complex (A6d). It is therefore likely that the compact appearance of the locus coeruleus in murid rodents is not a feature shared with primates and megachiropterans (and therefore lost in all other rodent species) and is an apomorphy specific to the murid rodents (Kruger et al., 2012). The cholinergic cortical neurons are a second murid rodent specific feature of the systems investigated (Bhagwandin et al., 2006; Kruger et al., 2012). In this sense, the compact locus coeruleus and the cortical cholinergic interneurons are likely to have evolved specifically within the murid rodent lineage, and do not affect the concluded alignment of the megachiropterans with the primates to the exclusion of all other mammals. The appearance of the compact portion of the locus coeruleus is a specific character creating the exclusive megachiropteran–primate phylogenetic link, although it might be argued that this character could have evolved in parallel in both lineages. Studies of other Euarchontoglires, such as hares and rabbits, tree shrews and the colugos (or flying lemurs) will determine whether this feature evolved independently in the megachiropteran and primate lineages, or whether it was inherited from a common megachiropteran–primate ancestor. The locus coeruleus is the main site for noradrenalin production in the brain and is involved in alterness/arousal as well as in the optimization of task performance and consolidation of long-term memories (Smeets and González, 2000; Aston-Jones and Cohen, 2005). To our knowledge, there are no specific behavioural or neural processing differences in the megachiropterans and primates compared to other mammals that would explain the presence of this exceptional arrangement of the locus coeruleus, but future studies may reveal differences related to this neural specialization. In addition to the presence of the A6c in the megachiropterans and primates, the absence of the A4 nucleus, the incipient nature of the A9v and the absence of the A15d nucleus in microchiropterans (Maseko and Manger, 2007; Kruger et al., 2010a), serve to distinguish the microchiropterans from both the megachiropterans and primates, and aligns them with the Soricidae. Thus, the variance in the nuclear complement of the catecholaminergic system strongly supports the hypothesis of chiropteran diphyly, and moreover, supports both the megachiropteran–primate and the microchiropteran-Soricidae phylogenetic relationships.

4.3. Serotonergic and orexinergic nuclei

Previous studies of the nuclear organization of the serotonergic system across mammals have shown that the organization of this system is very conservative from an evolutionary viewpoint, with all Eutherian mammals studied to date showing a similar nuclear complement (Dell et al., 2010). Variances in the nuclear complement of the serotonergic system have been observed in monotremes and marsupials, with the presence of hypothalamic serotonergic neurons, the lack of the caudal division of the dorsal raphe nuclear complex in the monotremes (Manger et al., 2002c), and the absence of the caudal ventrolateral serotonergic group in marsupials (Patzke et al., 2014). Although the three strepsirrhines possessed all serotonergic nuclei common to primates, and indeed all Eutherian mammals studied to date (Dell et al., 2010; Maseko et al., 2013; Calvey et al., 2013), the presence of a relatively large and clearly expressed lateral division of the dorsal raphe, a feature common to previously studied haplorhines as well as megachiropterans (Dell et al., 2010) may have some functional effect on the inhibition of REM sleep (Monti, 2011). Thus, the qualitative enlargement of a single nucleus of the serotonergic system appears to align the megachiropterans with the primates. This is a tenuous link that needs to be investigated in more detail, perhaps using quantitative stereological and allometric approaches.

The orexinergic system also appears to show a conservative organization across the mammalian species studied to date, and the organization of the orexinergic clusters in the three strepsirrhine species studied herein appear to follow the organization observed in most Eutherian mammals studied to date (Dell et al., 2013; Calvey et al., 2013). The one main exception of relevance to the problem of chiropteran phylogeny is the absence of the optic tract cluster of orexinergic neurons in the microchiropterans (Kruger et al., 2010b). This feature distinguishes the microchiropterans from all other mammals, including megachiropterans (Dell et al., 2013) and supports the hypothesis of chiropteran diphyly. It is presently unclear what specific functional implications the lack of these orexinergic neurons may have on the microchiropterans.

4.4. Primate and megachiropteran phylogenetic affinities

The present study clearly demonstrates that the organization, number and complement of nuclei belonging to the cholinergic, catecholaminergic, serotonergic and orexinergic systems in strepsirrhine primates is identical to that observed in the haplorhines, including humans. Thus, as proposed previously (Manger, 2005), there is a distinct primate order organization of the nuclei belonging these systems. While for the most part these nuclei are commonly found across many mammals (Dell et al., 2010; Calvey et al., 2013), the only species that share with primates the same full complement of nuclei are the megachiropterans. Of the 73 neural characters identified in strepsirrhine and haplorhine primates and in megachiropterans, only one specific feature links these species to the exclusion of all other mammals (although 5 characters distinguish the microchiropterans from the megachiropterans, see Table 1). When this neural characteristic (the presence of a compact portion of the locus coeruleus complex, A6c) is added to the extensive suite of neural features listed by Pettigrew et al. (1989) that links the megachiropterans to primates to the exclusion of the microchiropterans, as well as to the non-neural features supporting the megachiropteran–primate phylogenetic link, the data in favour of chiropteran diphyly and the relationship between megachiropterans and primates becomes well supported. There is also a sense in which the present data linking megachiropterans to primates are complementary, rather than mere additions. Martin (1986) dismissed this phylogenetic link by proposing convergent evolution of the midbrain visual trait, with independent evolution of this trait in both primates and megachiropterans under selection for visually directed behaviour in the "fine branch niche". Whatever one thinks about the

possibility of a megachiropteran manipulating such a niche, the present findings of completely non-visual features that link megachiropterans to primates refutes Martin's proposal of convergent evolution and broadens the conceptual basis of the "flying primate" hypothesis. While clearly more work is required to substantiate this proposed phylogenetic assignation, when considering the data that have been generated so far, the megachiropteran–primate link appears to be more parsimonious than chiropteran monophyly.

4.5. Why is chiropteran phylogeny of importance?

The extensive similarities of the neuromodulatory systems of megachiropterans and primates may be of importance for the translation of studies of animal models to the study of human mental function and dysfunction and human health in general. As a specific example, the locus coeruleus complex is of interest. Murid rodents, the most commonly used mammalian animal model (Manger et al., 2008), have what is likely to be an independently evolved structure of the locus coeruleus, while that of the megachiropterans and primates is more likely to be the result of a shared ancestry. The locus coeruleus complex is involved in many important neural functions, and the possibility that the compact and diffuse divisions of this complex are features specific and unique to the megachiropterans and primates, to the exclusion of rodents and all other mammals, cannot be ignored. The locus coeruleus optimizes task performance by incorporating cortical mechanisms involved in the evaluation of costs and benefits associated with task performance (Aston-Jones and Cohen, 2005). It also plays a very important role in mediating the sleep/wake cycle and may in fact be a primary factor that differentiates REM sleep from wakefulness (Aston-Jones and Cohen, 2005). This has important implications for human mental health, considering the well-known link between sleep disturbances and psychiatric disorders. For example, 80–90% of people suffering from depression have impaired sleep quality with insomnia increasing the risk for depression, anxiety and substance abuse (Smith and Aston-Jones, 2008). The severity of sleep disturbances correlates with the severity of the psychiatric disorder (Smith and Aston-Jones, 2008). In depression and mania, there is a total increase in the percentage of REM sleep as a function of total sleep time, and it is suggested that REM sleep is disinhibited in depression, narcolepsy and schizophrenia (Smith and Aston-Jones, 2008). The locus coeruleus complex plays a major role in mediating sleep cycles (Siegel, 2004). It is therefore likely that a megachiropteran would provide a more realistic animal model for the translation of relevant psychiatric testing to human when compared to the popular rat model. In addition, there is quite a gap between the visual organization of the primate brain and that of all other mammals, except megachiropterans. Understanding that phylogenetic gap, which includes complex thalamic lamination and new cortical areas, as well as the hemidecussated retinotectal pathway, requires a closer intermediate than a rodent, tree shrew or carnivore, a role that could be fulfilled by a megachiropteran sister to primates. In addition, both megachiropteran and microchiropteran bats can carry, with apparent impunity, a large number of viruses that are lethal to humans, such as Ebola, Hendra, Nipah and severe acute respiratory syndrome (SARS). Both kinds of chiropterans appear to have independently evolved more elaborate and more complex immune systems to deal with the increased cellular damage brought about by the load of reactive oxygen species that accompanies powered flight (Zhang et al., 2013). A consequence of the increased immunity may be impunity to many viruses. These observations are relevant to the conclusion that the two kinds of bats have separate, independent lineages, as the cellular and molecular mechanisms that provide the increased power and scope of their immune systems are also different in the way that they produce this outcome in the two kinds of bats (Zhang et al., 2013). Understanding how the chiropteran immune systems enable them to evade these viruses therefore involves an understanding of how the different megachiropteran and microchiropteran immune systems have each evolved to produce an independent but more powerful outcome in each case. While at this stage, the applicability of the megachiropterans as improved animal models of specific human mental functions and dysfunctions and general human health over murid rodents is still speculative, the data being generated in this and other comparative neurological studies, indicate that the megachiropterans might be a fruitful animal model to be employed in future studies of human mental dysfunction, general health and primate brain evolution. For these, and many other reasons, understanding chiropteran phylogeny is of more than just scientific interest.

Acknowledgments

This work was mainly supported by funding from the South African National Research Foundation (P.R.M.), by a fellowship within the Postdoctoral-Program of the German Academic Exchange Service, DAAD (N.P.), the Belgian co-operation service (DGD) at the Royal Museum for Central Africa (EG).

References

Arnason, U., Adegoke, J.A., Bodin, K., Born, E.W., Esa, Y.B., Gullberg, A., Nilsson, M., Short, R.V., Xu, X., Janke, A., 2002. Mammalian mitogenomic relationships and the root of the Eutherian tree. Proc. Natl. Acad. Sci. U.S.A. 99, 8151–8156.

Aston-Jones, G., Cohen, J.D., 2005. An integrative theory of locus coeruleus–norepinephrine function: adaptive gain and optimal performance. Annu. Rev. Neurosci. 28, 403–450.

Bhagwandin, A., Fuxe, K., Manger, P.R., 2006. Choline acetyltransferase immunoreactive cortical interneurons do not occur in all rodents: a study of the phylogenetic occurrence of this neural characteristic. J. Chem. Neuroanat. 32, 208–216.

Bhagwandin, A., Fuxe, K., Bennett, N.C., Manger, P.R., 2008. Nuclear organization and morphology of cholinergic, putative catecholaminergic and serotonergic neurons in the brains of two species of African mole-rat. J. Chem. Neuroanat. 35, 371–387.

Bhagwandin, A., Fuxe, K., Bennett, N.C., Manger, P.R., 2011. Distribution of orexinergic neurons and their terminal networks in the brains of two species of African mole rats. J. Chem. Neuroanat. 41, 32–42.

Bloch, J.I., Fisher, D.C., Gingerich, P.D., Gunnell, G.F., Simons, E.L., Uhen, M.D., 1997. Cladistic analysis and anthropoid origins. Science 278, 2134–2135.

Calvey, T., Patzke, N., Kaswera, C., Gilissen, E., Bennett, N.C., Manger, P.R., 2013. Nuclear organisation of some immunohistochemically identifiable neural systems in three Afrotherian species—*Potomogale velox*, *Amblysomus hottentotus* and *Petrodromus tetradactylus*. J. Chem. Neuroanat. 50, 48–65.

Dahlström, A., Fuxe, K., 1964. Evidence for the existence of monoamine-containing neurons in the central nervous system I. Demonstration of monoamine in the cell bodies of brainstem neurons. Acta Physiol. Scand. 62, 1–52.

Da Silva, J.N., Fuxe, K., Manger, P.R., 2006. Nuclear parcellation of certain immunohistochemically identifiable neuronal systems in the midbrain and pons of the Highveld molerat (*Cryptomys hottentotus*). J. Chem. Neuroanat. 31, 37–50.

Dell, L.-A., Kruger, J.-L., Bhagwandin, A., Jillani, N.E., Pettigrew, J.D., Manger, P.R., 2010. Nuclear organization of cholinergic, putative catecholaminergic and serotonergic systems in the brains of two megachiropteran species. J. Chem. Neuroanat. 40, 177–195.

Dell, L.A., Kruger, J.L., Pettigrew, J.D., Manger, P.R., 2013. Cellular location and major terminal networks of the orexinergic system in the brain of two megachiropterans. J. Chem. Neuroanat. 53, 64–71.

Dwarika, S., Maseko, B.C., Ihunwo, A.O., Fuxe, K., Manger, P.R., 2008. Distribution and morphology of putative catecholaminergic and serotonergic neurons in the brain of the greater canerat, *Thryonomys swinderianus*. J. Chem. Neuroanat. 35, 108–122.

Gallyas, F., 1979. Silver staining of myelin by means of physical development. Neurol. Res. 1, 203–209.

Gravett, N., Bhagwandin, A., Fuxe, K., Manger, P.R., 2009. Nuclear organization and morphology of cholinergic, putative catecholaminergic and serotonergic neurons in the brain of the rock hyrax, *Procavia capensis*. J. Chem. Neuroanat. 38, 57–74.

Gravett, N., Bhagwandin, A., Fuxe, K., Manger, P.R., 2011. Distribution of orexin-A immunoreactive neurons and their terminal networks in the brain of the rock hyrax, *Procavia capensis*. J. Chem. Neuroanat. 41, 86–96.

Hökfelt, T., Martenson, R., Björklund, A., Kleinau, S., Goldstein, M., 1984. Distributional maps of tyrosine-hydroxylase-immunoreactive neurons in the rat brain. In: Björklund, A., Hökfelt, T. (Eds.), Handbook of Chemical Neuroanatomy, Classical Neurotransmitters in the CNS, Part 1, vol. 2. Elsevier, Amsterdam, pp. 277–379.

Hutcheon, J.M., Kirsch, J.A.W., Pettigrew, J.D., 1998. Base-compositional biases and the bat problem, III. The question of microchiropteran monophyly. Philos. Trans. R. Soc. B: Biol. Sci. 353, 607–617.

Jabbari, K., Bernadri, G., 2004. Body temperature and evolutionary genomics of vertebrates: a lesson from the genomes of *Takifgu rubripes* and *Tetraodon nigroviridis*. Gene 333, 179–181.

Jameson, N.M., Hou, Z.-C., Sterner, K.N., Weckle, A., Goodman, M., Steiper, M.E., Wildman, D.E., 2011. Genomic data reject the hypothesis of a prosimian primate clade. J. Hum. Evol. 61, 295–305.

Kay, R.F., Ross, C., Williams, B.A., 1997a. Anthropoid origins. Science 275, 797–804.

Kay, R.F., Ross, C., Williams, B.A., Johnson, D., 1997b. Cladistic analysis and anthropoid origins. Response Sci. 278, 2135–2136.

Kirsh, J.A.W., Pettigrew, J.D., 1998. Base-compositional biases and the bat problem II. DNA-hybridization trees based on AT- and GC-enriched tracers. Philos. Trans. R. Soc. B: Biol. Sci. 353, 381–388.

Kruger, J.L., Dell, L.A., Bhagwandin, A., Jillani, N.E., Pettigrew, J.D., Manger, P.R., 2010a. Nuclear organization of cholinergic, putative catecholaminergic and serotonergic systems in the brains of five microchiropteran species. J. Chem. Neuroanat. 40, 210–222.

Kruger, J.-L., Dell, L.-A., Pettigrew, J.D., Manger, P.R., 2010b. Cellular location and major terminal networks of the orexinergic system in the brains of five microchiropteran species. J. Chem. Neuroanat. 40, 256–262.

Kruger, J.-L., Patzke, N., Fuxe, K., Bennett, N.C., Manger, P.R., 2012. Nuclear organization of cholinergic, putative catecholaminergic, serotonergic and orexinergic systems in the brain of the African pygmy mouse (*Mus minutoides*): organizational complexity is preserved in small brains. J. Chem. Neuroanat. 44, 45–56.

Limacher, A.M., Bhagwandin, A., Fuxe, K., Manger, P.R., 2008. Nuclear organization and morphology of cholinergic, putative catecholaminergic and serotonergic neurons in the brain of the Cape porcupine (*Hystrix africaeaustralis*): Increased brain size does not lead to increased organizational complexity. J. Chem. Neuroanat. 36, 33–52.

Manger, P.R., 2005. Establishing order at the systems level in mammalian brain evolution. Brain Res. Bull. 66, 282–289.

Manger, P.R., Rosa, M.G., Collins, R., 2001. An architectonic comparison of the ventrobasal complex of two Megachiropteran and one Microchiropteran bat: implications for the evolution of *Chiroptera*. Somatosens Mot. Res. 18, 131–140.

Manger, P.R., Fahringer, H.M., Pettigrew, J.D., Siegel, J.M., 2002c. Distribution and morphology of serotonergic neurons in the brain of the monotremes. Brain Behav. Evol. 60, 315–332.

Manger, P.R., Cort, J., Ebrahim, N., Goodman, A., Henning, J., Karolia, M., Rodrigues, S., Strkalj, G., 2008. Is 21st century neuroscience too focused on the rat/mouse model of brain function and dysfunction? Front. Neuroanat. 2, 5.

Martin, R.D., 1986. Are fruit bats primates? Nature 320, 482–483.

Maseko, B.C., Manger, P.R., 2007. Distribution and morphology of cholinergic, catecholaminergic and serotonergic neurons in the brain of Schreiber's long-fingered bat, *Miniopterus schreibersii*. J. Chem. Neuroanat. 34, 80–94.

Maseko, B.C., Bourne, J.A., Manger, P.R., 2007. Distribution and morphology of cholinergic, putative catecholaminergic and serotonergic neurons in the brain of the Egyptian Rousette flying fox, *Rousettus aegyptiacus*. J. Chem. Neuroanat. 34, 108–127.

Maseko, B.C., Patzke, N., Fuxe, K., Manger, P.R., 2013. Architectural organization of the African elephant diencephalon and brainstem. Brain Behav. Evol. 82, 83–128.

Matsui, A., Rakotondraparany, F., Munechika, I., Hasegawa, M., Horais, S., 2009. Molecular phylogeny and evolution of prosimians based on complete sequences of mitochondrial DNAs. Gene 441, 53–66.

Meredith, R.W., Janecka, J.E., Gatesy, J., Ryder, O.A., Fisher, C.A., Teeling, E.C., Goodbla, A., Eizirik, E., Simao, T.L., Stadler, T., Rabosky, D.L., Honeycutt, R.L., Flynn, J.J., Ingram, C.M., Steiner, C., Williams, T.L., Robinson, T.J., Burk-Herrick, A., Westerman, M., Ayoub, N.A., Springer, M.A., Murphy, W.J., 2011. Impacts of the Cretaceous terrestrial revolution and KPg extinction on mammal diversification. Science 334, 521–524.

Monti, J.M., 2011. Serotonin control of sleep-wake behaviour. Sleep Med. Rev. 15, 269–281.

Moon, D.J., Maseko, B.C., Ihunwo, A., Fuxe, K., Manger, P.R., 2007. Distribution and morphology of catecholaminergic and serotonergic neurons in the brain of the highveld gerbil, *Tatera brantsii*. J. Chem. Neuroanat. 34, 134–144.

Ni, X., Gebo, D.L., Dagosto, M., Meng, J., Tafforeau, P., Flynn, J.J., Beard, F.C., 2013. The oldest known primate skeleton and early haplorhine evolution. Nature 498, 60–64.

Olson, L., Fuxe, K., 1972. Further mapping out of central noradrenaline neuron systems: projections of the "subcoeruleus" area. Brain Res. 43, 289–295.

Patzke, N., Bertelsen, M.F., Fuxe, K., Manger, P.R., 2014. Nuclear organization of cholinergic, catecholaminergic, sertonergic and orexinergic systems in the brain of the Tasmanian devil (*Sarcophilus harrisii*). J. Chem. Neuroanat. 61–62, 94–106.

Pettigrew, J.D., 1986. Flying primates? Megabats have the advanced pathway from eye to midbrain. Science 231, 1304–1306.

Pettigrew, J.D., Kirsch, J.A.W., 1995. Flying primates revisited—DNA hybridization with fractionated, GC-enriched DNA. S. Afr. J. Sci. 91, 477–482.

Pettigrew, J.D., Kirsch, J.A.W., 1998. Base-compositional biases and the bat problem I. DNA-hybridization melting curves based on AT- and GC-enriched tracers. Philos. Trans. R. Soc. B: Biol. Sci. 353, 369–379.

Pettigrew, J.D., Jamieson, B.G.M., Robson, S.K., Hall, L.S., McNally, K.I., Cooper, H.M., 1989. Phylogenetic relations between microbats, megabats and primates (Mammalia: Chiroptera and Primates). Philos. Trans. R. Soc. Lond. 325, 489–559.

Pettigrew, J.D., Maseko, B.C., Manger, P.R., 2008. Primate-like retinotectal decussation in an echolocating megabat, *Rousettus aegyptiacus*. Neuroscience 153, 226–231.

Pieters, R.P., Gravett, N., Fuxe, K., Manger, P.R., 2010. Nuclear organization of cholinergic, putative catecholaminergic and serotonergic nuclei in the brain of the eastern rock elephant shrew, *Elephantulus myurus*. J. Chem. Neuroanat. 39, 175–188.

Rosa, M.P.G., Pettigrew, J.D., Cooper, H.M., 1996. Unusual pattern of retinogeniculate projections in the controversial primate, *Tarsius*. Brain Behav. Evol. 48, 121–129.

Siegel, J.M., 2004. The neurotransmitters of sleep. J. Clin. Psychiatry 65, 4–7.

Smeets, W.J.A.J., González, A., 2000. Catecholamine systems in the brain of vertebrates: new perspectives through a comparative approach. Brain Res. Rev. 33, 308–379.

Smith, R.J., Aston-Jones, G., 2008. Noradrenergic transmission in the extended amygdala: role in increased drug-seeking and relapse during protracted drug abstinence. Brain Struct. Funct. 213, 43–61.

Steinbusch, H.W.M., 1981. Distribution of serotonin-immunoreactivity in the central nervous system of the rat—cell bodies and terminals. Neuroscience 6, 557–618.

Törk, I., 1990. Anatomy of the serotonergic system. Ann. N.Y. Acad. Sci. 600, 9–35.

Woolf, N.J., 1991. Cholinergic systems in mammalian brain and spinal cord. Prog. Neurobiol. 37, 475–524.

Zhang, G., Cowled, C., Shi, Z., Huang, Z., Bishop-Lilly, K.A., Fang, X., Wynne, J.W., Xiong, Z., Baker, M.L., Zhao, W., Tachedjian, M., Zhu, Y., Zhou, P., Jiang, X., Ng, J., Yang, L., Wu, L., Xiao, J., Feng, Y., Chen, Y., Sun, X., Zhang, Y., Marsh, G.A., Crameri, G., Broder, C.C., Frey, K.G., Wang, L.F., Wang, J., 2013. Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. Science 339, 456–460.