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Nuclear organisation of some immunohistochemically identifiable neural systems in three Afrotherian species—*Potomogale velox*, *Amblysomus hottentotus* and *Petrodromus tetradactylus*

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ABSTRACT

The present study describes the organisation of the cholinergic, catecholaminergic, and serotonergic neurons in the brains of the giant otter shrew, the Hottentot golden mole and the four-toed sengi, and the orexinergic (hypocretinergic) system in the giant otter shrew and four-toed sengi. The aim of the present study was to investigate the possible differences in the nuclear complement of these neural systems in comparison to previous studies on other Afrotherian species and mammalian species in general. Brains of the golden mole, sengi and giant otter shrew were coronally sectioned and immunohistochemically stained with antibodies against cholineacetyl-transferase, tyrosine hydroxylase, serotonin and orexin-A.

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; 3V, third ventricle; 4V, fourth ventricle; 5n, trigeminal nerve; 7n, facial nerve; 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; ACN, cholinergic neurons of the amygdala; Amyg, amygdala; AON, anterior olfactory nucleus; AP, area postrema; B9, supralemniscal serotonergic nucleus; bic, brachium of the inferior colliculus; C1, rostral ventrolateral medullary tegmental group; C2, rostral dorsomedial medullary nucleus; C3, rostral dorsal midline medullary nucleus; C, caudate nucleus; ca, cerebral aqueduct; Cb, cerebellum; cc, corpus callosum; CCN, cholinergic neurons of the cerebral cortex; Cl, claustrum; CLi, caudal linear nucleus; CN, deep cerebellar nucleus; Co, cochlear nuclei; CoCN, cholinergic neurons of the cochlear nuclear complex; csc, commissure of the superior colliculi; CVL, caudal ventrolateral serotonergic group; dh, dorsal horn of spinal cord; df, dorsal funiculus of spinal cord; 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; EPL, external plexiform layer of olfactory bulb; EW, Edinger–Westphal nucleus; f, fornix; fr, fasciculus retroflexus; GC, central grey matter; GCLi, inner granular cell layer of olfactory bulb; GCLo, outer granular cell layer of olfactory bulb; GL, glomerular layer of olfactory bulb; GP, globus pallidus; Hbc, habenular commissure; Hbl, lateral habenular nucleus; Hbm, medial habenular nucleus; hc, hippocampal commissure; HCN, cholinergic neurons of the hippocampus; 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; ICCN, cholinergic neurons of the inferior colliculus; io, inferior olivary nucleus; IP, interpeduncular nucleus; Is.Call/TOL, islands of Calleja and the olfactory tubercle; LDT, laterodorsal tegmental nucleus; LGv, ventral lateral geniculate nucleus; LOT, lateral olfactory tract; LV, lateral ventricle; MB, mammillary bodies; Mc, main cluster of orexinergic neurons; MCL, mitral cell layer of olfactory bulb; mcp, middle cerebellar peduncle; MnR, median raphe nucleus; mlf, medial longitudinal fasciculus; mtf, medullary tegmental field; N.Acc, nucleus accumbens; N.Amb, nucleus ambiguus; N.Bas, nucleus basalis; NEO, cerebral neocortex; OB, olfactory bulb; OC, optic chiasm; OCN, cholinergic neurons of olfactory bulb; ONL, olfactory nerve layer of olfactory bulb; OT, optic tract; OTc, optic tract cluster of orexinergic neurons; OV, olfactory ventricle; P, putamen nucleus; PBg, parabigeminal nucleus; PC, cerebral peduncle; pc, posterior commissure; PCCN, cholinergic neurons of the piriform cortex; PIR, piriform cortex; PPT, pedunculopontine nucleus; PTa, pretectal area; py, pyramidal tract; PVG, periventricular grey matter; PVL, periventricular layer of olfactory bulb; pVII, preganglionic motor neurons of the superior salivatory nucleus or facial nerve; pIX, preganglionic motor neurons of the inferior salivatory nucleus; R, reticular nucleus of the dorsal thalamus; Rmc, magnocellular division of red nucleus; RMg, raphe magnus nucleus; ROb, raphe obscurus nucleus; RPa, raphe pallidus nucleus; RVL, rostral ventrolateral serotonergic group; S, septal nuclear complex; SC, superior colliculus; SCCN, cholinergic neurons of the superior colliculus; scp, superior cerebellar peduncle; Sep.L, lateral septal nucleus; Sep.M, medial septal nucleus; so, superior olivary nucleus; spV, spinal trigeminal tract; vh, ventral horn of spinal cord; VPO, ventral pontine nucleus; xscp, decussation of the superior cerebellar peduncle; zi, zona incerta; Zic, zona incerta cluster of orexinergic neurons.

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The majority of nuclei revealed in the current study were similar among the species investigated, to other Afrotherian species, and to mammals generally, but certain differences in the nuclear complement highlighted phylogenetic interrelationships. The golden mole was observed to have cholinergic interneurons in the cerebral cortex, hippocampus, olfactory bulb and amygdala. The four-toed sengi had cholinergic neurons in both colliculi and in the cochlear nucleus, but lacked the catecholaminergic A15d group in the hypothalamus. In both the golden mole and the four-toed sengi, the locus coeruleus (A6d group) was made up of few neurons. The golden mole also exhibited an unusual foreshortening of the brain, such that a major (mesencephalic?) flexure in the brainstem was evident.

1. Introduction

The Afrotherian mammalian cohort comprise what may be considered to be a very unusual mammalian grouping, one whose relationships have mainly been resolved on molecular rather than morphological grounds (e.g., van Dijk et al., 2001; Arnason et al., 2008; Hallström and Janke, 2008; Prasad et al., 2008; Asher et al., 2010; Dumbacher et al., 2012; McCormack et al., 2012). Within the Afrotheria are species that are fully aquatic (such as manatees and dugongs), extremely large (elephants), fossorial (golden moles), semi-aquatic (otter shrews), insectivorous (aardvarks, elephants shrews and tenrecs) and omnivorous (hyraxes). These species present with a range of body sizes, phenotypes, habitats and life histories, and are considered an ancient radiation of the Eutherian mammals. In this sense, the study of neural systems that are in general quite conservative in their evolution (e.g., Dell et al., 2010) is of interest, as a record of the changes, or indeed lack of changes, may reflect the potential evolutionary plasticity/malleability of the mammalian brain.

The cholinergic, catecholaminergic and serotonergic systems have been studied previously in two species belonging to the Afrotheria – the rock hyrax, *Procavia capensis* (Gravett et al., 2009) and the eastern rock elephant shrew, *Elephantulus myurus* (Pieters et al., 2010). While for the most part, these systems were similar to those reported in other mammals, several specific differences were noted. In the rock hyrax, the anterior nuclei of the dorsal thalamus were found to contain cholinergic neurons, there were cholinergic parvocellular neurons forming a shell around the typical laterodorsal tegmental and penduclopontine tegmental nuclei, and the locus coeruleus proper was observed to be made up of very few cells (Gravett et al., 2009). In contrast to the rock hyrax, cholinergic neurons were observed in both superior and inferior colliculi and the cochlear nuclei, and the catecholaminergic anterior hypothalamic group (A15d) was missing from the elephant shrew (Pieters et al., 2010). Observations of the orexinergic neurons in the rock hyrax showed a typically mammalian orexinergic system in this species apart from a dense innervation of the anterior nuclei of the dorsal thalamus (Gravett et al., 2011).

In the present study we extend the observations made on these systems in the Afrotheria by examining, using immunohistochemical means, the cholinergic, catecholaminergic and serotonergic in the brains of the giant otter shrew (*Potomogale velox*), the Hottentot golden mole (*Amblysomus hottentotus*) and the four-toed sengi (*Petrodromus tetradactylus*) and the orexinergic system in the brains of the giant otter shrew and four-toed sengi. The giant otter shrew is a semi-aquatic member of the Tenrecidae that is found in the swamps, streams and forest pools of central Africa. The Hottentot golden mole is a small fossorial golden mole found in the KwaZulu-Natal and Eastern Cape region of South Africa and inhabits a range of ecosystems from temperate to tropical dry forests, dry savanna, through to urban areas and introduced vegetation. The four-toed sengi is a large elephant shrew found in tropical to subtropical moist montane forests as well as moist savanna throughout large areas of sub-Saharan Africa.

As far as the authors are aware, the systems described in the current study have not been examined in any of these three species. In addition to the prior studies of Afrotherian species, the data from the current study provides a broader base for comparison within the Afrotheria and across mammals in general. This may help to elucidate the manner in which systems level changes occur across mammalian brains as features such as brain size, phenotype, life histories, phylogenetic relationships, and time since evolutionary divergence, change, and lead to a better understanding of the partition between the phylogenetic and functional signals they may carry (Manger, 2005).

2. Materials and methods

Brains from *P. velox*, *A. hottentotus* and *P. tetradactylus* were collected for the present study. Permits were obtained from the relevant wildlife authorities in the Democratic Republic of Congo and South Africa for the capture and euthanasia of the animals from their natural habitat. All animals were handled according to the guidelines of the University of the Witwatersrand Animal Ethics Committee. Each animal was weighed, anaesthetised and subsequently euthanized with weight appropriate doses of sodium pentobarbital (200 mg sodium pentobarbital/kg, i.p.). In the current study a single *P. velox* (body mass – 540 g; brain mass – 3.46 g; an adult male), two *A. hottentotus* (body masses – 72 g, 86 g; brain masses – 1.3 g, 1.2 g; both adult males), and three *P. tetradactylus* (body masses – 132 g, 138 g, 124 g; brain masses – 3.01 g, 2.80 g, 2.95 g; all adult males) were used. 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. A one in six series of sections, cut at 50 μm thickness in the coronal plane, was used for Nissl, myelin, choline acyltransferase (ChAT), tyrosine hydroxylase (TH), serotonin (5HT) and orexin (hypocretin/OxA). An additional golden mole brain was sectioned in the sagittal plane to demonstrate the flexure of the brainstem in this species (Fig. 1). Sections used for the Nissl series were

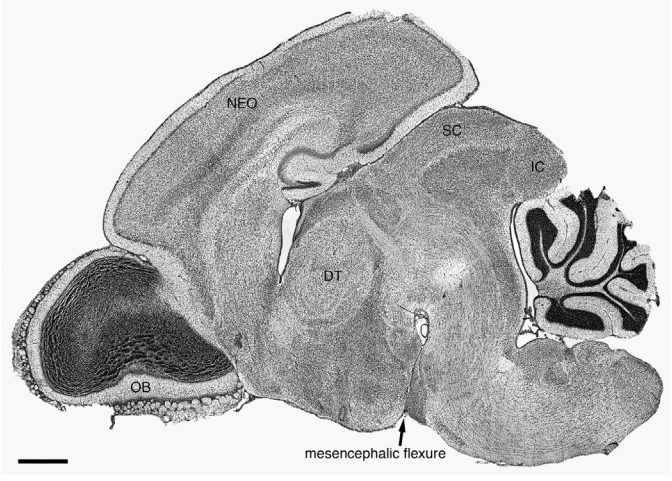

Fig. 1. Photomontage of a Nissl-stained parasagittal section of the brain of the Hottentot golden mole showing the unusual appearance of the diencephalon and brainstem. It appears that a foreshortening and widening of the cranium is related to the large (mesencephalic?) flexure of the subtelencephalic brain, resulting in the pons lying beneath the midbrain, and the inferior colliculus lying above the cerebellum. Scale bar = 1 mm. See list for abbreviations.