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Neurochemical organization and morphology of the sleep related nuclei in the brain of the Arabian oryx, *Oryx leucoryx*

Joshua G. Davimes[a], Abdulaziz N. Alagaili[b], Nigel C. Bennett[c], Osama B. Mohammed[b], Adhil Bhagwandin[a], Paul R. Manger[a], Nadine Gravett[a,*]

[a] School of Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown, 2193, Johannesburg, South Africa
[b] KSU Mammals Research Chair, Department of Zoology, King Saud University, Riyadh 11451, Saudi Arabia
[c] SARChI Chair for Mammalian Behavioural Ecology and Physiology, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa

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ABSTRACT

The Arabian oryx, *Oryx leucoryx*, is a member of the superorder Cetartiodactyla and is native to the Arabian Desert. The desert environment can be considered extreme in which to sleep, as the ranges of temperatures experienced are beyond what most mammals encounter. The current study describes the nuclear organization and neuronal morphology of the systems that have been implicated in sleep control in other mammals for the Arabian oryx. The nuclei delineated include those revealed immunohistochemically as belonging to the cholinergic, catecholaminergic, serotonergic and orexinergic systems within the basal forebrain, hypothalamus, midbrain and pons. In addition, we examined the GABAergic neurons and their terminal networks surrounding or within these nuclei. The majority of the neuronal systems examined followed the typical mammalian organizational plan, but some differences were observed: (1) the neuronal morphology of the cholinergic laterodorsal tegmental (LDT) and pedunculopontine tegmental (PPT) nuclei, as well as the parvocellular subdivision of the orexinergic main cluster, exhibited Cetartiodactyl-specific features; (2) the dorsal division of the catecholaminergic anterior hypothalamic group (A15d), which has not been reported in any member of the Artiodactyla studied to date, was present in the brain of the Arabian oryx; and (3) the catecholaminergic tuberal cell group (A12) was notably more expansive than previously seen in any other mammal. The A12 nucleus has been associated functionally to osmoregulation in other mammals, and thus its expansion could potentially be a species specific feature of the Arabian oryx given their native desert environment and the need for extreme water conservation.

Abbreviations: III, oculomotor nucleus; Vmot, motor nucleus of trigeminal nerve; Vsens, sensory nucleus of trigeminal nerve; 3V, third ventricle; 4V, fourth ventricle; 5n, trigeminal nerve; 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; Arc, hypothalamic arcuate nucleus; B9, supralemniscal serotonergic nucleus; C, caudate nucleus; ca, cerebral aqueduct; CLi, caudal linear nucleus; 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; f, fornix; fr, fasciculus retroflexus; GC, central gray matter; GiCRt, gigantocellular reticular nucleus; GP, globus pallidus; Hyp.d, dorsal hypothalamic cholinergic nucleus; Hyp.l, lateral hypothalamic cholinergic nucleus; Hyp.v, ventral hypothalamic cholinergic nucleus; IC, inferior colliculus; ic, internal capsule; IP, interpeduncular nucleus; Is.Call/TOL, islands of Calleja/olfactory tubercle; LDT, laterodorsal tegmental nucleus; Ll, lateral lemniscus; 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.Bas, nucleus basalis; OT, optic tract; OTc, optic tract cluster of orexinergic neurons; P, putamen nucleus; PBg, parabigeminal nucleus; PC, cerebral peduncle; PCRt, parvicellular reticular nucleus; PIR, piriform cortex; PPT, pedunculopontine tegmental nucleus; Pvc, parvocellular orexinergic cluster; R, thalamic reticular nucleus; Rmg, raphe magnus nucleus; RtTg, reticulotegmental nucleus of the pons; SC, superior colliculus; scp, superior cerebellar peduncle; Sep.L, lateral septal nucleus; Sep.M, medial septal nucleus; SON, supraoptic nucleus; STN, subthalamic nucleus; VPO, ventral pontine nucleus; xscp, decussation of the superior cerebellar peduncle; zi, zona incerta; Zic, zona incerta cluster of orexinergic neurons.

* Corresponding author.
E-mail address: Nadine.Gravett@wits.ac.za (N. Gravett).

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1. Introduction

The Arabian oryx, *Oryx leucoryx*, is the smallest member of the genus *Oryx*, and is grouped within the order Cetartiodactyla, which comprises cetaceans (whales and dolphins) and artiodactyls (even-toed ungulates). Arabian oryx are adaptive heterotherms, allowing them to survive in the harsh climatic conditions of the deserts and steppes of the Arabian Peninsula (Ostrowski et al., 2003). Given the environment occupied by the Arabian oryx under natural conditions, the question naturally arises as to whether there are any special neurological adaptations that have evolved to enable the Arabian oryx to regulate sleep.

The examination of sleep in natural conditions, especially under extreme circumstances has received very little attention in the literature. Understanding the ecology of a species, its sleep physiology and the underlying neuronal architecture regulating such patterns could provide an improved understanding of the evolution of sleep and its functions. For species that reside in desert environments a range of conditions may act as stress factors that affect survival, and may lead to specific adaptations and behaviours. Factors such as food quantity and quality, availability of water and extreme ambient temperatures are aspects of daily life in the desert that may alter adaptive patterns and timing of sleep (Degen et al., 1997; Schmidt-Nielsen, 1997).

The Arabian oryx has been previously examined in terms of its ecology and morphophysiological adaptations to the aforementioned environmental extremes. Adaptations such as lower total evaporative water loss rates, adaptive heterothermy and selective brain cooling are among several physiological responses to high temperatures (Williams et al., 2001; Ostrowski et al., 2003; Hetem et al., 2010, 2012a). Additionally the Arabian oryx has been shown to exhibit seasonal shifts in the timing of daily inactivity patterns, switching between winter diurnality and summer crepuscularity/nocturnality (Seddon and Ismail, 2002; Hetem et al., 2012b; Davimes et al., 2016). Despite these studies, no examination has been conducted on the underlying neuronal architecture that has been implicated in sleep control in other mammals for the Arabian oryx.

The sleep related neuronal systems are comprised of nuclei that extend from the level of the basal forebrain to the pontine region and are comprised of nuclei that are cholinergic, catecholaminergic, serotonergic and orexinergic as well as GABAergic interneurons. The cholinergic nuclei located within the basal forebrain and pons are primarily involved in cortical activation and are mostly active during wakefulness and REM sleep (for reviews, see Harris, 2005; Jones, 2005; Siegel, 2006). The catecholaminergic nuclei are predominantly involved in sleep state homeostasis, wakefulness and REM sleep. The norepinephrine containing neurons of the locus coeruleus (LC) complex discharge most during wakefulness, have reduced discharge rates during non-REM sleep and cease to discharge during REM sleep (Jones, 2005). The midbrain serotonergic nuclei are most active during reduced states of arousal and regulate behaviours such as grooming and muscle tone. This system shows reduced activity during non-REM sleep and the neurons cease to discharge during REM sleep (Jones, 2005). The orexinergic neurons, located within the hypothalamus, play an integral role in the maintenance of arousal through its wide spread projections to the histaminergic, norepinephrine and serotonergic systems (for review, see Siegel, 2004). The GABAergic neurons and terminal networks found throughout and projecting to the aforementioned nuclei are thought to promote non-REM sleep by inhibiting those neurons that are involved in arousal. Certain groups of GABAergic neurons have a maximal discharge rate at the onset of sleep, continue to discharge during non-REM sleep and in some instances also continue to discharge during REM sleep (Siegel, 2004).

The order Artiodactyla is the fifth most speciose mammalian order, with over 220 species (Grzimek, 1990, 2003; Nowak, 1999), but despite this diversity, only a few studies have described certain aspects of the systems that have been implicated in sleep control within this order. The nuclei of the cholinergic, catecholaminergic, serotonergic and orexinergic systems have been described in the giraffe (Bux et al., 2010; Dell et al., 2012), the cholinergic system has been described in the Goettingen miniature pig (Mahady et al., 2016), a partial description of the cholinergic, and full descriptions of the catecholaminergic and serotonergic systems have been provided for sheep (Ferreira et al., 2001; Iqbal et al., 2001), and aspects of the catecholaminergic and orexinergic systems have been described in the cow and pig (Tillet and Thibault, 1989; Østergaard et al., 1992; Kitahama et al., 1994; Tillet, 1994; Leshin et al., 1995a,b, 1996; for review, see Tillet and Kitahama, 1998; Ettrup et al., 2010). While, for the most part, the nuclear organization of these systems can be thought of as typically mammalian, certain features have been identified that could be described as specific features of the Artiodactyl brain. In the giraffe it has been noted that the neurons of the cholinergic laterodorsal tegmental (LDT) nucleus are larger than those of the pedunculopontine tegmental (PPT) nucleus (Bux et al., 2010), whilst the opposite was reported for the Goettingen mini pig (Mahady et al., 2016). The orexinergic neurons of the hypothalamus, while having the three clusters generally observed in mammals, form an additional medially located parvocellular cluster not observed in most other mammals, but is seen in the Cetartiodactyls studied to date, including the harbour porpoise, minke whale and hippopotamus, and also in the Afrotherian African elephant (Dell et al., 2012, 2016a, 2016b, 2016c; Maseko et al., 2013).

While the organization of these nuclei appears to be predictable across species within the same mammalian order (for review, see Manger, 2005), the pressure of surviving within the harsh Arabian desert may have led to the evolution of specific specializations within the brain of the Arabian oryx. Thus, the aim of the current study is twofold: (1) to provide the first complete description of the systems that have been implicated in sleep control in the brain of an Artiodactyl; and (2) to determine whether the harsh desert environment has led to the evolution of any specific neural novelties related to these nuclei in the Arabian oryx.

2. Materials and methods

2.1. Brain acquisition

Two adult female Arabian oryx (average body mass of 65 kg, average brain mass of 166 g, not pregnant, not lactating), from the National Wildlife Research Centre near Taif in the Kingdom of Saudi Arabia, that were part of a planned management cull, were overdosed on the same day using sodium pentobarbital (200 mg/kg i.v.) under permission from the Saudi Wildlife Authority. All animals were handled according to the guidelines of the University of the Witwatersrand Animal Ethics Committee (clearance number 2008/36/1), which parallel those of the National Institute of Health (NIH) for the care and use of animals in scientific experimentation. The brains of these animals were perfusion fixed *via* the internal carotid arteries with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4, 4 °C) (Manger et al., 2009). The brains were then removed from the skulls (Fig. 1) and post-fixed for 48 h in 4% paraformaldehyde in 0.1 M PB at 4 °C. The brains were then transferred to a 30% sucrose in 0.1 M PB solution at 4 °C until equilibrated (approximately 7 days), and then placed in an antifreeze solution for the same length of time. The brains were stored in the antifreeze solution at −20 °C until immunohistochemical processing.