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Research report

Neuroanatomical investigation of the gonadotrophin-releasing hormone 1 system in the seasonally breeding Cape dune mole-rat, *Bathyergus suillus*

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ABSTRACT

The gonadotrophin-releasing hormone 1 (GnRH1) system has been investigated immunohistochemically in Cape dune mole-rats (*Bathyergus suillus*), subterranean rodents that normally display severe aggression towards conspecifics. These animals breed seasonally and show a reduced mean plasma level of luteinising hormone during the non-breeding season. GnRH1-immunoreactive (ir) cell bodies and processes are found in the septal/preoptic area and the mediobasal hypothalamus; the cell bodies are found in equal measure in these two regions. Dense aggregations of GnRH1-ir fibres are present in the organum vasculosum of the lamina terminalis and the external zone of the median eminence. The total number of detectable GnRH1-ir cell bodies does not differ between the sexes or within the sexes between breeding and non-breeding seasons. Similarly there is no difference in the distribution of detectable GnRH1-ir cell bodies in male and female mole-rats in and out of the breeding season. Although the average size of GnRH1-ir cell bodies does not differ between the seasons in males, their size in females is significantly smaller in the non-breeding season. Whether this reduced size reflects reduced GnRH1 synthesis remains to be determined.

1. Introduction

The Cape dune mole-rat, *Bathyergus suillus,* is a subterranean, seasonally breeding rodent that normally displays severe xenophobia towards conspecifics [11]. During the breeding season, males use seismic communication in the form of hind foot drumming to advertise their intention to mate. The female responds to the male by drumming at a different frequency. The male proceeds to burrow towards the female, brief copulation ensues and the male subsequently returns to his own burrow system [2,3]. The breeding period appears to be restricted to May through to September [9,10], a period that coincides with winter rainfall in the Cape Province of South Africa.

In Cape dune mole-rats, mean circulating levels of luteinising hormone (LH) display significant seasonal differences within each sex, the concentration being lower during the non-breeding season. Nevertheless, the magnitude of the LH response to exogenous GnRH1 does not vary seasonally in either males or females [9]; this suggests that there is no down-regulation of pituitary GnRH1 receptors during the non-breeding season.

The GnRH1 neuronal system has been previously investigated in various social mole-rats, such as the Damaraland mole-rat, *Cryptomys damarensis* [12], the common mole-rat, *Cryptomys hottentotus hottentotus*, and the highveld mole-rat, *Cryptomys hottentotus pretoriae* [6]. To date there has been no characterisation of the GnRH1 system in any of the solitary African mole-rat species.

In mammals, GnRH1-ir cells are found in the forebrain, at sites extending from the olfactory placode, through the preoptic/septal area, to the mediobasal hypothalamus [15]. The degree of caudal migration varies between species. State-dependent changes in the GnRH1-ir system have been identified in various species. Thus, the number of detectable GnRH1-ir cell bodies changes at the time of puberty in the Djungarian hamster, *Phodopus sungorus* [20], after ovulation in the little brown bat, *Myoptois licifugus* [1], and in response to mating in the female musk shrew, *Suncus murinus* [5]. In the seasonally breeding Syrian hamster, *Mesocricetus auratus*, the number of GnRH1-ir cell bodies remains stable, but their size increases during photoperiodic suppression of the reproductive axis [19].

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Relatively little is known about the reproductive physiology of Cape dune mole-rats; breeding under laboratory conditions is precluded due to their typically aggressive behaviour. The present study was designed to characterize the GnRH1 system of these animals in terms of distribution, number and size of the GnRH1-ir cell bodies within the forebrain of both sexes during the breeding and non-breeding seasons.

2. Materials and methods

2.1. Animals

As part of a pest control program at the Cape Town International Airport (33°58′S 18°37′E), 8 female and 6 male Cape dune mole-rats were captured in the breeding season (July, 2004) and 6 of each sex in the non-breeding season (January, 2005). Body mass during the breeding season ranged from 520 g to 835 g with a mean of 723 g ± 52 g (S.E.M.) for females ($n = 8$) and 390–1920 g with a mean of 953 g ± 261 g for males ($n = 6$). Out of the breeding season, body mass ranged from 680 g to 980 g with a mean of 821 g ± 44 g for females ($n = 6$) and 840–1400 g with a mean of 1090 g ± 101 g for males ($n = 6$). All experimental procedures were approved by the ethics committee of the University of Pretoria (AUCC 040702-015).

Shortly after capture, animals were euthanazed with an overdose of halothane anaesthetic (Zeneca, Johannesburg, South Africa), and perfused through the ascending aorta with 200 ml saline, followed by approximately 200 ml of buffered 4% paraformaldehyde (PFA) solution. Brains were removed and stored in 2% PFA at 4 °C until processing. Prior to sectioning, the brains were placed in a 30% sucrose solution for 96 h. The tissue was cut with a cryostat (at 25 μm) in the coronal plane. Sections were collected into 6 rostro-caudal series, placed in anti-freeze solution and stored at −20 °C.

2.2. Histology

One set of sections for each animal was treated immunohistochemically to reveal GnRH1-immunoreactivity. Sections were washed in PBS (45 min), followed by Triton X-100 (BDH Chemical Company, UK; 0.5% in PBS; 30 min) and PBS (45 min). They were placed in a 0.1% H_2O_2 solution (30 min), followed by a PBS wash (30 min). After pre-treatment in 2% normal donkey serum (30 min), sections were placed in rabbit anti-GnRH1 serum (Incstar Corporation, USA; 1:30,000; 72 h at 4 °C). Subsequently sections were washed in PBS (2 h) and incubated in the secondary antibody, biotinylated donkey anti-rabbit IgG (Jackson Immunoresearch Laboratories Inc., USA; 1:750 for 2 h at room temperature); this was followed by a PBS wash (45 min). Thereafter the sections were exposed to an avidin-biotin complex (Elite Kit; Vector Laboratories, Peterborough, UK; 1:1000 for 90 min). After being washed in PBS, the sections were rinsed several times with Tris buffer (0.1 M, pH 7.6; 30 min). They were then incubated in 0.05% 3,3′-diaminobenzidine tetrahydrochloride, 0.15% nickel ammonium sulphate and 0.005% hydrogen peroxide in Tris buffer (15 min). The reaction was terminated by rinsing the sections in Tris buffer. Increasing dilutions of the primary antibody led to a commensurate attenuation of the immunoreactive signal. No immunoreactivity was observed when the primary or secondary antibody was omitted or when the primary antibody had been pre-treated with GnRH1 (5 μg/ml). Several sets of sections were Nissl-stained with toluidine blue to provide a guide to the general cytoarchitectonics of the Cape dune mole-rat brain.

2.3. Analysis

GnRH1-ir cell bodies were counted in every sixth section from the rostral medial septum to the posterior hypothalamus. Only cell bodies with a visible nucleus were counted. The number of cell bodies detected was multiplied by six to establish the total number of GnRH1-ir cell bodies for each brain. Image analysis software (ImageJ version 1.30; National Institutes of Health, Bethesda, MD, USA) was used to determine the size of the perikaryon of 20 randomly selected GnRH1-ir cells for each animal, according to the method of Robinson et al. [14]. Statistica (Ver 6; Statsoft, Tulsa, OK, USA) was used for all statistical analyses. Non-parametric data were analysed by Kruskal–Wallis Analyses of Variance (ANOVA); parametric data were analysed by two-way ANOVAs followed by the Tukey HSD *post hoc* test. Results are presented as the mean and S.E.M. The significance level was assumed at $p < 0.05$.

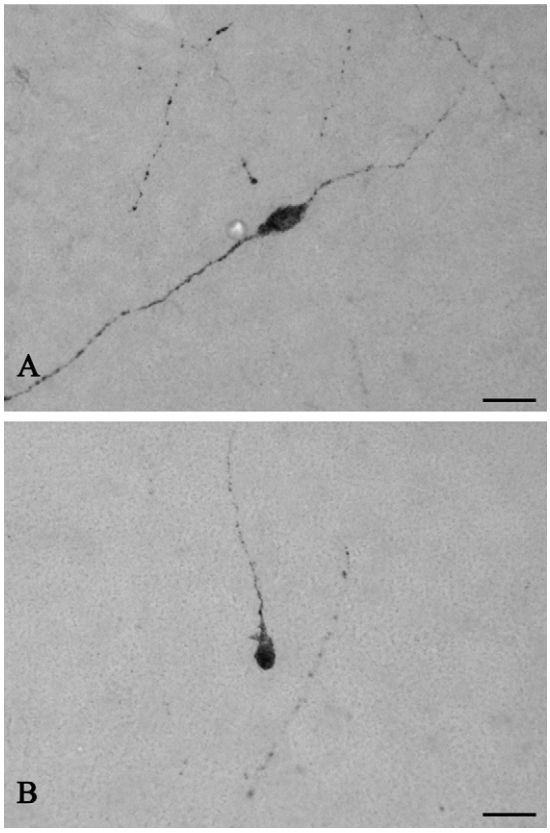

Fig. 1. Photomicrographs of gonadotrophin-releasing hormone 1-immunoreactive cell bodies in a Cape dune mole-rat: (A) bipolar cell body, (B) unipolar cell body. Scale bars = 20 μm.

3. Results

GnRH-1-ir cell bodies in Cape dune mole-rats are found to be spindle-shaped and either unipolar or bipolar (Fig. 1A and B).They are distributed diffusely from the medial septum (Fig. 2A) and preoptic area (Fig. 2B and C) to the mediobasal hypothalamus (Fig. 2H–K). Dense aggregations of GnRH1-ir processes are found at two sites: the organum vasculosum of the lamina terminalis (OVLT; Fig. 2C) and the external zone of the median eminence (ME; Fig. 2G–L).

There is no significant difference between the numbers of GnRH1-ir cell bodies detected in females in the breeding season (1061.28 ± 191.66; $n = 8$) and out of the breeding season

Table 1
The mean (±S.E.M.) number, relative distribution and size of gonadotrophin-releasing hormone 1-immunoreactive (GnRH1-ir) cell bodies in female and male Cape dune mole-rats during the breeding and non-breeding seasons

	Total number of GnRH1-ir cell bodies in the PSA and MBH	Percentage of GnRH1-ir cell bodies in the PSA and MBH which are located in the PSA	Size of GnRH1-ir cell bodies (μm²)
Females:			
Breeding season ($n = 8$)	1061.28 ± 191.66	43.6 ± 5.4	89.15 ± 1.93
Non-breeding season ($n = 6$)	1558.02 ± 221.31	50.7 ± 6.3	74.07 ± 2.23*
Males:			
Breeding season ($n = 6$)	1557.00 ± 129.64	47.7 ± 4.5	89.38 ± 4.39
Non-breeding season ($n = 6$)	1615.98 ± 129.64	48.7 ± 4.7	84.05 ± 4.74

PSA, preoptic/septal area; MBH, mediobasal hypothalamus. '*', significantly smaller ($p < 0.001$) than in females during the breeding season.