A neuroanatomical and neuroendocrinological study into the relationship between social status and the GnRH system in cooperatively breeding female Damaraland mole-rats, *Cryptomys damarensis*

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Abstract

The gonadotrophin-releasing hormone (GnRH) system in female Damaraland mole-rats, *Cryptomys damarensis*, has been investigated to map the distribution of GnRH-immunoreactive (GnRH-IR) structures in the brain of this species and to assess whether changes in this system may mediate the inhibitory effect of social cues on fertility. The distribution of GnRH-IR cell bodies and fibres was similar to that of other mammals, forming a loose continuum along a septo-preoptico-infundibular pathway. GnRH-IR cell bodies were more abundant in the vicinity of the organum vasculosum of the lamina terminalis than in the medial basal hypothalamus. GnRH-IR cells and fibres were also found in the subfornical organ. The cell bodies were typically unipolar or bipolar. No differences were found in the morphology or size of the cell bodies or in the number of cells between non-reproductive females and reproductive females living together in a colony. However, GnRH concentrations, measured in the brain by radioimmunoassay, were significantly higher in non-reproductive females than in reproductive females; this finding was complemented by the reduced immunoreactivity for GnRH in the median eminence and proximal pituitary stalk of reproductive females. In contrast, the concentrations of GnRH measured by radioimmunoassay in non-reproductive and reproductive males did not differ. These results are consistent with the hypothesis that GnRH release is inhibited in the non-reproductive females but not in the non-reproductive males of this species.

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Introduction

Reduced activity of the gonadotrophin-releasing hormone (GnRH) system underlies, at least in part, the observed anovulation and/or gonadal regression in various forms of natural infertility. GnRH priming studies (during which the GnRH receptors are exposed to increased levels of their ligand) in socially suppressed marmoset monkeys, *Callithrix jacchus* (Ruiz *et al.* 1986), in naked mole-rats, *Heterocephalus glaber* (Faulkes *et al.* 1990) or in seasonally anoestrous sheep (McLeod *et al.* 1982) demonstrate an increase in the sensitivity of the pituitary gonadotrophs to GnRH and ultimately to the initiation of ovulation; this suggests a hypothalamic site for the reproductive suppression which can be bypassed by exogenous administration of the releasing hormone. Immunocytochemical investigation of the GnRH system has revealed that

morphological parameters of GnRH-immunoreactive (GnRH-IR) neurones (for example, their size and/or number) can vary according to sex in the springbok, *Antidorcas marsupialis* (Robinson *et al.* 1997), following gonadectomy in rats (King *et al.* 1987), following puberty in the Djungarian hamster, *Phodopus sungorus* (Yellon & Newman 1991) and in as little as 1 h following first mating in the female musk shrew, *Suncus murinus* (Dellovade *et al.* 1995a). Differences in GnRH-IR neurones have also been identified between pre-ovulatory and post-ovulatory conditions in the little brown bat, *Myotis lucifugus* (Anthony *et al.* 1989) and between territorial and non-territorial, subordinate male cichlid fish, *Haplochromis burtoni* (Francis *et al.* 1993). The dynamic nature of these alterations in morphological characteristics of GnRH-IR neurones illustrates the plasticity of the GnRH system in various species. Differences in the immunoreactivity or

morphology of GnRH neurones may reflect an alteration in the functioning (synthesis and/or release) of the GnRH system.

Damaraland mole-rats, *Cryptomys damarensis*, are eusocial, subterranean rodents that inhabit arid regions of south-western Africa. Breeding is limited to a single reproductive pair in colonies that may contain as many as 41 individuals (Jarvis & Bennett 1993). The non-reproductive females exhibit a socially induced infertility while in the colony, but start ovulating spontaneously in the absence of the breeding pair (Molteno & Bennett 2000). Anovulation in non-reproductive females is characterised by a reduced pituitary sensitivity to a single, exogenous GnRH challenge; given the autoregulatory action of GnRH on its receptors on the gonadotrophs, this may be due to an inhibition of GnRH release. The initiation of ovulation in non-reproductive females removed from the breeding pair is accompanied by a significantly greater plasma luteinising hormone (LH) response to exogenous GnRH administration. Breeding females exhibit significantly greater GnRH-stimulated LH secretion than non-reproductive females (Bennett *et al.* 1996, Molteno & Bennett 2000). Thus the social environment has a clear effect on the reproductive neuroendocrine system of non-reproductive females. The effect of the social environment on reproductive function does not appear to be comparable in non-reproductive males, their pituitary sensitivity to GnRH being no different from that of reproductive males (Molteno & Bennett 2000).

This study is the first to investigate the effect of the social environment on the GnRH system in a cooperatively breeding mammal, *C. damarensis*, by means of immunocytochemistry and radioimmunoassay. The study was designed to characterise the morphology and distribution of the GnRH-IR system in the brain of this species and to determine whether the inhibitory effects of social cues on fertility in non-reproductive female mole-rats are induced through changes in function and/or morphology of the GnRH system.

Materials and Methods

Animals

Damaraland mole-rats were trapped at Hotazel, Northern Cape Province, South Africa (27°17' S; 22°58' E). All animals were field-caught and brought back to the laboratory and habituated for 2 months prior to experimentation. Further unrelated animals were paired to establish reproductive groups. All animals were housed in the same constant-temperature room, received the same husbandry and were fed the same food. These animals acclimatise well to laboratory conditions and begin (or continue) breeding successfully in the laboratory. Females rendered reproductive in the laboratory by pairing unrelated males and females were classified as reproductive once the female had given birth to at least one litter. Colonies were housed

in plastic crates (1 × 0.5 × 0.5 m) with nesting boxes, wood-shavings and shredded paper towelling for nesting material. The animals were maintained at a constant temperature (25 °C), in continuous darkness and were fed and cleaned under dim red light. Animals were provided with freshly chopped vegetables daily. For use in the immunocytochemical study, female mole-rats were assigned to one of three groups (see below), according to their reproductive status and the social environment.

Group 1

Reproductive females (RFs) were easily identifiable by their large teats and perforated vaginas. From a practical perspective, the social structure of the species (one reproductive female per colony) meant that a limited number of reproductive females were available and it was difficult to obtain large numbers of reproductive females in a specific reproductive phase.

Group 2

Non-reproductive females (NRFs) were housed in functionally complete, wild-caught colonies.

Group 3

Removed non-reproductive females (rNRFs) were maintained in the absence of the breeding pair. These reproductively non-functional colonies were maintained in the laboratory for 11 weeks prior to the females being killed. No sexual activity occurs in these colonies partly as a result of an inhibition to breed with kin.

For radioimmunoassay of brain GnRH content, forebrains were obtained from RFs and NRFs, and also from reproductive males (RMs) and non-reproductive males (NRMs).

GnRH immunohistochemistry

Perfusion and tissue processing

RFs, NRFs and rNRFs ($n = 7$ for each group) were anaesthetised using halothane followed by intramuscular injection of 30 mg ketamine/kg and 5 mg rompun/kg. Individuals were perfused through the aorta with approximately 150 ml 0.1 M PBS, followed by approximately 300 ml 4% paraformaldehyde in 0.1 M PBS. Solutions were filtered immediately prior to use. Heads were removed and immersed in 4% paraformaldehyde overnight; brains were removed and placed sequentially in 15 and 30% sucrose at 4 °C until they sank. They were then frozen on dry-ice and stored at -70 °C until sectioning. The brains were sectioned coronally at 30 µm using a cryostat. Every fifth section from the rostral junction of the hemispheres to the posterior hypothalamus was processed. After washing in 0.1 M PBS, free-floating sections were treated with 0.5% Triton X-100 (BDH Chemical Company, Poole, UK), to increase antibody penetration, and 0.02%

hydrogen peroxide for 30 min to block endogenous peroxidase activity. Sections were placed in 2% normal donkey serum for 30 min to minimise non-specific binding of the antibodies. They were then incubated in mouse anti-GnRH antibody (1:1000; kindly supplied by Dr D Silversides) overnight and, after washing in 0.1 M PBS, in biotinylated goat anti-mouse IgG (1:1000; Vector Laboratories, Peterborough, UK) for 2 h at room temperature. Following treatment with Avidin Biotin Complex (Elite Kit, Vector Laboratories) for 90 min, the sections were washed in 0.1 M PBS and then in 0.05 M Tris buffer (Trizma 7.6, Sigma). A blue-black reaction product was formed by a 6 min incubation of sections in a solution containing nickel ammonium sulphate, 3'-diaminobenzidine and hydrogen peroxide. Immunohistochemical controls included incubation of sections following immunoneutralization of the antibody with GnRH, or omission of either the primary or the secondary antibody; these procedures result in the loss of all immunoreactivity.

Analysis

Distribution, morphology, size and number of immunoreactive GnRH perikarya in the forebrain extending from the rostral preoptic area to the posterior hypothalamus were determined and compared between RFs, NRFs and rNRFs. The number of processes deriving from all GnRH cells in every fifth section from the preoptic area of each female was determined. The size of the perikaryon of 20 randomly selected GnRH-IR cells in the preoptic area of each animal was established according to the method described by Robinson *et al.* (1997) using image analysis software (Scion Image Beta 3b, Frederick, Maryland, USA). The total number of GnRH cells was counted for each animal. Statistical comparisons were made using both non-parametric Kruskal-Wallis tests and parametric ANOVA.

To establish the area occupied by GnRH-IR fibres in the median eminence, computer-assisted image analysis was undertaken on matched sections from RFs and NRFs (three sections per animal; $n = 5$ animals for each group) using the thresholding and area measurement procedures in the ImageJ 1.26t program (W Rasband, National Institutes of Health, Bethesda, MD, USA). The mean tissue background value was established according to the grey scale (0 = black; 255 = white). The area covered by pixels with a grey scale value that fell below 80% of the background value was automatically measured.

GnRH measurement

GnRH extraction

Following decapitation, brains were removed from RFs, NRFs, RMs and NRMs ($n = 10$ for each group) and frozen on dry-ice. GnRH was extracted according to the method of King & Millar (1986). Prior to radioimmunoassay, whole brains were lyophilised and their dry weights deter-

mined. Lyophilised brains were minced with a sterile blade and processed individually in 5 ml 2 M acetic acid for 3 min using a polytron homogeniser. All steps were performed at 4°C. The homogenates were centrifuged at 18 000 *g* for 60 min and the supernatant fluid was freeze-dried overnight. Individual brain extracts were reconstituted in 4 ml TF solution (2% triethylamine, 1.05% formic acid, 96.95% dH₂O; pH 3.2). Extracts were sonicated for 3 min and centrifuged at 10 000 r.p.m. for 15 min. The supernatants were concentrated overnight using a Savant speed-vac concentrator connected to a freeze-drier and reconstituted in 1 ml gel-PBS buffer (PBS containing 0.1% gelatin) for radioimmunoassay.

GnRH radioimmunoassay

Concentrations of GnRH extracted from individual mole-rat brains were determined by radioimmunoassay based on the method described by King & Millar (1986). GnRH antiserum 678, raised in rabbits (J A King, Department of Chemical Pathology, University of Cape Town, South Africa), requires both the NH₂- and COOH-termini for binding and cross-reacts with all seven known forms of GnRH (King *et al.* 1994). Synthetic mammalian GnRH (mGnRH) (R P Millar, Medical Research Council, Edinburgh, Scotland) was used as the standard and ¹²⁵I-labelled mGnRH, iodinated using the chloramine-T method (Greenwood *et al.* 1963), was used as the tracer.

Serial doubling dilutions of mGnRH were prepared in gel-PBS over the range 0.48–1000 pg/ml. The ¹²⁵I-labelled mGnRH tracer was used at approximately 15 000 c.p.m. per 100 µl in a total assay volume of 500 µl. The mGnRH standard preparations and samples of GnRH extract, assayed at two dilutions as a routine test for parallelism, were assayed in duplicate. NRM samples were assayed in crude form, and following purification through Sep-pak columns (Waters Associates) to determine whether purification was required. Following overnight incubation, bound and free peptides were separated using 200 ml Pharmacia Decanting Suspension (Kabi Pharmacia Diagnostics, Uppsala, Sweden). After 30 min the reaction was terminated by adding 1 ml dH₂O. Tubes were centrifuged at 3000 r.p.m. at 4°C and the pellet containing the bound fraction was counted for 1 min on a gamma counter (Packard Riastar 5400, Meriden, CT, USA).

Assay validation

The suitability of the assay for measuring GnRH from mole-rat brain extract was assessed by testing the parallelism between serial doubling dilutions of synthetic mGnRH standard and both crude extract and extract purified through Sep-pak columns (Waters Associates). Following logit-log transformation of the data, the slopes of the curves were tested for parallelism, using the *Statistica* computer package (Statsoft, Tulsa, OK, USA). The slopes of the three curves were not significantly different (ANOVA, $F_{2,14} = 0.14$, $P > 0.05$). Sep-pak purification

did not improve the effectiveness of the assay to measure GnRH and yielded lower values than crude samples. The intra- and inter-assay coefficient of variations were 9.6 ($n = 14$) and 6.6% ($n = 6$) respectively. GnRH concentrations were analysed by two-way ANOVA with sex and reproductive status as independent factors ($n = 9$ for each group). *Post-hoc* analysis was carried out by Tukey's Honest Significant Difference (HSD) test.

Results

Morphology and distribution of GnRH cell bodies

The GnRH-IR cell bodies in *C. damarensis* are distributed in a loose continuum along a septo-preoptico-infundibular pathway (Fig. 1*a–d* and *f*; Fig. 2*a*). Within the medial septal region, cells containing GnRH immunoreactivity are predominantly oriented with their long axis in the dorso-ventral direction; more caudally, this axis is generally oriented mediolaterally and rostrocaudally. A high proportion of the total number of GnRH cell bodies is found from the rostrocaudal level of the medial septum and organum vasculosum of the lamina terminalis (OVLt) to the medial preoptic area (MPOA) (Fig. 1*b* and *c*). Dorsally these cells are located close to the midline; ventrally they are distributed more laterally (Fig. 1*a–c*). GnRH cells are also observed in the subfornical organ (Fig. 2*c*). Relatively few of the total number of GnRH cells are located in the mediobasal hypothalamus (MBH) caudal to the supra-chiasmatic nuclei (Fig. 1*f*) (14.7% in RFs, 13.2% in NRFs and 15.6% in rNRFs). A few GnRH cells are found caudal to the MBH, reaching sites adjacent to the mammillary recess of the third ventricle (Fig. 2*a*).

GnRH-IR cell bodies in *C. damarensis* are typically unipolar or bipolar cells with a perikaryon approximately 7–12 μm in diameter (Fig. 2*d* and *e*). GnRH cells totalling 341 in RFs, 378 in NRFs and 409 in rNRFs were analysed. In a small proportion of these cells distinct processes were not observed, probably due to the plane of sectioning; these were classified as non-polar (Fig. 2*d*). Kruskal–Wallis analysis revealed that the mean number of cells per female that were unipolar ($H = 0.2$, $P > 0.05$, $n = 21$) or bipolar ($H = 1.0$, $P > 0.05$, $n = 21$) was not significantly different between the three groups (Fig. 3). Only two GnRH cells with multiple processes were observed in this study. The GnRH cell bodies and proximal processes possess a generally smooth contour (Fig. 2*d* and *e*); although immunoreactive processes were occasionally visible in the vicinity of GnRH dendrites, appositions between GnRH perikarya were not apparent.

Morphology and distribution of GnRH fibres

GnRH-IR fibres typically display varicosities approximately 1.0 μm in diameter (Fig. 2*d*). Although GnRH fibres are apparent in the olfactory bulb, the major tract of fibres follows the septo-preoptico-infundibular continuum. A plexus of GnRH-IR fibres is also observed within the subfornical organ (SFO) (Fig. 2*c*). Fibres course in a dorso-ventral direction from the medial septum and in a rostro-caudal direction along the ventral midline (Fig. 1*a–c* and Fig. 2*b*). A dense network of immunoreactive fibres is observed around and within the OVLt (Fig. 1*b*). This dense network of fibres continues primarily along the midline in a very dense aggregation ventral to the third ventricle (Fig. 1*c*). Caudal to this region evidence of an optic chiasm is minimal due to the vestigial state of the optic nerves in this species; nevertheless, immunoreactive fibres and perikarya are found within the membranous structure forming the floor of the third ventricle at this level (Fig. 1*d*). Further caudally, a smaller number of GnRH fibres can be seen beneath the third ventricle, medial to the suprachiasmatic nuclei (Fig. 1*e*). The general distribution of GnRH fibres extends into more lateral regions as they approach the rostral median eminence (ME) (Fig. 1*f* and *g*); caudal to this level the fibres form an extensive arching pattern from the borders of the third ventricle throughout the mediobasal regions, achieving a dense aggregation in the external zone of the rostral median eminence (Fig. 1*h–j*). Within the major part of the ME, GnRH fibres are principally located in its lateral margins (Fig. 1*k–m*). The internal zone of the ME in these animals is remarkably thick medially; it protrudes into the third ventricle and is surrounded by deep lateral recesses of that ventricle (Fig. 1*k–l*). The dense GnRH immunoreactivity in the external zone of the ME continues caudal to the pituitary stalk, as revealed in the parasagittal plane (Fig. 2*b*). As the pituitary stalk starts to form, dense areas of GnRH immunoreactivity are found around its immunonegative central core (Fig. 1*n*). The mean area ($\pm$ S.E.M.) occupied by GnRH fibres in the median eminence was less ($P < 0.01$) in RFs ($0.0178 \pm 0.00159 \text{ mm}^2$, $n = 5$) than in NRFs ($0.0246 \pm 0.0112 \text{ mm}^2$, $n = 5$); examples illustrating this difference are presented for the RF (Fig. 1*o*) and NRF (Fig. 1*n*) conditions.

Number and size of cell bodies

A large variation in the number of GnRH cells in individual forebrains was observed from the confluence of the

Figure 1 Coronal sections (*a–n*; rostral to caudal) from the brain of a non-reproductive female (NRF) and (*o*) from the brain of a reproductive female (RF) showing GnRH immunoreactivity in: (*a*) the medial septum (MS) and rostral preoptic area; (*b*) the MS and region of the organum vasculosum laminae terminalis (OVLt); (*c*) the medial preoptic area and in a densely immunoreactive site caudal to the OVLt and ventral to the third ventricle (3V); (*d*) the membranous structure which (in the absence of a detectable optic chiasm) forms the rostral floor of the 3V (the immunoreactivity is revealed in the higher magnification insets); (*e*) the region medial to the supra-chiasmatic nuclei (SCN); (*f–g*) the retrochiasmatic area rostral to the median eminence (ME); (*h–m*) a dense band of fibres forming the external zone of the ME. Fibres surrounding a central immunonegative core in the pituitary stalk (PS) typically show stronger immunoreactivity in non-reproductive than in reproductive females (*n* and *o*). Arrows indicate selected GnRH perikarya (*a–d*, *f*). Scale bar denotes 200 μm ; PT, pars tuberalis.

Figure 2 Distribution and morphology of GnRH-immunoreactive cell bodies and fibres in non-reproductive female Damaraland mole-rats. (a) An immunoreactive perikaryon (arrow) and immunoreactive fibres in the posterior median eminence at the level of the mammillary recess of the third cerebral ventricle (MR 3V) and surrounding the pituitary stalk (PS). (b) The densely immunoreactive external zone of the median eminence continues caudal to the pituitary stalk as shown in a parasagittal section. (c) Dense fibres with GnRH immunoreactivity in the subfornical organ (SFO) and a GnRH perikaryon (arrow). (d) Medium-power photomicrograph showing GnRH bipolar (bp), unipolar (up) and non-polar (np) GnRH cells. (e) High-power photomicrograph showing the smooth contour of the perikaryon and proximal dendrites of a bipolar GnRH neurone. Scale bars denote length in micrometres; PT, pars tuberalis.

two hemispheres rostrally, to the posterior hypothalamus caudally (Fig. 4); this ranged from 76 to 173 in RFs (mean 124.57 ± 14.33), from 74 to 172 in NRFs (mean 130.43 ± 36.11) and from 79 to 234 in rNRFs (mean

134.0 ± 49.23). The number of GnRH cell bodies did not differ between the RF, NRF and rNRF groups in the whole forebrain ($H_2 = 0.12$, $P > 0.05$, $n = 21$) or in the preoptic area ($H_2 = 0.27$, $P > 0.05$, $n = 21$) or in the mediobasal

Figure 3 Mean ($\pm$ S.E.M.) number of non-polar, unipolar or bipolar GnRH cells detected in every fifth section taken through the preoptic region of females that were housed in the presence of the breeding pair and either reproductive (solid bars; $n = 7$) or non-reproductive (hatched bars; $n = 7$), or housed in the absence of the breeding pair and still in a non-reproductive condition (open bars; $n = 7$).

Figure 4 Mean ($\pm$ S.E.M.) number of GnRH immunoreactive neurones detected in every fifth section taken through the forebrain from the confluence of the two hemispheres rostrally, to the posterior hypothalamus caudally (open bars; $n = 7$), through the preoptic area (hatched bars; $n = 7$) or through the medial basal hypothalamus (solid bars; $n = 7$). RF, reproductive females; NRF, non-reproductive females; rNRF, non-reproductive females removed from the breeding pair.

Figure 5 Mean ($\pm$ S.E.M.) concentrations of GnRH in the brain of reproductive females ($n = 9$), non-reproductive females ($n = 9$), reproductive males ($n = 9$) and non-reproductive males ($n = 9$). A two-way ANOVA revealed a significant interaction between sex and reproductive status ($F_{1,32} = 12.02$, $P = 0.002$). Reproductive females had significantly lower GnRH concentrations than non-reproductive females ($P = 0.008$) and reproductive males ($P = 0.036$) (Tukey's HSD test).

hypothalamus ($H_2 = 0.56$, $P > 0.05$, $n = 21$; Fig. 2). Given that only every fifth section was processed, the corrected mean total number of GnRH cell bodies per brain over all three groups of females was calculated as 648.33 ± 43.36 . There was also a large variation in GnRH cell body size; this ranged from 40.6 to $154.2 \mu\text{m}^2$ (mean = $89.7 \pm 2.0 \mu\text{m}^2$) in RFs, from 47.9 to $140.4 \mu\text{m}^2$ (mean = $86.0 \pm 1.7 \mu\text{m}^2$) in NRFs and from 36.4 to $168.2 \mu\text{m}^2$ in rNRFs (mean = $88.3 \pm 1.9 \mu\text{m}^2$); no significant differences were detected between the three groups (ANOVA, $F = 0.97$, $P > 0.05$, $n = 420$).

Brain GnRH concentrations

Neither sex ($F_{1,32} = 0.33$, $P = 0.571$) nor reproductive status ($F_{1,32} = 2.02$, $P = 0.165$) alone had a significant effect on GnRH. However, a significant interaction term ($F_{1,32} = 12.02$, $P = 0.002$) reflected the fact that reproductive females had significantly lower GnRH concentrations than non-reproductive females ($P = 0.008$) and reproductive males ($P = 0.036$) (Fig. 5).

Discussion

The distribution of GnRH-IR cell bodies and fibres in the brain of *C. damarensis* forms a loose continuum along a septo-preoptico-infundibular pathway. Although this is typical of mammals, interspecific differences in the distribution of GnRH neurones have been observed, especially with respect to the degree to which they are present caudal to the preoptic area (Silverman *et al.* 1994). This variability may be associated with their unusual migration from the olfactory placode (Schwanzel-Fukuda & Pfaff 1989). In the opossum, *Monodelphis domestica*, GnRH cell bodies do not migrate into the preoptic area (Schwanzel-Fukuda *et al.* 1988), but in the rat, they do not reach into

the MBH (Witkin *et al.* 1982, Merchenthaler *et al.* 1984). In contrast, in the mink approximately 7% of GnRH cell bodies are located in the POA and 80% in the MBH (Ntouni *et al.* 1994). Nevertheless, in most mammals in which GnRH cell bodies migrate into the MBH these cells usually constitute a relatively small percentage of the total number of such cells (Silverman *et al.* 1994). This is true of the Damaraland mole-rat, in which only approximately 14% of the total number of GnRH-IR cell bodies are found in the MBH. The intraspecific comparisons carried out in the present study did not identify variations in the distribution of GnRH-IR cells and fibres between RFs, NRFs and rNRFs.

As found in all mammalian species studied to date, two main areas of dense GnRH-IR fibres, the OVLT and the ME, were observed in the mole-rat (Fig. 1b and Fig. 1j–m respectively). GnRH-IR fibres together with cell bodies were also found in another circumventricular organ, the SFO (Fig. 2c); this site has been reported to contain GnRH fibres with extrinsic cells of origin in rats (Witkin *et al.* 1982). The diameter of the GnRH cell body in *C. damarensis* is similar to that found in other small mammals, including white-footed mice ($8 \mu\text{m}$; Glass 1986) and rats ($10 \mu\text{m}$; Witkin *et al.* 1982), and smaller than that reported for sheep (15 – $20 \mu\text{m}$; Lehman *et al.* 1986) or springboks (10 – $20 \mu\text{m}$; Robinson *et al.* 1997). The mean total number of neurones calculated per Damaraland mole-rat (648 ± 43) is similar to that observed in the Syrian hamster (650 – 750 ; Jennes & Stumpf 1980) or the Djungarian hamster (300 – 400 ; Yellon & Newman 1991). In larger species including ungulates (Lehman *et al.* 1986, Robinson *et al.* 1997) and primates (Marshall & Goldsmith 1980, Silverman *et al.* 1982) these neurones number in the thousands.

The GnRH neurones in the Damaraland mole-rat are predominantly unipolar and bipolar, as found in many mammals including the rat (Witkin *et al.* 1982, Merchenthaler *et al.* 1984), white-footed mouse (Glass 1986), Syrian hamster (Jennes & Stumpf 1980) and mink (Ntouni *et al.* 1992). In contrast, those found in the sheep (Lehman *et al.* 1986), springbok (Robinson *et al.* 1997) and rhesus monkey (Silverman *et al.* 1982) have been reported to have a more complex morphology. It should be noted that the number of bipolar neurones identified in the present study may have been underestimated; many of the apparently unipolar GnRH cells in the rat brain have been shown to be bipolar using silver–gold intensification of the immunocytochemical staining (Merchenthaler *et al.* 1984). In the male Djungarian hamster, differential changes in the incidence of these morphological subtypes have been observed during sexual maturation (Yellon & Newman 1991). Thus, the number of unipolar, but not bipolar cells, in the diagonal band of Broca (DBB) and medial preoptic area nearly doubles peripubertally; subsequently, between puberty and full adulthood, the number of bipolar cells in the DBB and lateral preoptic area also doubles. It has been speculated that change in the

incidence of these cell types is related to differential GnRH storage and release during development (Yellon & Newman 1991). Nevertheless, in the present study, the number of unipolar and bipolar cells was not found to vary with reproductive status.

No significant differences in GnRH-IR neuroanatomy were found between reproductively active and inactive Damaraland mole-rats. Similar results were observed for seasonally anoestrous and reproductive sheep (Lehman *et al.* 1986) and for reproductive and bachelor male springboks (Robinson *et al.* 1997). These findings are in contrast to those for seasonally breeding female white-footed mice, in which the number of cell bodies and the optical density of their GnRH immunoreactivity increase in the preoptic area and anterior hypothalamus following exposure to an inhibitory, short photoperiod (Glass 1986). In the seasonally breeding Syrian hamster, Urbanski *et al.* (1991) did not find differences in the number of GnRH-IR neurones in individuals maintained under long or short day length regimens, but did find that the area of the cell body was larger in short-day maintained animals. This suggests that an alteration in the GnRH system, leading to an accumulation of GnRH in the cell bodies, may occur within some species in response to the inhibitory environmental cues that affect reproduction. Indeed, in the present study radioimmunoassay revealed significant differences in brain GnRH content between NRFs and RFs. The finding that the reproductively inhibited NRFs exhibit higher GnRH levels than RFs suggests that an inhibition of GnRH release may underlie the social suppression of reproductive functions in female *C. damarensis*. Evidence for a comparable process in the male members of this species was not obtained, supporting the hypothesis that NRMs exhibit reproductive inhibition that is largely behavioural (Bennett *et al.* 1996).

The question arises as to where the increased levels of GnRH accumulate. GnRH is transported from the site of synthesis in the cell body to the nerve terminals where it is released into the portal vessels in the ME. The results of the present study indicate no differences between the RF and NRF groups in the size or number of GnRH-IR cell bodies, but a significantly higher concentration of GnRH in the NRF group; these findings would be consistent with an accumulation of the peptide in the terminals rather than the cell bodies within the NRF group. This hypothesis is supported by the more intense level of GnRH immunoreactivity observed in the NRF group at the level of the median eminence and proximal pituitary stalk (e.g. Fig. 1*n* compared with Fig. 1*o*). A similar precedent exists, since a higher density of GnRH-IR fibres in the ME has been observed in seasonally breeding female white-footed mice maintained under an inhibitory, short photoperiod (Glass 1986). Furthermore, studies on musk shrews have shown that mating behaviour that fails to induce ovulation is associated with a higher level of median eminence GnRH than that found after successful reflex ovulation (Dellovade *et al.* 1995*b*).

The results of the present study are consistent with inhibition of GnRH release from the ME, and consequent accumulation of GnRH in the terminals, being one of the factors contributing to the suppression of ovarian function in socially suppressed female mole-rats. The GnRH system may also be inhibited at the level of gene transcription, mRNA translation or post-translational processing. Future research directions will involve the use of antibodies directed against pro-GnRH and together with *in situ* hybridization for GnRH mRNA we intend to gain further insights into the regulatory processes that mediate the effect of social environment on fertility in the Damaraland mole rat.

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