CHAPTER 5:

NEUROANATOMY AND NEUROENDOCRINOLOGY OF THE GnRH SYSTEM OF CAPE MOLE-RATS AND THE NATAL MOLE-RATS

Abstract

This study mapped the distribution and morphology of the GnRH neuronal systems of Cape and Natal mole-rats and the GnRH-immunoreactivity of the median eminence was quantified. A comparison was made between the winter and summer seasons in both species and also between dominant and subordinate animals in the Natal mole-rat species.

Although the Cape mole-rat is larger than the Natal mole-rat, it has a smaller number of GnRH neurons. No differences were found in the number or size of these neurons across season in either species or reproductive status in the Natal mole-rat. In both species, the size of GnRH-ir neurons was similar in the different seasons and no difference was detected according to reproductive status.

The GnRH neurons and fibres are loosely distributed along the septo-preoptico-infundibular pathway in both species. Dense clusters of fibres are visible in the area of the organum vasculosum of the lamina terminalis and the median eminence. The species differed with regard to the incidence of GnRH-ir neurons along the rostral-caudal axis of the brain. In the Cape mole-rat, almost 90% of the GnRH-ir perikarya are present in the medial septum or preoptic area, and only 10% in the mediobasal hypothalamus. In the Natal mole-rat, a much larger proportion (40%) of the total GnRH-ir neurons is located in the mediobasal hypothalamus with the remainder in the medial septum or preoptic area. In the Cape mole-rat females there is no difference in GnRH-immunoreactivity in the median eminence in and out of the breeding season. In the Natal mole-rat, dominant animals of both sexes had significantly less GnRH-immunoreactivity in the median eminence than the

subordinate animals. This suggests that GnRH is retained in the median eminence in these subordinate, behaviourally suppressed animals.

Behaviourally, both of these species display regulated reproduction, breeding in the Cape mole-rat is restricted to a specific part of the year, while subordinate individuals of the Natal mole-rats are reproductively suppressed by dominant animals. However, neuroendocrinologically, this is only reflected in subordinate animals of the Natal mole-rat.

Introduction

Gonadotropin releasing hormone (GnRH) is a decapeptide that serves both as a hormone and a neurotransmitter in the mammalian brain. It is an essential part of the reproductive process since it is responsible for the release of luteinising hormone (LH) and follicle stimulating hormone (FSH) from the pituitary, and has been found to promote reproductive behaviour (Dellovade *et al*. 1998).

Most vertebrate species possess two or more forms of GnRH in separate cell populations. GnRH-1 was first characterised in mammals. Only two different forms of mammalian GnRH-1 have been reported; guinea-pig GnRH-1 differs by two amino acids from all other known mammalian forms (Jiminez-Liñan *et al.* 1997). Recently it has been found that the GnRH-1 gene sequence in highveld mole-rats shows similarities to that of the guinea-pig, but the translated peptide corresponds to the standard mammalian form (Kalamatianos *et al.* 2005).

GnRH-1 neurons concerned with reproductive regulation originate from the medial olfactory placode early in mammalian development, from where they migrate along the nervus terminalis to colonise the forebrain in and around the preoptic area (POA) and mediobasal hypothalamus (MBH) (Schwanzel-Fukuda & Pfaff 1989). These neurons project to the median eminence where the GnRH is stored in terminals before being released into the pituitary portal system. It is then transported to the anterior pituitary gland, where it acts upon surface receptors of gonadotropes to stimulate the production and secretion of LH and FSH.

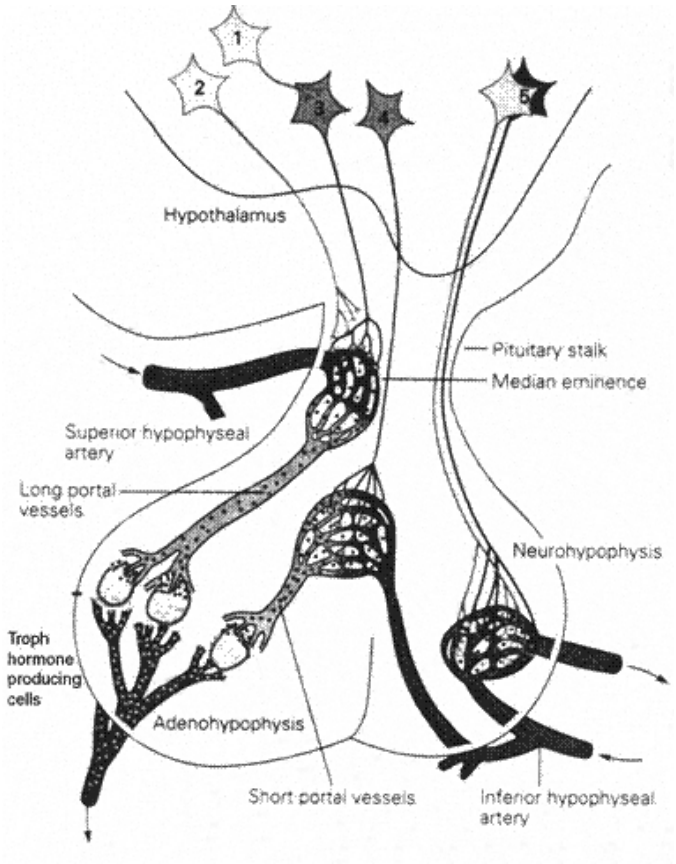

Figure 5.1: The pathway of the GnRH peptide from the perikarya to the anterior pituitary.

In this study, we have characterised the morphology and distribution of the GnRH-1 system of the solitary Cape mole-rat and social Natal mole-rat. The research was designed to determine whether there are inter- and/or intra-species differences in the GnRH-1 systems of breeding and non-breeding members of these species.

Methods

Female Cape mole-rats (6 in the breeding season, 7 out of the breeding season) and male and female Natal mole-rats (7 dominant females, 6 subordinate females, 8 dominant males, 7 subordinate males) were used for this experiment.

Refer to chapter 2 for immunocytochemical procedure.

After the sections were mounted on gelatinised slides and cover slipped, they were analysed under a light microscope.

Analyses

The distribution of GnRH-ir processes was established and the total number of GnRH-ir cell bodies was counted in every sixth section from the confluence of the two hemispheres rostral to the posterior hypothalamus caudally. Only cell bodies with a visible nucleus were counted. The data were corrected for sampling rate of one in six sections. Image analysis software (ImageJ version 1.30, National Institutes of Health, USA) was used to determine the size of the perikaryon of 20 randomly distributed GnRH neurons for each animal.

Additionally, the density of GnRH immunoreactivity in the median eminence was quantified according to the method of Robinson *et al*. (1997).

Results

- **Morphology and distribution of GnRH cell bodies**

Cape mole-rat

In the Cape mole-rat, GnRH-ir cell bodies are typically spindle-shaped with smooth contours (Plate 5.1 a, b). The majority of these cells are unipolar or bipolar, although a very small number of multipolar cells are present. Some cells without apparent processes are observed, most likely due to the plane of sectioning.

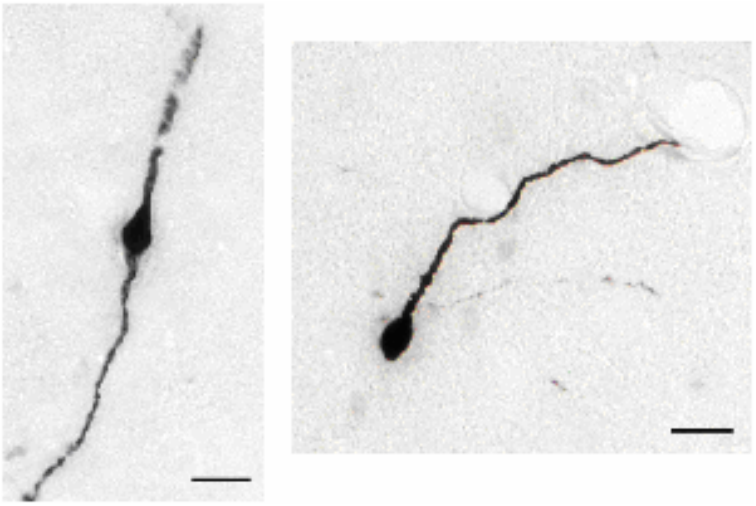

Plate 5.1(a). Bipolar cell body; **(b)**. Unipolar cell body. Scale bars = 20μm.

GnRH-ir perikarya are distributed loosely along the septo-preoptico-infundibular pathway. The majority of the GnRH-ir perikarya are located in the

medial septum (MS) and preoptic area (POA) (females BS, 87%, females OBS, 90%), and a smaller number of GnRH cell bodies are found further caudal in the mediobasal hypothalamus (MBH) (females BS, 13%, females OBS, 10%) (Figure 5.2). Very few GnRH-ir cell bodies are found around the suprachiasmatic nucleus (SCN).

No differences were observed in morphology or distribution of the GnRH cells between female Cape mole-rats in and out of the breeding season.

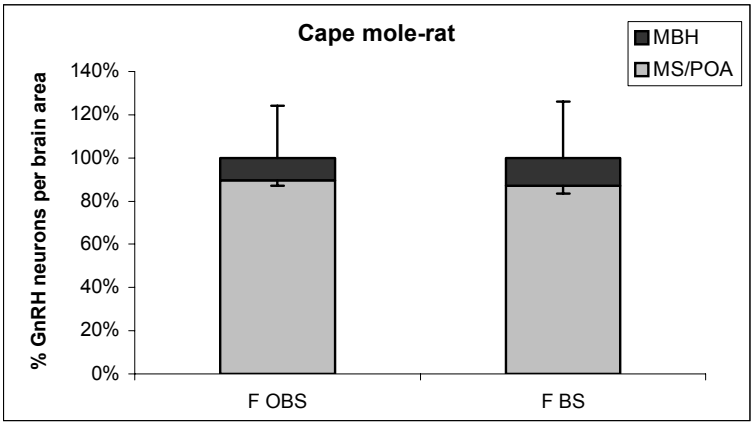

Figure 5.2: The relative distribution of GnRH-ir perikarya in the medial septum (MS)/ preoptic area (POA) and the mediobasal hypothalamus (MBH) in the brain of the Cape mole-rat. F OBS – female, out of breeding season, F BS – female breeding season.

Natal mole-rat

In terms of shape, contour and polarity, the GnRH-ir neurons in the Natal mole-rat resemble those of the Cape mole-rat. In the Natal mole-rat, the majority of GnRH-ir cell bodies are situated in the MS/POA (BF, 67%, SF, 68%, BM, 62%, SM, 60%). Nevertheless, a significant proportion of the total GnRH-ir cell bodies are present in the MBH (RF, 33%, SF, 32%, RM, 38%,

SM, 40%) (Figure 5.3). GnRH cell bodies are present around but not in the SCN. GnRH neurons are seen in the hypothalamus as far caudal as the level of the median eminence and pituitary stalk, but not in those structures.

As in the Cape-mole-rat, GnRH-ir neurons are predominantly located in the MS/POA, but in the Natal mole-rat, the proportion of GnRH-ir cells occurring in the MBH is larger than that in the Cape mole-rat.

No differences in the morphology or distribution are seen between dominant and subordinate females, or between dominant and subordinate males. Male mole-rats have a slightly higher percentage of GnRH neurons in the MBH than females, but not significantly so.

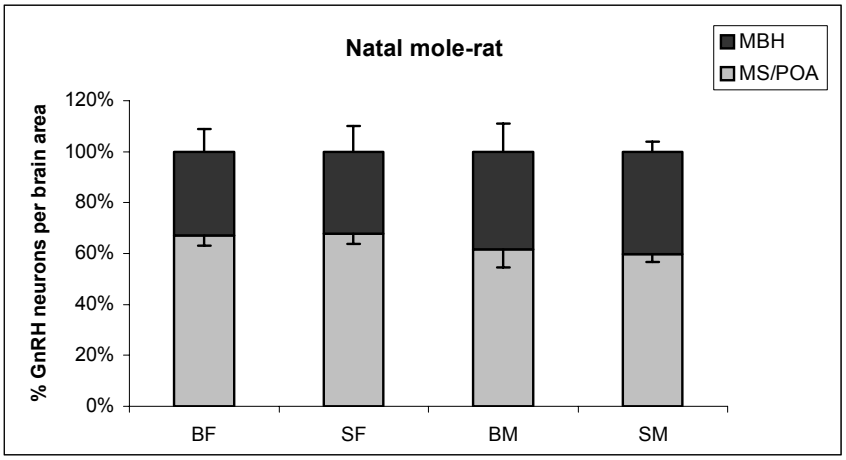

Figure 5.3: The relative distribution of GnRH perikarya in the medial septum/preoptic area and mediobasal hypothalamus in the brain of the Natal mole-rat. BF - breeding females, SF - subordinate females, BM - breeding males, SM - subordinate males. MS – medial septum, POA – preoptic area, MBH – mediobasal hypothalamus.

- **Morphology and distribution of GnRH-ir processes**

Cape mole-rat

The GnRH-ir processes of the Cape mole-rat have a characteristic beaded appearance. They are present along the septo-preoptico-infundibular continuum from the medial septum to the median eminence. In the rostral areas of the medial septum, sparse fibres are observed in the midline, growing denser and more widespread as they proceed caudally to the anterior commissure. In this area, most fibres and cell bodies are located between the anterior commissure and the ventral limit of the brain. Some fibres are observed in the subfornical organ (SFO), but no cell bodies. A dense concentration of immunoreactive fibres is present in and around the organum vasculosum of the lamina terminalis (OVLT). These processes progress caudally around and within the vestigial optic chiasm. GnRH-ir fibres become diffuse rostral to the suprachiasmatic nuclei (SCN). Fibres are seen ventral and lateral to the SCN, none are found in the SCN. Caudal to the SCN, fibres begin forming a thin ventral aggregation as they proceed towards the median eminence. Further caudally, these fibres become denser arching towards the base of the third ventricle at the level of the median eminence. The internal part of the median eminence protrudes into the third ventricle, resulting in a bicornate recess at the base of the ventricle. GnRH-ir processes are particularly dense within the lateral margins of the median eminence.

The GnRH-ir immunoreactivity in the median eminence of the Cape mole-rat females in the breeding season is not significantly different from those out of the breeding season (Mann Whitney U-test, n1=4, n2=4, U=7, Z=-0.288, p=0.77). (Figure 5.4, Plate 5.2)

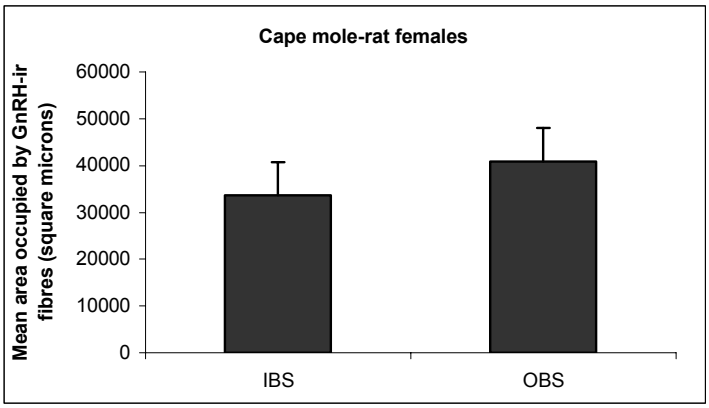

Figure 5.4: Mean area of GnRH immunoreactivity in the median eminence of female Cape mole-rats. IBS – in breeding season, OBS – out of breeding season.

Natal mole-rat

As in the Cape mole-rat, GnRH-ir fibres of the Natal mole-rat also have a beaded appearance. The general distribution of GnRH-ir fibres in the Natal mole-rat is very similar to that of the Cape mole-rat.

The Natal mole-rat appears to have a lower number of fibres in the area of the optic chiasm than the Cape mole-rat, while the area around the SCN in the Natal mole-rat has a higher density of GnRH-ir fibres than in the Cape mole-rat. The form of the median eminence differs between the two species. In the Natal mole-rat the median eminence does not appear to have the same protrusion into the third ventricle as the Cape-mole-rat.

In both the male and female Natal mole-rats, the GnRH-ir immunoreactivity of the median eminence was lower in the dominant animals compared to the subordinate animals. This difference was significant in the female animals (Mann Whitney U-test, n1=6, n2=6, U=1, p=0.006), and in the males (Mann Whitney U-test, n1=7, n2=8, U=8, p=0.02). (Figure 5.5, Plate 5.3, 5.4)

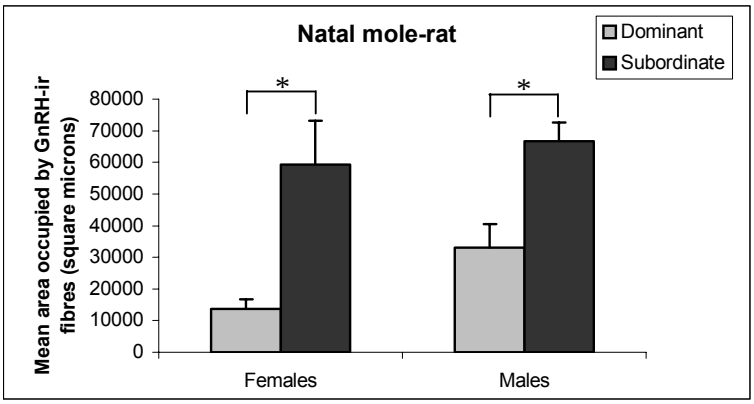

Figure 5.5: Mean area of the GnRH immunoreactivity in the median eminence of breeding and subordinate Natal mole-rats. (*=p<0.05)

- **Number and size of the GnRH cell bodies**

Cape mole-rats

The mean total number of GnRH-ir neurons observed in the Cape mole-rat brains was 423±35. There was no significant difference in number of GnRH-ir neurons between female Cape mole-rats in (394±58.6) and out (449.1±45.4) of the breeding season. (Mann-Whitney U-test, n1=6, n2=7, U=14.5, p=0.35) (Figure 5.6).

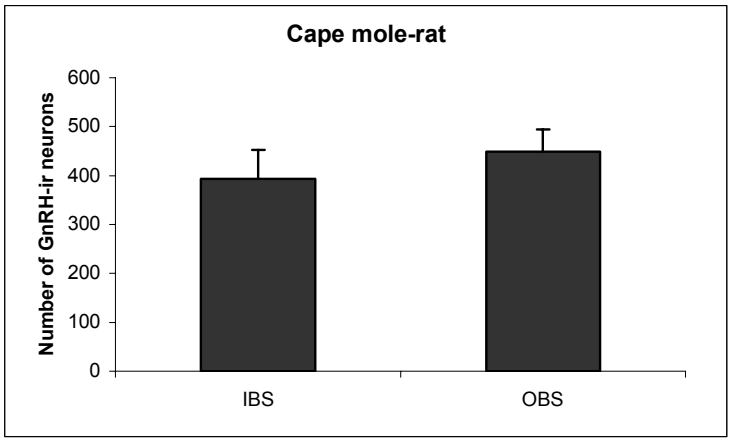

Figure 5.6: Comparison between the mean numbers of GnRH-ir neurons of female Cape mole-rats in (F BS) and out (F OBS) of breeding season.

Plate legends:

Plate 5.2: Rostrocaudal coronal sections from the brain of female Cape mole-rat (*Georychus capensis*) showing GnRH immunoreactive staining (a) in the medial septum (MS) and rostral preoptic area, (b) in the MS and region of the organum vasculosum lamina terminalis (OVLT), (c) in the medial preoptic area and a densely innervated site caudal to the OVLT and ventral to the third ventricle (3V), (d) in the structure forming the floor of the 3V in place of the optic chiasm, (e) in the region of the suprachiasmatic nucleus (SCN), (f-g) in the mediobasal hypothalamus (MBH) caudal to the SCN, (h-i) in the rostral median eminence (ME), (j-l) in the medial and caudal ME of female mole-rats out of breeding season (OBS), (m-o) in the medial and caudal ME of female mole-rats in the breeding season (IBS). Arrows (→) indicate GnRH perikarya.

Plate 5.3: Rostrocaudal coronal sections from the brain of female Natal mole-rats *(Cryptomys hottentotus natalensis*) showing GnRH immunoreactive staining (a) in the medial septum (MS) and rostral preoptic area, (b) in the MS and region of the organum vasculosum lamina terminalis (OVLT), (c) in the medial preoptic area and a densely innervated site caudal to the OVLT and ventral to the third ventricle (3V), (d) in the structure forming the floor of the 3V in place of the optic chiasm, (e) in the region of the suprachiasmatic nucleus (SCN), (f-g) in the mediobasal hypothalamus (MBH) caudal to the SCN, (h-i) in the rostral median eminence (ME), (j-l) in the ME and pituitary stalk (PS) of non-breeding females, (m-o) in the ME and PS of breeding females. Arrows (→) indicate GnRH perikarya.

Plate 5.4: Rostrocaudal coronal sections from the brain of male Natal mole-rats *(Cryptomys hottentotus natalensis*) showing GnRH immunoreactive staining (a) in the medial septum (MS) and rostral preoptic area, (b) in the MS and region of the organum vasculosum lamina terminalis (OVLT), (c) in the medial preoptic area and a densely innervated site caudal to the OVLT and ventral to the third ventricle (3V), (d) in the structure forming the floor of the 3V in place of the optic chiasm, (e) in the region of the suprachiasmatic nucleus (SCN), (f-g) in the mediobasal hypothalamus (MBH) caudal to the SCN, (h-i) in the rostral median eminence (ME), (j-l) in the ME and pituitary stalk (PS) of non-breeding males, (m-o) in the ME and PS of breeding males. Arrows (→) indicate GnRH perikarya.

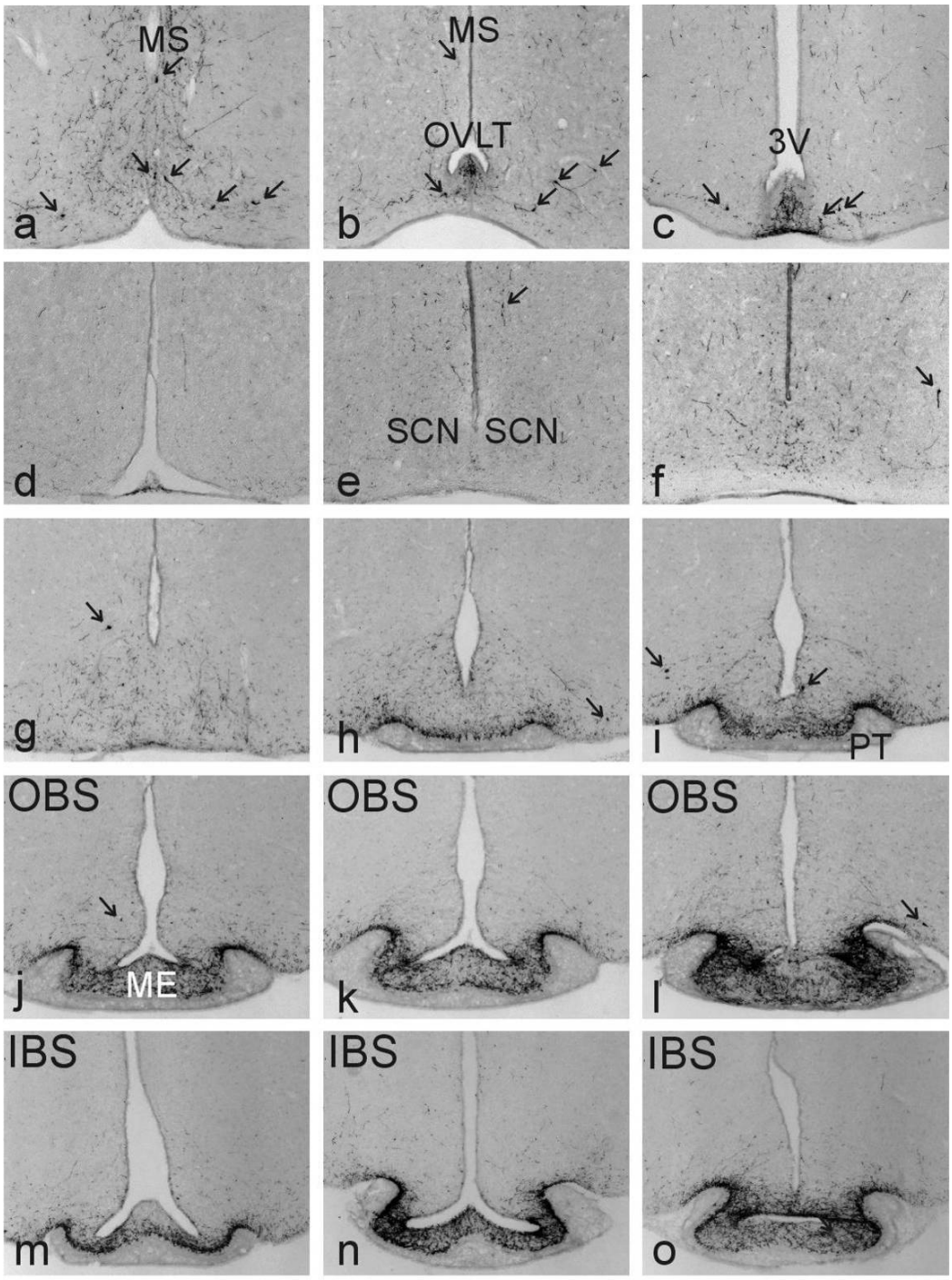
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Plate 5.2

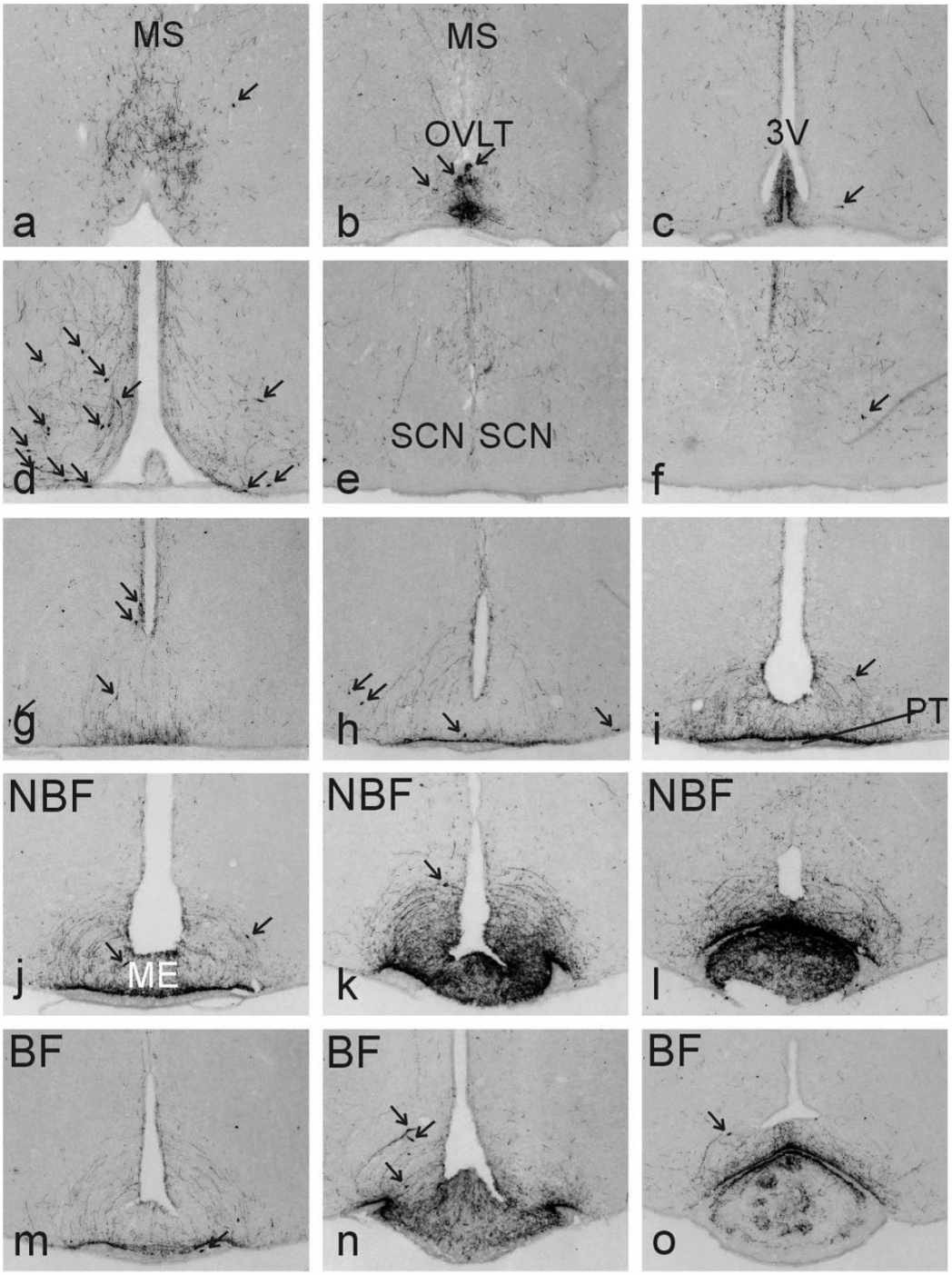
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Plate 5.3

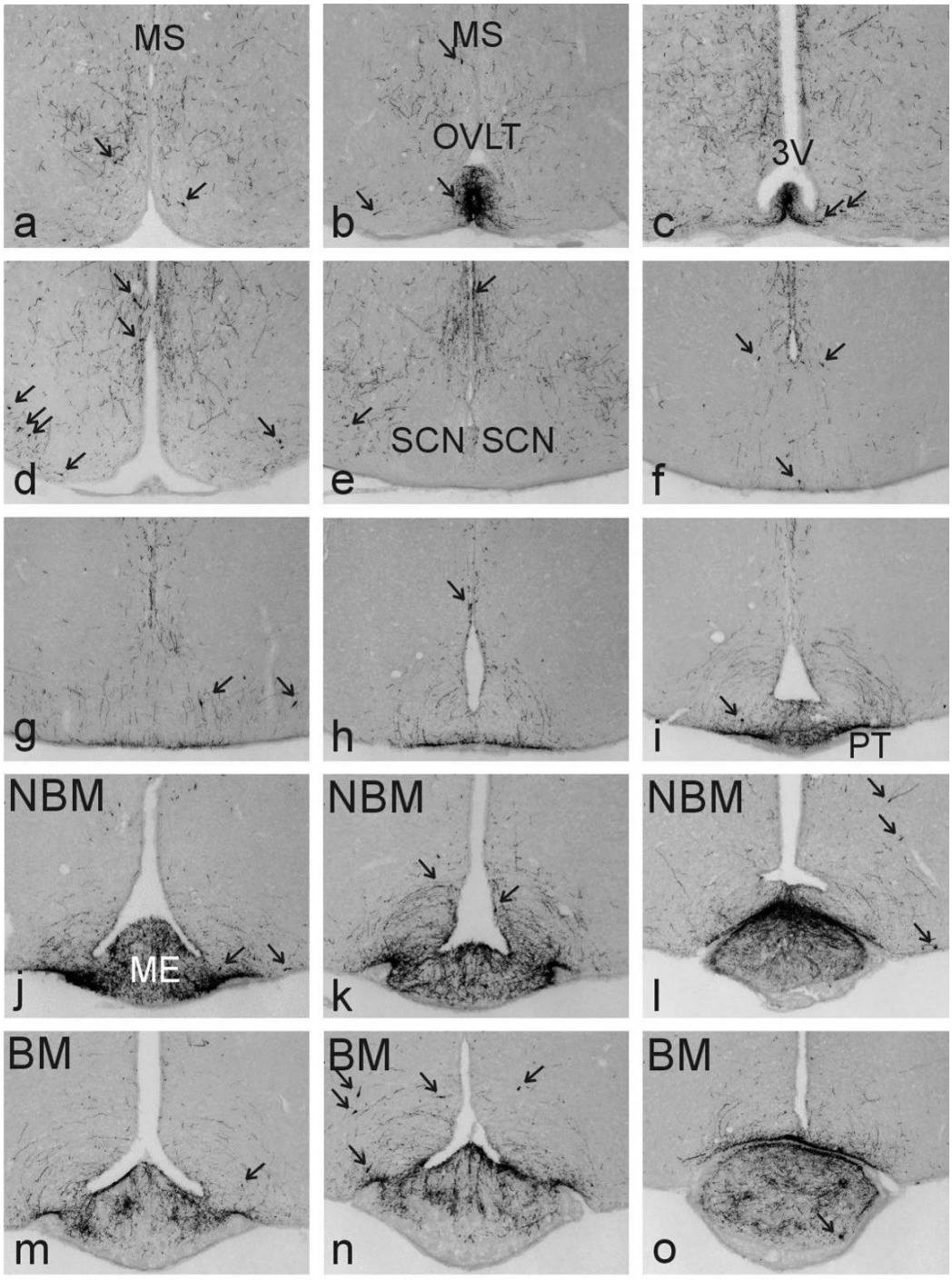
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Plate 5.4

The size of GnRH perikarya in female Cape mole-rats in the breeding season ranged from 88.77μm^2 to 113.23μm^2 (mean: 102.77±3.83μm^2); the range for the females out of the breeding season was 95.45μm^2 to 119.32μm^2 (mean: 109.93±3.11μm^2). There was no significant seasonal difference in the size of these cell bodies in the female Cape mole-rats (MWU, n1=6, n2=7, U=10, Z=1.571, p=0.116) (Figure 5.7).

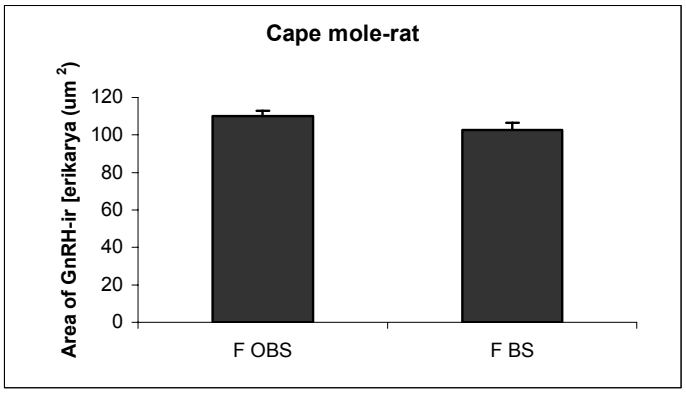

Figure 5.7: Mean size of GnRH-ir perikarya in the Cape mole-rat. F OBS – female, out of breeding season, F BS – female breeding season.

Natal mole-rat

The mean total number of GnRH cell bodies in the Natal mole rat was calculated as 721.07±41.1. No significant difference was observed in the number of cell bodies in dominant (females: 654±98.5; males: 714±72.7) and subordinate (females: 801±76.1; males: 733.7±89.2) Natal mole-rats of either sex (Females: n1=6, n2=7, U=13, Z=-1.143, p=0.253, males: n1=7, n2=8, U=27.5, Z=-0.0579, p=0.954) (Figure 5.8).

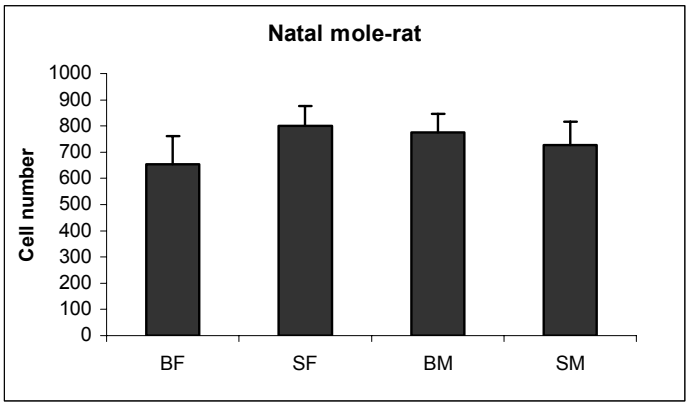

Figure 5.8: Comparison between the mean numbers of GnRH-ir neurons in the Natal mole-rat. BF – breeding females, SF – subordinate females, BM – breeding males, SM – subordinate males.

The Natal mole-rat has significantly more GnRH cell bodies than the Cape mole-rat (Mann Whitney U test, n1=18, n2=28, U=62, Z=4.276, p=0.000019).

The GnRH cell body size for breeding female Natal mole-rats ranged from 86.29 to 119.16μm^2 (mean: 100.47$\pm$5.1μm^2) and the range for the subordinate females was 86.09 to 113.77μm^2 (mean: 101.63$\pm$4.18μm^2). There was no significant difference in the neuron size between the breeding and subordinate females (MWU, n1=7, n2=6, U=21, Z=0, p=1).

The cell body size for breeding males varied from 86.29 to 113.24μm^2 (mean: 101.51$\pm$2.81μm^2) and subordinate males varied from 96.85 to 120.35μm^2 (mean: 105.52$\pm$3.63μm^2). Similarly, there was no significant difference between the breeding and subordinate males (MWU, n1=7, n2=8, U=22, Z=-0.694, p=0.479). Neither was there a significant difference between breeding

males and females (MWU, n1=7, n2=8, U=8, Z=0, p=1), or subordinate males and females (MWU, n1=7, n2=6, U=16, Z=-0.714, p=0.475) (Figure 5.9).

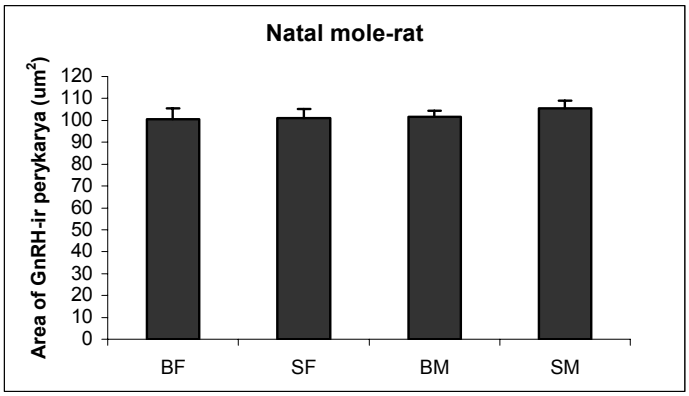

Figure 5.9: Difference in GnRH-ir perikarya size of the Natal mole-rat. BF - breeding females, SF - subordinate females, BM - breeding males, SM - subordinate males.

Discussion

The morphology and distribution of the GnRH systems of the solitary Cape mole-rat and the social Natal mole-rat is described as well as a quantification of the GnRH content in the median eminence. This is discussed and compared with regard to breeding season and reproductive status.

GnRH-ir neurons in both the Cape mole-rat and the Natal mole-rat are predominantly unipolar or bipolar. This finding is consistent with that described for the common mole-rat, the highveld mole-rat and the Damaraland mole-rat (DuToit *et al*. 2006, Molteno *et al*. 2004), as well as several other small mammal species (bat – Fernandez *et al*. 1992, white footed mouse - Glass *et al*. 1986; rat – Witkin *et al*. 1982; hamster - Yellon *et al.* 1990, Yellon & Newman 1991). While larger mammals tend to have more complex neuronal morphology (sheep - Wood *et al.* 1992, springbok -

Robinson *et al*. 1997, pony - Melrose *et al*., 1994), the importance thereof is not fully understood. It is thought that neurons with a more complex morphology have a greater potential of association formation, hence can be influenced by a greater number of neural inputs (Robinson *et al*. 1997).

In some seasonally breeding species, there is a seasonal change in the morphology of the GnRH neurons. In sheep a larger number of dendritic processes and a higher innervation of these processes are present during the period of anoestrous (Lehman *et al*. 1986, Xiong *et al*. 1997). In contrast, neither in the Cape mole-rat nor the Natal mole-rat, were any seasonal changes in the GnRH cell body morphology observed.

A higher number of GnRH-ir cell bodies are present in the brains of the Natal mole-rat compared with those of the Cape mole-rat (721±41 vs. 423±35). It has been proposed that the total number of GnRH-ir cell bodies is related to body size (Yellon & Newman, 1991). The Cape mole-rat does not conform to this trend in that, although this mole-rat is rather larger than the other mole-rat species currently investigated (mean body sizes: Cape mole-rat 180g; common mole-rat 60-80g, highveld mole-rat 90-110g, Natal mole-rat 100g, Damaraland mole-rat 130g; Jarvis & Bennett, 1991,1993), the number of GnRH-ir cell bodies is lower than most. The opposite situation occurs in the case of the highveld mole-rat, where there is an unusually high number of GnHR-ir neurons present for its body size (Du Toit *et al*. 2006). The number of GnRH cell bodies present in the Natal mole-rat brains is roughly equivalent to the number found in the Damaraland mole-rat (648±33; Molteno *et.al*. 2004) and the common mole-rat (605±60; DuToit *et.al*. 2006), but less than half of that found in the highveld mole-rat (1489±183; DuToit *et.al.* 2006). There is thus considerable interspecies variation in the GnRH cell body numbers of mole-rats. The mean total numbers of GnRH neurons of other small mammals

such as Djungarian hamsters (300-400, Yellon *et al*. 1990) and Syrian hamsters (650-750, Jennes & Stumpf 1980) fits well within this range, while larger mammalian species possess GnRH cell numbers of several thousands (Lehman *et al*. 1986, Robinson *et al*. 1997, Silverman *et al*. 1982).

There was no seasonal change in the size or number of GnRH-ir neurons in the Cape mole-rat or the Natal mole-rat. Similarly, the total number of GnRH-ir cell bodies in other seasonally breeding species such as the Djungarian hamster, prairie vole and Japanese wood mouse, were found to be similar in the breeding and non-breeding seasons (Yellon & Newman 1991, Kriegsfeld & Nelson 1999, Kuwahara *et al.* 2000). In contrast, the detectable GnRH-ir neuron population in the gerboa, a hibernating rodent, reduces by up to 55% in the POA in the autumn (El Quezzani *et al*. 2000).

GnRH perikarya in the Natal and Cape mole-rats were found to be of similar size. The GnRH cell body sizes of the highveld and common mole-rats were found to be slightly larger (Du Toit *et al*. 2006), whereas those of the Damaraland mole-rat had smaller perikarya (Molteno *et al*. 2004).

The GnRH cell body size and numbers did not differ between the reproductive and non-reproductive animals of either sex in the Natal mole-rat. This trend is consistent with those findings of other social mole-rat species with a reproductive division of labour (Du Toit *et al*. 2006, Molteno *et al.* 2004). Reproductive and non-reproductive springbok likewise have similar sized GnRH neurons (Robinson *et al*. 1997).

In the female Cape mole-rats, the size of the GnRH cell bodies did not differ in and out of the breeding season. Similarly, the season did not affect the GnRH-ir cell body size or number in social, seasonally breeding mole-rats (Du

Toit *et al*. 2006). In some seasonally breeding species, however, the secretion of GnRH is suppressed during the non-breeding season; this is associated with an increase in the net GnRH in the brain and an enlargement of the GnRH neurons (Syrian hamster - Urbanski *et al*. 1991, Japanese wood mouse – Kuwahara *et al.* 2000).

Although inter-specific differences are observed, characteristically the GnRH system is distributed in a loose continuum along the septo-preoptico-infundibular pathway in mammals (El Ouezzani *et al*. 2000, Davis *et al*. 1985, Caldani *et al*. 1988, Robinson *et al*. 1997). The degree of caudal migration of GnRH neurons during ontogenetic development varies within species, resulting in the relative presence of the neurons in the preoptic area and basal hypothalamus (Silverman *et al*. 1994). In general, GnRH cell bodies are similarly distributed in all mole-rat species investigated (this study, DuToit *et al*. 2006, Molteno *et al*. 2004). In both the Cape and the Natal mole-rat, GnRH perikarya are found throughout the medial septum, ventral and horizontal limbs of the diagonal band of Brocca and preoptic area to the mediobasal hypothalamus. Although there are large inter-species differences within the Bathyergidae, the majority of GnRH-ir cell bodies are found in the preoptic area, rostral in the region of the ventral diagonal band of Broca, and further caudally below the anterior commissure (Cape mole-rat:~90%, Natal mole-rat:~65%). The remainder of the GnRH perikarya are found further caudal in the mediobasal hypothalamus scattered around the perimeter of the third ventricle and around the SCN (not within). Although fibres are observed in the subfornical organ, no cell bodies are observed in this structure in either of the species.

Reproductive status does not appear to affect the distribution or morphology of GnRH neurons in the Natal mole-rat. Similarly, the GnRH systems of the

common mole-rat, highveld mole-rat and Damaraland mole-rat do not show alterations associated with social status in its distribution or morphology (Van der Walt 2003, Molteno *et al*. 2004).

Two main areas of dense innervation occur, namely the OVLT and the median eminence. In the Cape mole-rat, the GnRH fibres in the median eminence are not significantly more densely out of the breeding season. This suggests that the GnRH production is not affected by seasonal changes. Thus GnRH is produced and secreted during both the breeding and non-breeding seasons.

In the Natal mole-rat, the GnRH-ir fibre distribution in the median eminence was significantly higher in the non-breeding animals. This implies that the subordinate animals manufacture GnRH, but show an inhibition of its release into the portal blood capillaries to the pituitary.

Seasonally breeding species undergo a down-regulation of reproductive activity during the non-breeding season (Gerlach & Aurich 2000), and this is usually reflected in their reproductive physiology. Hypothalamic sensitivity towards steroid hormones alters, resulting in retention of GnRH in the median eminence, which inhibits the release of LH in the pituitary that in turn is responsible for the inhibition of gonadal hormones and gamete production.

Seasonally breeding mole-rats do not appear to undergo complete cessation of reproduction. Previous studies revealed that according to gonadal anatomy and hormonal responses to stimulation, the reproductive system is active during the non-breeding season (Hart & Bennett 2006, Spinks *et al*. 1997, 2000, Van der Walt *et al*. 2001, Janse van Rensburg *et al*. 2002). The hormonal profiles of the Cape mole-rat are consistent with what has been

found in other bathyergid species (previous chapter). Hence, not surprisingly, there are no differences in the neuroanatomy (neuron numbers and size) and neuroendocrinology (GnRH-immunoreactivity in the median eminence) of the Cape mole-rat brain in and out of the breeding season. Therefore, the Cape mole-rat does not conform to the classical depiction of a seasonal breeding mammal, but it compares well with other seasonal breeding bathyergids (Spinks *et al*. 1997, 1999, Janse van Rensburg *et al*. 2002).

Subordinate members of social mole-rat colonies are reproductively quiescent, but not sterile (Bennett *et al*. 1999). Social mole-rat species display a spectrum of socially induced infertility (Bennett *et al*. 1997). The degree and mechanism of reproductive suppression varies among different species. In the highly inbred naked mole-rat, subordinate animals are physiologically suppressed from breeding. Ovulation is blocked in subordinate females, and sperm production and motility are suppressed in males (Faulkes *et al*. 1990a, 1991). The pituitary response to a GnRH challenge is significantly less than in dominant animals (Faulkes *et al*. 1990b). All other mole-rat species are outbreeding (Bennett & Faulkes 2000), and mechanisms of suppression tend more towards incest avoidance as the degree of sociality in species subsides. The Damaraland mole-rat can be classified as eusocial, female subordinates are physiologically suppressed like in the naked mole-rat, while males do not show any physiological constraints that prevent them from reproducing (Bennett *et al*. 1994, Bennett *et al*. 1996). The Natal mole-rat has two closely related sister species, the common mole-rat and the highveld mole-rat. Whereas subordinate animals from both these species exhibit a significant response to a GnRH challenge (Spinks *et al*. 2000, Van der Walt *et al*. 2001), the difference of the response to exogenous GnRH was significant between dominant and subordinate females in the highveld mole-rat (Van der Walt *et al*. 2001). Also, social status was reflected in the density of GnRH-

immunoreactivity in the highveld mole-rat, but not the common mole-rat (Du Toit *et al*. 2006). It therefore appears that the degree of sociality-induced infertility of the Natal mole-rat lies somewhere between these two species. While there is no difference in the magnitude of the response to exogenous GnRH (previous chapter), there is an unambiguous difference in the density of the GnRH-immunoreactivity in the median eminence between dominant and subordinate animals of both sexes.

Behaviourally, both species under investigation display a form of regulated reproduction, breeding in the Cape mole-rat is restricted to a specific part of the year, while subordinate individuals of the Natal mole-rats repressed from reproduction, presumably as a result of incest taboos. However, neuroendocrinologically, reproductive regulation is only reflected in subordinate animals of the Natal mole-rat.