# Reproductive status affects the expression of prolactin receptor mRNA in the brain of female Damaraland mole-rats

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**Short title:** Breeding status affects prolactin receptor expression

## Highlights

- Plasma prolactin levels similar in breeding and nonbreeding Damaraland mole-rats.
- Reproductive suppression associated with differential neural Prlr gene expression.
- Elevated Prlr levels in choroid plexus and arcuate nucleus of suppressed females.

**Abstract**

The eusocial Damaraland mole-rat (*Fukomys damarensis*) represents an extreme example of reproductive skew, in that reproduction is completely blocked in female subordinate group members. It is thought that in these animals normal GnRH secretion from the hypothalamus is disrupted. Prolactin, a peptide hormone secreted from the anterior pituitary gland, has been implicated in a wide variety of functions. Well documented in rodents is its role in mediating lactational infertility. Elevated circulating prolactin levels, such as during lactation, are associated with reduced GnRH release into the portal blood and with a reduction in the frequency and amplitude of LH pulses. The present study aimed at investigating whether such a mechanism could act in reproductively suppressed female Damaraland mole-rats. By means of *in situ* hybridisation we studied the distribution and gene expression of the prolactin receptor (*Prlr*) in wild-caught female Damaraland mole-rats with different reproductive status. Substantial *Prlr* expression was found in several brain regions, with highest levels in the choroid plexus and moderate expression in the preoptic and tuberal hypothalamus. While in reproductive and non-reproductive females plasma prolactin levels were very low and not significantly different, quantification of the *Prlr* hybridisation signal revealed significant differences in relation to reproductive status. Reproductively suppressed females had increased expression of *Prlr* in the choroid plexus and in the arcuate nucleus (ARC) when compared to reproductive females. This suggests higher local prolactin levels in the brain of suppressed females. Together with previous findings, it could indicate that prolactin inhibits ARC kisspeptin neurons, which then would lead to reduced activation of GnRH neurons in such females.

**KEYWORDS**: prolactin receptor, social status, *in situ* hybridization, *Fukomys damarensis*, prolactin

## 1. Introduction

In mammals, prolactin receptors have been found in a wide variety of tissues and consequently, numerous biological functions of prolactin have been described (Kelly et al. 1991; Bole-Feysot et al. 1998; Freeman et al. 2000). The neuroendocrine functions of prolactin include the stimulation of maternal behaviour, suppression of fertility, suppression of stress response, regulation of food intake and body mass and activation of hypothalamic dopaminergic neurons to regulate its own secretion (Grattan and Kokay 2008). Well documented in humans and rodents is the suppression of reproduction during lactation, where high circulating levels of prolactin are associated with an inhibition of gonadotropin secretion and leading to anoestrus or amenorrhoea. Similar effects are also observed in hyperprolactinemic non-lactating females (McNeilly 1980, 2001).

In group-living mammals, reproductive suppression, where dominant individuals inhibit the reproductive capabilities of subordinates via behavioural and other interactions, is commonly found (Keller and Reeve 1994). Extremes of high reproductive skew are represented by societies with a single breeding pair and helpers, such as in callitrichid primates (Abbott 1984), mongooses and meerkats (Rasa 1973, Doolan and Macdonald 1997) and African mole-rats (Bennett and Faulkes 2000). The mole-rats of the family Bathyergidae exhibit a wide range of social behaviour and restricted reproduction has been found in several species (Bennett and Faulkes 2000). Highly social Damaraland mole-rats (*Fukomys damarensis*) represent an extreme example of reproductive skew, in that reproduction is completely blocked in female subordinate group members (Bennett 1994, Bennett et al. 1994). This blockade results from an inhibition of ovulation, which is caused by a disruption in the normal GnRH secretion from the hypothalamus (Molteno et al. 2004). Despite the potential for prolactin in being involved in the mechanism of reproductive suppression in highly social African mole-rats, its possible role remains elusive.

In rats and mice, elevated circulating levels of prolactin (hyperprolactinemia) are associated with a reduction in the frequency and amplitude of LH pulses and a suppression of GnRH release into the portal blood (Koike et al. 1984, Cohen-Becker et al. 1986, Fox et al. 1987, Grattan et al. 2007). The reduced activity of the GnRH neurons is considered the principal cause of infertility. This suppressive effect of prolactin is most likely mediated indirectly through prolactin-sensitive afferents such as kisspeptin because only a small percentage of GnRH neurons express the prolactin receptor (Prlr, Grattan et al. 2007, Kokay et al. 2011). Prolactin secretion is under the control of dopamine, which is produced in several distinct hypothalamic dopaminergic neuron populations that express prolactin receptor. Increased prolactin levels lead to the activation of the tuberoinfundibular dopaminergic neurons (TIDA) and to increased dopamine synthesis, which after transport to the anterior pituitary binds to type-2 dopamine receptors and inhibits prolactin secretion. During lactation, the state of physiological hyperprolactinemia, this feedback mechanism is not functional and the elevated prolactin level does not activate TIDA neurons (Grattan and Kokay 2008).

Kisspeptin, a product of the *Kiss1* gene, is a strong activator of GnRH neurons and is considered essential for reproduction and the timing of puberty onset in mammals. *Kiss1*-expressing neuron populations have been found mainly in two regions of the hypothalamus, the anteroventral periventricular nucleus (AVPV) and in the arcuate nucleus (ARC, Smith et al. 2006). *Prlr* is expressed in both *Kiss1* neuron populations (Kokay et al. 2011). In lactating rats, when prolactin levels are elevated, there is a pronounced upregulation of Prlr protein and downregulation of *Kiss1* mRNA and protein expression in the ARC (Pi and Grattan 1999a, Yamada et al. 2007, Araujo-Lopes et al. 2014). Moreover, chronic infusion of prolactin in female mice has been found to suppress oestrus cyclicity, GnRH and LH release and to reduce hypothalamic *Kiss1* mRNA expression (Sonigo et al. 2012). These data indicate that the prolactin-induced suppression of GnRH release is mediated by inhibition of *Kiss1* neurons.

In Damaraland mole-rats we have previously shown that reproductive status affects the neuroendocrine phenotype of females with reproductively suppressed females having reduced gene expression of steroid hormone receptors in several preoptic-hypothalamic brain regions, including the AVPV and the ARC. Moreover, such females also exhibit reduced *Kiss1* expression in the ARC compared to reproductively active females (Voigt et al. 2014, Voigt and Bennett 2018). In another highly social African mole-rat, the naked mole-rat (*Heterocephalus glaber*), transcriptome profiling revealed reduced expression of genes involved in dopamine metabolism in reproductively suppressed subordinate females, which remain in a pre-pubertal anovulatory state (Mulugeta et al. 2017). The present study in Damaraland mole-rats aimed at investigating whether prolactin could play a role in the mechanism of reproductive suppression of subordinate females in this species. We measured circulating prolactin levels and we used *in situ* hybridization to determine the distribution and gene expression of *Prlr* in the forebrain of reproductively active and reproductively suppressed females.

## 2. Materials and Methods

### *2.1. Animals*

In the current study, adult female Damaraland mole-rats (*Fukomys damarensis*) were used. Data were obtained from nine reproductives (breeder) and nine nonreproductives (nonbreeder). The females came from colonies that were captured between April and July 2015. Mole-rats were captured near the village of Black Rock, Northern Cape, South Africa (27°7´S, 22°50´E) with Hickman live-traps under permission from Northern Cape Nature Conservation authorities. Prior to sacrifice, animals were housed for a maximum period of 12 weeks in captivity under a 12L:12D cycle at 25° in plastic containers (1.0m x 0.5m x 0.5m) containing wood shavings and they were fed on sweet potato, gem squash and apples. In each colony, the reproductive status was determined for all adults. Breeding females (syn. queens)

could be readily distinguished from non-reproductive females by the presence of a perforate vagina and prominent teats. None of the females were pregnant or lactating at the time of sacrifice. All animals were kept in their original colonies until sacrifice. At the time of brain collection, body mass of all animals was recorded to the nearest gram. All experimental procedures were approved by the University of Pretoria Animal Ethics Committee (EC003-12).

*2.2. Blood sampling and hormone assay*

Upon decapitation of the animal, trunk blood was collected into heparin-coated collection tubes. Blood samples were centrifuged, the plasma collected and frozen at –40°C until hormone assays were conducted. Plasma samples were assayed for prolactin using the commercially available enzyme-linked immunosorbent assay (Elabscience© Guinea pig prolactin ELISA kit, Catalogue No: E-EL-GP0358) according to the manufacturer's instructions. The prolactin assay had previously been validated for *F. damarensis* (Bennett et al. 2018). The sensitivity of the assay was 0.1 ng/ml, the detection range 0.16-10 ng/ml, and coefficient of variation for repeatability was < 10%.

*2.3. Brain histology*

Mole-rats were killed by decapitation using a small mammal commercially available guillotine, brains were dissected out of the skull, immediately frozen on dry ice and stored at -80°C until used. Before sectioning, brain mass was recorded to the nearest milligram. Frozen brains were cut on a cryostat into 20 µm coronal sections. The plane of the sections was adjusted to match as closely as possible the plane of the rat brain atlas (Paxinos and Watson 2007). Sections were mounted onto Superfrost Plus slides (Menzel-Gläser, Braunschweig, Germany). *In situ* hybridization was carried out on adjacent series of sections for the localization of prolactin receptor (*Prlr*) mRNA.

*2.4. Cloning of cDNA probes*

Based on sequence information available from Damaraland mole-rat, PCR was used to amplify a fragment of the *Prlr* gene. Total RNA was extracted from the mole-rat hypothalamus by using the RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany). The synthesis of first-strand cDNA was done with SUPERSCRIPT III Reverse Transcriptase (Invitrogen, Karlsruhe, Germany) and random primers. The resulting RNA-DNA hybrids were subsequently used in PCR to generate pieces of the appropriate gene. The forward primer was 5'-CCAGGAACCAACGGAGGACT-3' and the reverse primer was 5'-AGCCACTGCCCAGACCATAA-3'. PCR was carried out for 40 cycles by using the following parameters: 94°C for 1 minute, 55°C for 45 seconds, 72°C for 1 minute. The amplified fragment was purified and cloned into the pCRII TOPO vector using the TOPO-TA cloning kit (Invitrogen, Karlsruhe, Germany). Resultant clones were sequenced to verify the authenticity and fidelity of the amplification. The cloned *Prlr* sequence [GenBank: MF944110] is 624bp in length and shows 78% homology with mouse prolactin receptor [GenBank: NM_011169.5]. Different isoforms of the prolactin receptor have been described in several species including rat and mouse, which differ mainly in the composition of the cytoplasmic domain (Bole-Feysot et al. 1998). Our *Prlr* sequence covers the extracellular and transmembrane domains (exons 4-8), which are common to short and long forms. Therefore, both isoforms are detected with our probe.

*2.5. In situ hybridization*

The expression of prolactin receptor in brain sections was detected with antisense RNA probes labeled with $^{35}$S-CTP as described before (Voigt et al. 2014). Labeling of the probes with $^{35}$S-CTP (1250 Ci/mmol; Perkin Elmer, Rodgau, Germany) was performed using the Riboprobe System (Promega). Our *in situ* hybridization procedure followed a previously published protocol (Whitfield et al. 1990) with modifications as described elsewhere (Gahr

and Metzdorf 1997). For signal detection, sections were exposed to autoradiographic film (Kodak Biomax MR, Rochester, NY, USA) for 7 weeks. Brain sections from reproductive and non-reproductive animals were run through the entire procedure at the same time and placed on each autoradiographic film to avoid any possible effect of small differences in procedures on the observed group differences. Control sections processed with the sense probes were obtained from two female mole-rats and were labelled by the same procedure as described above. Autoradiograms from these sections showed no signal. These control data will therefore not be discussed below.

*2.6. Data analysis*

Images from autoradiograms were scanned with an Epson Perfection V750 Pro scanner connected to a PC running the image analysis software Image J 1.43u (NIH, USA; see http://rsb.info.nih.gov/ij/). Before acquisition the system was calibrated by using a calibrated optical density step tablet (T2115CC; Stouffer Industries, Inc., Mishawaka, IN, USA) and a calibration curve was fitted with the Rodbard function of Image J [y=d+(a-d)/(1+(x/c)^b)]. This calibration was applied to all images and it extended beyond the darkest spot to be measured in the autoradiograms so that the signals that were measured never reached saturation. Regions of interest in each section (defined by the presence of a denser signal density than surrounding areas) were delineated on screen with the computer mouse and their average optical density (OD) was calculated by built-in functions of the software. Background optical density of the film was measured in a rectangular area ($1mm^2$) in the same image immediately ventral to the brain section of interest. Final OD measurements were obtained by subtracting the film background OD value from the OD value of the region of interest and represent the average measurement from both hemispheres. Brain regions were identified using the atlas of the rat (Paxinos and Watson 2007). Before analysis, sections of all animals were anatomically matched according to the anterior commissure (AC) at the level where it

reaches its largest extension. For quantification of the mRNA expression levels in the preoptic area (POA), the ARC, the ventrolateral part of the ventromedial nucleus (VMHvl) and the medial nucleus of the amygdala (MeA), adjacent sections along the rostro-caudal axis were measured throughout the extent of the labelling that was visible. Labelling in the POA comprised the AVPV and the medial preoptic nucleus (MPO). For anterior and posterior bed nuclei stria terminalis (aBST, pBST) and for the paraventricular nucleus (PVH), three consecutive sections were measured, respectively. For the choroid plexus (ChP), three consecutive sections at the level of the anterior commissure were measured.

### *2.7. Statistical analysis*

Statistical analyses were carried out using JMP software. Data are presented as means ± SEM. *Prlr* gene expression was analysed by using a REML-model with reproductive status (breeder vs. non-breeder) and brain region (POA, VMHvl, PVH, ARC, BST, MeA, ChP) as fixed factors and animal ID as random factor. Post hoc analyses were performed with the 'test slices' comparison in JMP. All tests were two-tailed and the significance level was fixed at $p<0.05$.

## 3. Results

### *3.1. Morphological measurements and plasma prolactin levels*

Females of different status differed significantly in body mass with breeders (115.8 ± 3.1g) being heavier than nonbreeders (83.3 ± 4.0g, $t=6.32$, $df=16$, $P<0.0001$). No significant group differences were found in brain mass ($t=1.24$, $df=16$, $P=0.23$). Plasma prolactin levels of female breeders and nonbreeders were in most animals below the detection limit of the assay. Detectable values were only found in three breeders (mean: 1.98±1.71 ng/ml, range: 0.27-5.40 ng/ml) and three nonbreeders (mean: 0.27±0.06 ng/ml, range: 0.17-0.37 ng/ml). The highest prolactin plasma level found was 5.40 ng/ml, which came from a breeding individual.

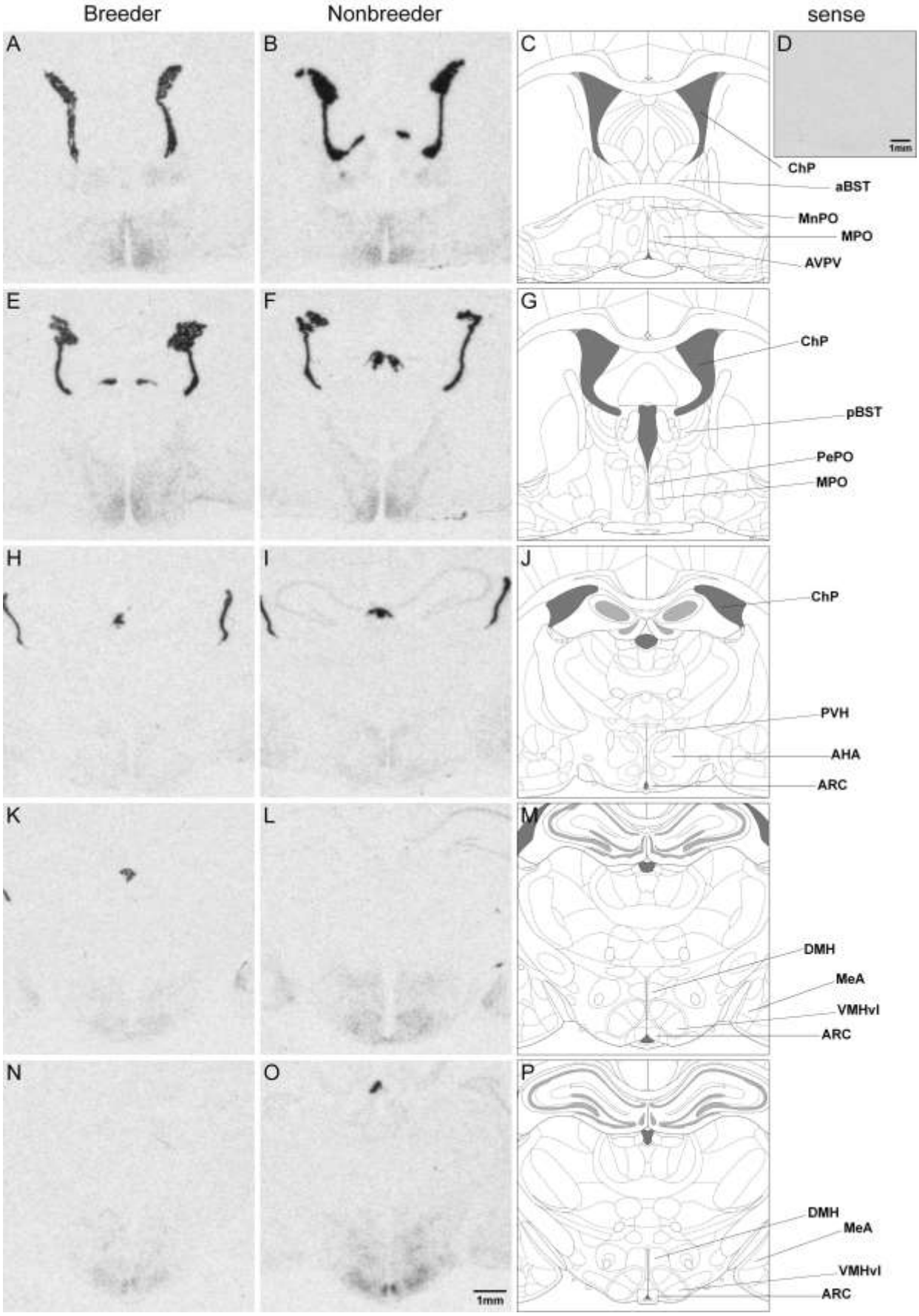


**Figure 1.** Brightfield photomicrographs of autoradiograms from coronal sections through the mole-rat brain illustrating the distribution of *Prlr* mRNA visualized by *in situ* hybridization. Panels illustrate sections from a

breeding female (A, E, H, K, N) and a reproductively suppressed female (B, F, I, L, O) from the level of the preoptic area to the caudal hypothalamus. D: a section hybridized with the sense probe is shown. Abbreviations: AHA, anterior hypothalamic area**;** ARC, arcuate nucleus; AVPV, anteroventral periventricular nucleus; aBST, bed nucleus of the stria terminalis, anterior part; pBST, bed nucleus of the stria terminalis, posterior part; ChP, choroid plexus; DMH, dorsomedial nucleus; MeA, medial amygdaloid nucleus; MnPO, median preoptic nucleus; MPO, medial preoptic nucleus; PePO, preoptic periventricular nucleus; PVH, paraventricular nucleus; VMHvl, ventromedial hypothalamic nucleus, ventrolateral part.

### *3.2. Distribution of prolactin receptor mRNA*

Within the hypothalamus, *Prlr* expression was most pronounced in the preoptic hypothalamus comprising AVPV and the MPO and in the tuberal hypothalamus comprising the ARC and the VMHvl. In other hypothalamic areas weak signals were found in the median preoptic nucleus (MnPO), the preoptic periventricular nucleus (PePO), the anterior hypothalamic area (AHA), the PVH and the dorsomedial nucleus (DMH). Outside the hypothalamus, very strong labelling of *Prlr* was visible in the ChP. Weaker signals occurred in the anterior and posterior part of the BST, the lateral septum and in the MeA (Fig. 1).

### *3.3. Gene expression related to reproductive status*

Measurement of the intensity of the *Prlr* hybridisation signal in the six different brain areas revealed significant group differences (Fig. 2). The analysis of the average optical density revealed no overall effect of status ($F_{1,16}$=4.39, p=0.053), but a significant effect of the brain region ($F_{6,96}$=398.07, p<0.001) and a significant interaction between status and region ($F_{6,96}$=3.53, p=0.003). Reproductively suppressed females had significantly elevated *Prlr* expression in the ChP and in the ARC compared to breeding females. No significant group differences were found in other brain regions.

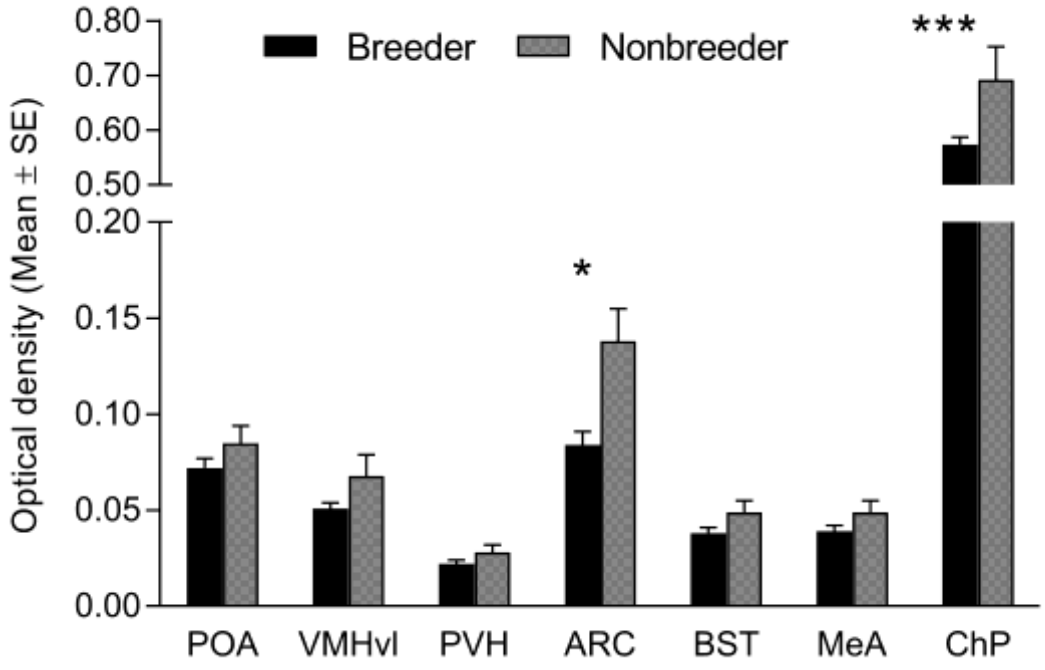


**Figure 2**. Average optical density of the hybridization signal for *Prlr* in different brain regions of breeding females and reproductively suppressed females. * $p<0.05$, *** $p< 0.001$.

## DISCUSSION

Here we report for the first time the mRNA expression patterns of *Prlr* in the brain of female Damaraland mole-rats. In this species, female subordinates, in the presence of the queen, experience strong physiological suppression to the extent of being anovulatory (Bennett 2011). While circulating prolactin levels are very low in both groups of females, by using *in situ* hybridization, we identify a reproductive status-related difference in *Prlr* expression in the ChP and in the ARC, which could play a role in the mechanism of suppression of reproductive behaviour.

### Prolactin levels

Studies in humans and rodents have shown that hyperprolactinemia, a condition of elevated circulating prolactin levels, causes a reduction of GnRH and LH secretion and leads to anovulation (Grattan 2015). The physiologically high level of prolactin during lactation is

maintained because the TIDA neurons in the ARC no longer release dopamine, which under normal conditions inhibits prolactin release (Romano et al. 2013). The hormone data from the present study in Damaraland mole-rats provide no evidence for elevated circulating prolactin levels indicative of hyperprolactinemia in reproductively suppressed females. In both groups of females, circulating prolactin levels were very low or undetectable. It is a limitation to our study that we were not able to assess the oestrous cycle stages using vaginal cytology, therefore, it must be assumed that breeders were in different stages of their oestrous cycle. In rats and mice, circulating prolactin levels peak during proestrous (Butcher et al. 1974; Michael 1976).

It is possible that the hormone level measured in the circulation does not reflect the hormone level in the brain and, thus, reproductively suppressed females could indeed maintain much higher local prolactin levels in the brain extracellular space and in the CSF than those found in the circulation. Prolactin can enter the brain not only from the peripheral circulation, but could reach the hypothalamus and act particularly on the ARC by diffusion from the fenestrated capillaries of the portal blood system in the median eminence. Very high prolactin levels (4900 ng/ml) are found in the portal plasma of rats while prolactin levels in the pheripheral circulation are low (25 ng/ml; Oliver et al. 1977). Moreover, prolactin can also be produced in the brain (Marano and Ben-Jonathan 2014). Analysis of the brain gene expression of tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, would indicate whether reproductive and non-reproductive females differ in the extent of inhibition by dopamine of prolactin release.

**Distribution of *Prlr* expression**

The distribution of the *Prlr* mRNA short form and long form has been previously described in the rat and mouse brain (Chiu and Wise 1994, Bakowska and Morrell 1997, Bakowska and Morrell 2003, Brown et al. 2010). The results of the present study in mole-rats, which reports

very high expression in the ChP and moderate expression in the preoptic and tuberal hypothalamus, largely agrees with these previous studies. Our probe was designed to detect both the long and short form of the prolactin receptor, therefore we cannot distinguish between their distribution. However, it has been shown that in most areas the distribution of the two isoforms is overlapping (Bakowska and Morrell 2003). One discrepancy to previous studies is the lack of expression in the supraoptic nucleus of mole-rats, which could be explained by the lower sensitiviy of the autoradiographic detection compared to that of silver grains in emulsion coated slides. Interestingly, the distribution of the *Prlr* gene matches to a large extent the distribution of steroid hormone receptors in the brain of the Damaraland mole-rat. Strong signals of androgen receptor (AR), estrogen receptor α (ERα) and progesterone receptor (PGR) mRNA expression were previously found in the AVPV, MPO, VMHvl, ARC, BST and MeA (Voigt et al. 2014, Voigt et al. 2016). In rats, oestradiol (E2) and progesterone are involved in the regulation of prolactin receptor gene expression in the brain (Sugiyama et al. 1994; Pi and Voogt 2002; Anderson et al. 2008) and this could also apply to mole-rats.

**Breeding status affects *Prlr* expression**

Quantification of the hybridization signal revealed significant upregulation of the *Prlr* gene in the ChP and in the ARC of reproductively suppressed females compared to breeding females despite very low and not significantly different circulating prolactin levels. Previous studies, administering radio-labelled prolactin to rats and mice have shown that high prolactin levels in the peripheral circulation result in increased prolactin uptake at the ChP, measured as the amount of radio-labelled prolactin found in the cerebrospinal fluid (Walsh et al. 1978; Mangurian et al. 1992; Brown et al. 2016). Moreover, during lactation, a physiological condition of chronically high plasma prolactin levels, *Prlr* mRNA and protein expression at the ChP are significantly increased (Pi and Grattan 1999a; Pi and Grattan 1999b; Augustine et

al. 2003; Tabata et al. 2012). Also, Prlr protein expression in the rostral and caudal ARC is increased during lactation (Pi and Grattan 1999b). In the light of these data, our findings of increased *Prlr* expression in the ChP and ARC of reproductively suppressed females could indicate that local prolactin levels in the brain and CSF are elevated in these females. Alternatively, the low circulating prolactin levels found in these females may reflect the higher uptake of the hormone by Prlr in the brain.

High prolactin levels, such as during lactation, have been shown to suppress *Kiss1* mRNA expression in the ARC and to lead to reduced LH release in rats and mice (Yamada et al. 2007; Sonigo et al. 2012; Araujo-Lopes et al. 2014; Brown et al. 2014). Prolactin acts directly on ARC *Kiss1* neurons as shown by coexpression of *Prlr* and by induction of pSTAT5 protein expression, a marker for the activation of the long form prolactin receptor in such neurons (Kokay et al. 2011; Araujo-Lopes et al. 2014). Furthermore, treatment of hypothalamic explants containing the median eminence, the ARC and VMH, with prolactin reduces GnRH release and this effect can be reversed by addition of kisspeptin (Sonigo et al. 2012). Kisspeptin is a strong activator of GnRH neurons and is considered essential for reproduction and the timing of puberty onset in mammals (Smith et al. 2006). In Damaraland mole-rats, it is thought that reproductive suppression in subordinate females results from an inhibition of GnRH release from the hypothalamus. In support of this hypothesis, we recently reported reduced *Kiss1* expression and fewer *Kiss1*-expressing cells in the ARC of suppressed compared to breeding females (Voigt and Bennett 2018). Together, these data could indicate that in the former elevated prolactin levels in the brain lead to activation of the *Prlr* and inhibition of *Kiss1* in ARC kisspeptin neurons, which in turn leads to reduced activation of GnRH neurons and reduced LH release from the pituitary. Future studies involving double-label *in situ* hybridization should confirm colocalization of *Prlr* and *Kiss1* in ARC of Damaraland mole-rats. We previously argued that a long-term negative feedback effect of E2 could be acting on the caudal ARC neurons of reproductively suppressed Damaraland mole-

rats, leading to decreased levels of *Kiss1* gene expression (Voigt and Bennett 2018) as has been proposed for seasonally anoestrus ewes (Smith et al. 2007). However, the present findings are not contradictory because there is increasing experimental evidence that besides high circulating prolactin levels additional factors must contribute to the suppression of kisspeptin neurons during lactation (Araujo-Lopes et al. 2014; Brown et al. 2014).

Neurons expressing *Prlr* also exist in the rostral preoptic area, being part of the A15 dopaminergic neurons, which coexpress kisspeptin (Clarkson and Herbison 2011; Brown et al. 2015). In mice, the increased prolactin level during lactation results in suppression of kisspeptin synthesis in these neurons and reduced stimulation of GnRH neurons in this area (Liu et al. 2014). The *Kiss1* neurons in the rostral preoptic area are critical for stimulating the preovulatory GnRH/LH surge. Therefore, one could assume that suppression of these neurons could explain anovulation in subordinate female Damaraland mole-rats. Although we previously found a region-specific downregulation of GnRH mRNA in the rostral preoptic area of reproductively suppressed females (Voigt and Bennett 2017), the lack of differences in *Kiss1* expression in this area (Voigt and Bennett 2018) together with a lack of *Prlr* upregulation in the present study makes it unlikely that these neuron populations play a major role in a possible prolactin-induced infertility.

In conclusion, we have shown that breeding status affects neural *Prlr* expression in female Damaraland mole-rats. Although we find no evidence for elevated circulating prolactin levels, the increased *Prlr* expression at the ChP and in the ARC suggest higher prolactin levels in the brain of suppressed females. We propose that a mechanism similar to lactation-induced infertility is acting in such females, whereby elevated brain prolactin levels reduce Kiss1 neuron activity in the ARC, leading to reduced activation of GnRH neurons and reduced LH release from the pituitary. Further experimental work is necessary to substantiate this hypothesis.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

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