The Curious Case of the Naked Mole-Rat: How Extreme Social and Reproductive Adaptations Might Influence Sex Differences in the Brain

Phoebe D. Edwards, Ilapreet Toor, and Melissa M. Holmes

Contents

Abstract Research in the neurobiology of sex differences is inherently influenced by the study species that are used. Some traditional animal research models, such as rats and mice, show certain sex differences in the brain that have been foundational to neurobiological research. However, subsequent work has demonstrated that these differences are not always generalizable, especially to species with different social structures and sex-associated roles or behaviors. One such example is the naked mole-rat (*Heterocephalus glaber*), which has an unusual social structure among mammals. Naked mole-rats live in large groups where reproduction is restricted to a dominant female, called the "queen," and often only one breeding male. All other animals in the group, the "subordinates," are socially suppressed from reproduction and remain in a prepubescent state as adults, unless they are removed from the presence of the queen. These subordinates show little to no sex differences in external morphology, neural morphology, or behavior. However, there are a suite of neurobiological differences between subordinate and breeding naked mole-rats. After naked mole-rats attain breeding status, many of the classically sexually differentiated brain regions increase in volume (paraventricular nucleus, medial amygdala, bed nucleus of the stria terminalis). There are additionally social status differences in sex hormone receptor expression in the brain, as well as other changes in gene expression, some of which also show sex differences – though not always in

P. D. Edwards, I. Toor, and M. M. Holmes (✉)
Department of Psychology, University of Toronto Mississauga, Mississauga, ON, Canada
e-mail: melissa.holmes@utoronto.ca

Curr Topics Behav Neurosci
https://doi.org/10.1007/7854_2022_310

the predicted direction based on other rodent studies. Data from naked mole-rats show that it is critical to consider the evolved social structure of a species when studying sex differences in the brain.

Keywords Cooperative breeding · Eusocial · Mole-rat · Plasticity · Sex difference · Social status

Sex differences in the brain are traditionally interpreted through the lens of sex differences in behavior. In many species, males and females differ in levels of competition for access to mating opportunities, their particular roles in reproduction, and the specific behaviors associated with these processes. Thus, it is not unreasonable to postulate that sex differences in the brain would correspond to these sex differences in behavior. Foundational work in traditional laboratory rodent models (rats, mice, guinea pigs) has provided support for this framework. For example, in rats and several other species, the sexually dimorphic nucleus of the medial preoptic area (SDN-POA) is significantly larger in males than in females (Greenberg and Trainor 2016). Accordingly, lesioning of this area in rats impairs male-typical sexual behaviors, including mounting, intromission, and ejaculation (De Jonge et al. 1989). There are also female-biased examples: in mice and rats, females have more cells involved in dopamine synthesis in the anteroventral periventricular nucleus (AVPV) of the brain, and this population of cells is functionally associated with levels of maternal behavior (Simerly 1989; Forger et al. 2004; Scott et al. 2015).

Such sex differences in the brain are predominantly shaped during critical developmental periods. Neural sex differences are often derived through an organizational-activational process, where the brain is organized by gonadal hormones early in development (prenatal and early postnatal life) and is then modulated by gonadal hormones in adulthood (Phoenix et al. 1959; Arnold 2009). Increasingly, puberty has been considered a second or extended critical period of brain organization, where circulating sex hormones alter structure and circuits in numerous areas of the brain (Sisk and Zehr 2005; Schulz et al. 2009). Puberty is of exceptional importance because of the confluence of gonadal activation, neural rewiring, and restructuring of the social environment (Sisk and Zehr 2005; Bell et al. 2013). Organizational and activational events are not entirely distinct from one another during this period, but rather part of a spectrum where organizational events can transition into activational events during puberty (Juraska et al. 2013). The influx of gonadal steroid hormones during puberty activates neural circuitry in a sex-specific manner, and also further contributes to the sculpting of sex differences in various brain regions (Sisk and Zehr 2005). For example, in the rat brain, sex differences in region volume in the AVPV, SDN-POA, and medial amygdala (MeA) are established through new cell birth during puberty, but these sex-specific changes in cell number can be attenuated by prepubertal gonadectomy (Ahmed et al. 2008).

However, the same neurobiological sex differences are not consistently present across species and may even be inconsistent among different strains of mice (Brown et al. 1999; Holmes et al. 2009). Some species display seasonal sex differences in the brain: common shrew (*Sorex araneus*) males have a larger hippocampal CA1 than

females in the summer, when they have larger home ranges than females, but not in the winter, when both sexes are less active (Lázaro et al. 2018). Similarly, there are seasonal sex differences in the hippocampal dentate gyrus in meadow voles (*Microtus pennsylvanicus*), where females have higher rates of cell proliferation and death than males in the non-breeding season, but not in the breeding season (Galea and McEwen 1999). Conditions in the social environment may also result in sex-specific changes in the brain. For example, absence of social contact can have sex-specific effects in Syrian hamsters (*Mesocricetus auratus*): isolated males have higher V1a receptor binding in the anterior hypothalamus compared to grouped males, whereas isolation does not affect V1a binding in females in this region (Ross et al. 2019). Similar examples can be found in birds. In the zebra finch (*Taeniopygia guttata*), mixed effects between sex, social context (exposure to novel or familiar animals), and behavioral phenotype influence activation of several dopaminergic populations (Kelly and Goodson 2015). Male zebra finches have more tyrosine hydroxylase/Fos double-labeled cells in the medial hypothalamus/preoptic area than females, though the interaction effects of social context and behavioral phenotype on this population are independent of sex (Kelly and Goodson 2015).

There are additionally striking interaction effects between social status and sex differences in the brain. Social hierarchies exist in many species, and an individual's social status within the hierarchy (being dominant or subordinate) results in different roles, behaviors, and often, physiological and reproductive changes (Sapolsky 2004). In some species, social dominance is simply associated with better chances of successful reproduction (e.g., Holekamp et al. 1996), whereas in others, subordinates will fully suppress sexual maturation because their chances of accessing reproductive opportunities are so low (e.g., Jarvis et al. 1994). Subordinates can also display alternative phenotypes, for example, subordinate males of some species of cichlid fish will appear and behave as females until they gain dominance, when they will switch to the male-typical coloration and territorial behaviors (Maruska and Fernald 2010).

Accordingly, social status can influence neurobiological sex differences in species-specific ways. Acquisition of dominance in female Syrian hamsters increases activation of serotonergic neurons in the anterior portion of the dorsal raphe nucleus relative to subordinate females, but there is no detectable effect of social status on this population in males (Terranova et al. 2016). In cooperatively breeding white-browed sparrow weavers (*Plocepasser mahali*), dominant males have larger HVC and RA regions in the forebrain (involved in song production) than do subordinate males. This is thought to be because the dominant males have an additional type of song that subordinate males and females do not sing (Voight and Gahr 2011). Though subordinate males still have larger HVC and RA regions than females, their mRNA expression of candidate genes in the HVC is more similar to females than to dominant males (Voight and Gahr 2011).

Thus, ample evidence indicates we must acknowledge that the neural sex differences seen across vertebrates are not rigid phenomena. Rather, they can be dynamic in response to social organization, reproductive strategy, and factors in the social environment. The variability in regard to neural sex differences demonstrates it is

essential to consider species-specific social organization and breeding strategy in order to understand both how and why sex differences in the brain evolve and persist. Studying species that differ in social organization captures a more multidimensional view of sex differences, facilitating interpretation of the significance and function of sex differences in the brain. Here, we focus on the naked mole-rat, a strange and remarkable eusocial rodent that shows minimal evidence of sexual differentiation. Naked mole-rats have evolved a social system so extreme in its taxon that it provides a powerful opportunity for investigation into the factors influencing sex differences.

1 Naked Mole-Rats: A Case for Social Status

Naked mole-rats are subterranean rodents native to sub-Saharan Africa. They reside in colonies typically containing 70–80 individuals, but can be upwards of 300, in an expansive burrow system (Jarvis 1981; Brett 1991; Jarvis et al. 1994). They are considered eusocial, with a reproductively active breeding caste consisting of a breeding female and 1–3 breeding males, and a reproductively suppressed subordinate caste (Jarvis 1981; Brett 1991). Social hierarchies seem to be maintained via aggressive interactions between queen and subordinates (biting, shoving, etc.), as the physical presence of the breeding female is required to maintain subordinate reproductive suppression (Faulkes et al. 1990, 1991; Reeve and Sherman 1991).

While queens have an ovarian cycle length of approximately 34 days, subordinate females have quiescent ovaries, low levels of luteinizing hormone, and consistently low progesterone levels, signalling a lack of ovulation (Faulkes et al. 1990). Once the suppression from the breeding female is removed, either by her death or by removal of the subordinate from the colony, ovulation activates in female subordinates (Faulkes and Abbott 1993; Smith et al. 1997). Male subordinates are also reproductively suppressed and show smaller testis size and low sperm quality in comparison with breeding males (Faulkes et al. 1991, 1994). However, there is continued spermatogenesis in subordinate males, making reproductive suppression less absolute than in females (Jarvis 1981). Subordinate animals are chronologically adults but rarely sexually mature. Given the importance of puberty in wiring and rewiring neural circuits involved in social cognition and reproductive behaviors, naked mole-rats provide an opportunity to study what would occur in the adult brain if puberty was never achieved.

Breeder naked mole-rats exhibit the same copulatory behaviors as other mammals, where males will mount and intromit, and females will perform lordosis during the peri-ovulatory phase of their ovulatory cycle (Jarvis 1991; Holmes and Goldman 2021). The breeders also exhibit a mutual genital nuzzling behavior that occurs throughout the female's ovulatory cycle, and also continues through pregnancy (Lacey et al. 1991). Mounting behavior in males can be eliminated via castration and rescued through testosterone treatment, but genital nuzzling is not completely eliminated by castration (Goldman et al. 2006). Furthermore, aggressive interactions

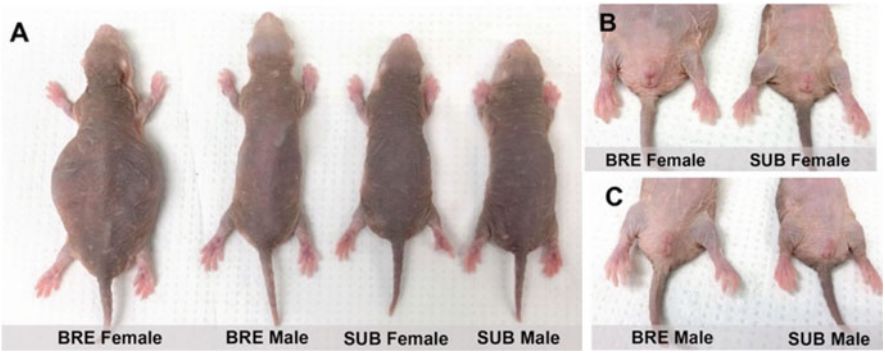

Fig. 1 Differences in naked mole-rat external morphology by status and sex. (**a**) Breeding (BRE) female and male on the left tend to be larger than subordinates (SUB). Subordinates have no sex differences in overall body size and appearance. (**b**) Genitalia of breeding female on the left compared to subordinate female on the right. The breeding females have enlarged genitals and a more obvious vaginal fold or vaginal perforation relative to the subordinate females. (**c**) Genitalia of breeding male on the left compared to subordinate male on the right. There are no status differences between breeding and subordinate males, and minimal sex differences between subordinate females and subordinate males. Modified from Faykoo-Martinez and Holmes (2021)

are also often performed by breeders, especially the breeding female. The breeding female initiates more shoving interactions than any other individual within the colony, including the breeding male, but is never the recipient of shoves (Clarke and Faulkes 2001). The onset of increased aggressive interactions is closely associated with caste transition, increases in urinary progesterone in females, and testosterone in males, following removal of previous breeders (Clarke and Faulkes 2001).

Subordinates rarely, if ever, perform copulatory or aggressive behaviors within the colony (Clarke and Faulkes 2001; Goldman et al. 2006). In fact, naked mole-rat subordinates show very little sex differences both behaviorally and anatomically. All subordinate individuals, regardless of sex, contribute to colony and litter care (Sherman et al. 1992). Task specialization is more closely associated with the body size of the individual rather than their sex (Sherman et al. 1992; Mooney et al. 2015a). There is also evidence that dispersal behavior is not sex biased in either wild or laboratory colonies, with males and females being equally likely to leave the colony (Braude 2000; Toor et al. 2020; c.f. O'Riain et al. 1996 – male-biased dispersal). In terms of external anatomy, there are no sex differences in size or weight between male and female naked mole-rats (Fig. 1). External genitalia is remarkably similar and can only be differentiated by an inconspicuous vaginal fold in females, and there is no difference in anogenital distance or phallus size/length (Fig. 1; Peroulakis et al. 2002).

The lack of sex differences in the naked mole-rat seems to be mediated by its unique social system. When comparing the eusocial mole-rat to its solitary cousin the silvery mole-rat, and its less-extreme eusocial cousin the Damaraland mole-rat, social structure was found to predict genital morphology within the Bathyergidae family with naked mole-rats having no sex differences in genitalia and associated

muscular, silvery mole-rats having sex differences, and Damaraland mole-rats being intermediate (Seney et al. 2009).

2 Brain Morphology: Status Differences, Not Sex Differences

The lack of sex differences in naked mole-rats (especially subordinates) is also reflected in their neural morphology (Table 1). Regions of the brain that are typically sexually differentiated in laboratory rats and mice have been examined in both breeding and subordinate naked mole-rats. These regions include the bed nucleus of the stria terminalis (BST), the paraventricular nucleus of the hypothalamus (PVN), the ventromedial nucleus of the hypothalamus (VMH), and the medial amygdala (MeA). In laboratory rats and guinea pigs, males have more neurons in the BST, and this region is implicated in sexual behavior, aggressive behavior, and response to social challenges (Forger et al. 2004; Greenberg and Trainor 2016). The PVN is critically involved in stress axis reactivity, as it secretes corticotropin-releasing factor (CRF), and has additional projections to the pituitary. In rats, males show post-puberty differences in PVN cellular activation in response to stress, whereas females show post-puberty differences in CRF expression (Viau et al. 2005). The VMH is involved in copulatory behavior and is larger in male rats, with a greater density of cells (De Vries and Simerly 2002). Male rats also have a larger MeA, which is functionally associated with olfactory investigation and sexual arousal (Cooke et al. 1999).

In naked mole-rats, none of these regions significantly differ in volume, cell number, or cell size between males and females (Holmes et al. 2007). This is true in both subordinates and breeding animals. However, all of these regions have status differences: breeders, regardless of sex, differ from subordinates. The BST, PVN, and MeA are larger in volume in breeders than in subordinates. The VMH shows no status differences in regional volume, but contains more cells within breeders, with a marginally larger cell size (Holmes et al. 2007). The increase in volume in the PVN occurs when subordinates are removed from reproductive suppression and paired with an opposite sex animal (Fig. 2; Holmes et al. 2011), essentially when the animal undergoes reproductive maturation. In contrast, the increase in volume in the BST does not happen at initial pairing and reproductive maturation, but rather once the animal successfully reproduces (Holmes et al. 2011). Further, these status differences are not due to an overall change in brain morphology in breeders, as other regions unrelated to sexual behavior remain unchanged. The suprachiasmatic nucleus, which controls circadian rhythms, and the anterior cortical amygdaloid nucleus, which receives olfactory input, do not differ in volume, cell number, or cell size between subordinates and breeders, nor is there a difference in cortical thickness (Holmes et al. 2007).

Table 1 Naked mole-rat brain status differences and sex differences discovered to date

	Region	Measure	Method	Sex differences	Status differences	Reference
Brain morphology	Anterior cortical amygdaloid nucleus	Cell size and number	Thionin staining	No difference	No difference	Holmes et al. (2007)
		Region volume		No difference	No difference	
	Bed nucleus of the stria terminalis (BST)	Cell size and number		No difference	No difference	
		Region volume		No difference	Larger in breeders	
	Medial amygdala (MeA)	Cell size and number		No difference	No difference	
		Region volume		No difference	Larger in breeders	
	Onuf's nucleus motoneurons	Cell size and number	Kluver-Barrera staining	No difference	More cells in breeders, fewer small cells in breeders	Seney et al. (2006)
	Paraventricular nucleus (PVN)	Cell size and number	Thionin staining	No difference	No difference	Holmes et al. (2007)
		Region volume		No difference	Larger in breeders	
	Suprachiasmatic nucleus of the hypothalamus (SCN)	Cell size and number		No difference	No difference	
		Region volume		No difference	No difference	
	Ventromedial nucleus of the hypothalamus (VMH)	Cell size and number		No difference	More cells in breeders, marginally larger cell size	
		Region volume		No difference	No difference	
Oxytocin receptors	BST	Oxytocin receptors	Autoradiography	No difference	No difference	Mooney et al. (2015b)
	Hippocampus			No difference	No difference	
	Major islands of Calleja			No difference	No difference	

(continued)

Table 1 (continued)

	Region	Measure	Method	Sex differences	Status differences	Reference
	MeA			More OTR in males	No difference	
	NAcc			No difference in subordinates, marginally lower OTR in breeding females relative to breeding males	No difference	
	VMH			No difference	No difference	
Reproductive axis	BST	Androgen receptors	Immuno-histochemistry	No difference	Less AR in breeders	Holmes et al. (2008)
	MeA			More AR in males	Less AR in breeders	
	PVN			No difference	Less AR in breeders	
	SCN			No AR present in this region	No AR present in this region	
	Ventral portion of the premammillary nucleus (PMv)			More AR in males	Less AR in breeders	
	VMH			More AR in males in VMHdm and VMHdl but no difference in VMHc	Less AR in breeders	
	Whole right hemisphere of the diencephalon	Androgen receptors (*Ar*)	qPCR	Higher levels in males	Higher levels in breeding males than subordinate males	Swift-Gallant et al. (2015)
		Aromatase (*Cyp19a1*)		Higher levels in females	Higher levels in breeding females than subordinate females	
		Estrogen receptors (*Esr1*)		Higher levels in females	Higher levels in breeding females than subordinate females	
		Progesterone receptors (*Pgr*)		–	Higher levels in breeding females than subordinate females; lower levels in breeding males than subordinate males	

Reproductive onset related	NAcc	Kisspeptin (*Kiss1*)	qPCR	Marginally higher levels in males than in females	No difference	Faykoo-Martinez et al. (2018)
		Neuropeptide VF precursor (*Npvf*)		Higher levels in males than in females	No difference	
		Tachykinin 3 receptor (*Tac3r*)		Higher levels in males than in females	No difference	
	PVN	Kappa opioid receptor (*Kor*)		Marginally higher levels in males than in females	No difference	
		RFRP receptor (*Gpr147*)		Higher levels in males than in females	No difference	
Stress axis	Arcuate nucleus	Corticotropin-releasing factor receptor 2 (*Crfr2*)	qPCR	No difference	Higher levels in subordinates than in breeders	Faykoo-Martinez et al. (2018)
	Hippocampus	Glucocorticoid receptor (*Nr3c1*)		No difference	No difference between subordinates or breeders, but higher levels in pubescent animals.	
	MeA	Corticotropin-releasing factor receptor 2 (*Crfr2*)		No difference	Higher levels in subordinates than in breeders	
	NAcc	Corticotropin-releasing factor receptor 2 (*Crfr2*)		No difference	No difference between subordinates or breeders, but higher levels in pubescent animals	
	PVN	Corticotropin-releasing factor receptor 2 (*Crfr2*)		Marginally higher levels in breeding males relative to breeding females, no sex difference in subordinates	Higher levels in breeding males than in subordinates, no status difference in females	

(continued)

Table 1 (continued)

	Region	Measure	Method	Sex differences	Status differences	Reference
		Glucocorticoid receptor (*Nr3c1*)		No difference	No difference between subordinates or breeders, but higher levels in pubescent females	
	Whole brain, amygdala	Corticotropin-releasing factor receptor 2	Autoradiography	Higher levels in females	No difference	Beery et al. (2016)
	Whole brain, amygdala, piriform cortex	Corticotropin-releasing factor receptor 1		No difference	Higher levels in subordinates	

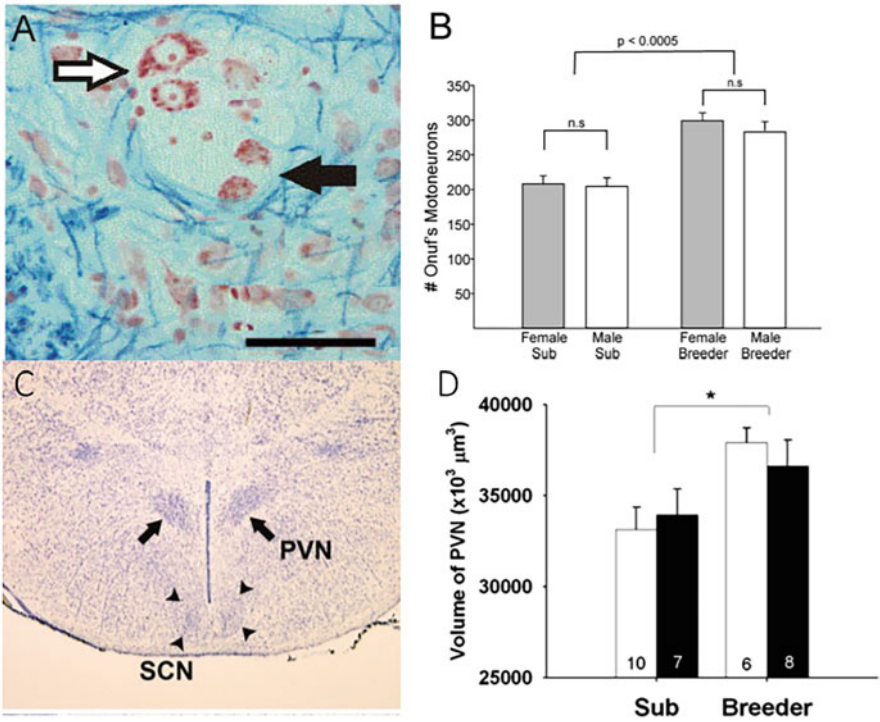

Fig. 2 Examples of neurobiological status differences and lack of sex differences in naked mole-rats. (**a**) Photomicrograph of a Kluver-Barrera-stained portion of Onuf's nucleus. Motoneurons are marked by the white arrow, and "small cells" are marked by the black arrow. Scale bar = 50 µm. (**b**) Mean (± SEM) counts of motoneurons in Onuf's nucleus by sex and status. Breeders and subordinates differed in cell number but had no detectable sex differences. (**c**) Photomicrograph of a thionin-stained coronal section of the PVN (**d**) Mean (± SEM) regional volume of the PVN by sex and status, with males as black bars and females as white bars. Breeders and subordinates differed in volume while no significant sex differences or sex and status interactions were detected. Modified from Seney et al. (2006) and Holmes et al. (2007)

Lack of sex differences in volume and cell number also extend to other parts of the nervous system. In the mammalian spinal cord, the population of motoneurons that innervate the muscles of the phallus is called Onuf's nucleus (ON motoneurons). The homologous regions in rats and mice are referred to as the spinal nucleus of the bulbocavernosus and the dorsolateral nucleus. Because this area is associated with the muscles of the phallus, it displays sex differences in a wide variety of mammals including rats (Breedlove and Arnold 1980), humans (Forger and Breedlove 1986), dogs (Forger and Breedlove 1986), Mongolian gerbils (*Meriones unguiculatus*; Ulibarri et al. 1995), and Asian musk shrews (*Suncus murinus*; Polak and Freeman 2010). Yet, in naked mole-rats, there is no sex difference in number or size of ON motoneurons (Fig. 2; Peroulakis et al. 2002; Seney et al. 2006). This is true for both subordinates and breeders, however breeders have more large ON motoneurons than

subordinates, regardless of sex (Seney et al. 2006). This again emphasizes the predominance of status differences, rather than sex differences, in this species.

3 Brain Gene and Receptor Expression: Status Differences and Sex Differences

Though naked mole-rats, as of yet, have no identified sex differences in brain morphology, they display some sex differences, as well as status differences, in gene and receptor expression in the brain (Table 1**;** Fig. 3). Brain steroid hormone receptor expression and circulating steroid hormone levels do show some sex differences in this species. Although subordinates do not have sex differences in progesterone or testosterone, breeding females have elevated progesterone levels relative to males and subordinate females, and breeding males have elevated testosterone levels relative to females and subordinate males (Clarke and Faulkes 1997, 1998; Zhou et al. 2013; Swift-Gallant et al. 2015; Faykoo-Martinez et al. 2018). Thus, sex differences in progesterone and testosterone levels are latent until adult subordinates undergo reproductive maturation, whereafter sex differences in gonadal steroid hormones become pronounced. Estradiol is more variable as subordinate females can have high estradiol levels, comparable to non-pregnant breeding females (Zhou et al. 2013). However, estradiol levels are elevated in breeding females when they initially become reproductively active (4 weeks after separation from the natal colony) and during pregnancy (Swift-Gallant et al. 2015; Edwards et al. 2021). The estradiol levels in subordinates are positively associated with queen levels and increase during her pregnancy. This is possibly by social transmission of queen hormones or due to reduced queen aggression during pregnancy which may allow subordinates to begin to sexually mature during that time (Watarai et al. 2018; Edwards et al. 2021). Breeding males and gonadectomized animals of both sexes can also have high and variable estradiol levels, potentially adrenal in origin (Zhou et al. 2013; Swift-Gallant et al. 2015).

The sex differences in neural androgen receptor levels in naked mole-rats are generally consistent with expectations based on other species, with males having higher levels than females. Quantification of brain androgen receptors with immunohistochemistry has demonstrated that male naked mole-rats have more androgen receptors than females in three particular regions of the brain: the MeA, VMH, and premammillary nucleus (Holmes et al. 2008). Though this sex effect appeared more pronounced in breeders, no significant sex by reproductive status interaction effect was detected (Holmes et al. 2008). When androgen receptor (*Ar*) mRNA expression is quantified from the right hemisphere of the diencephalon (pooled tissue), again male naked mole-rats are found to have higher androgen receptor expression than females (Swift-Gallant et al. 2015). Status differences in androgen receptors are also abundant. Regardless of sex, subordinates surprisingly have higher levels of androgen receptor protein expression in the BST, PVN, VMH, and MeA relative to

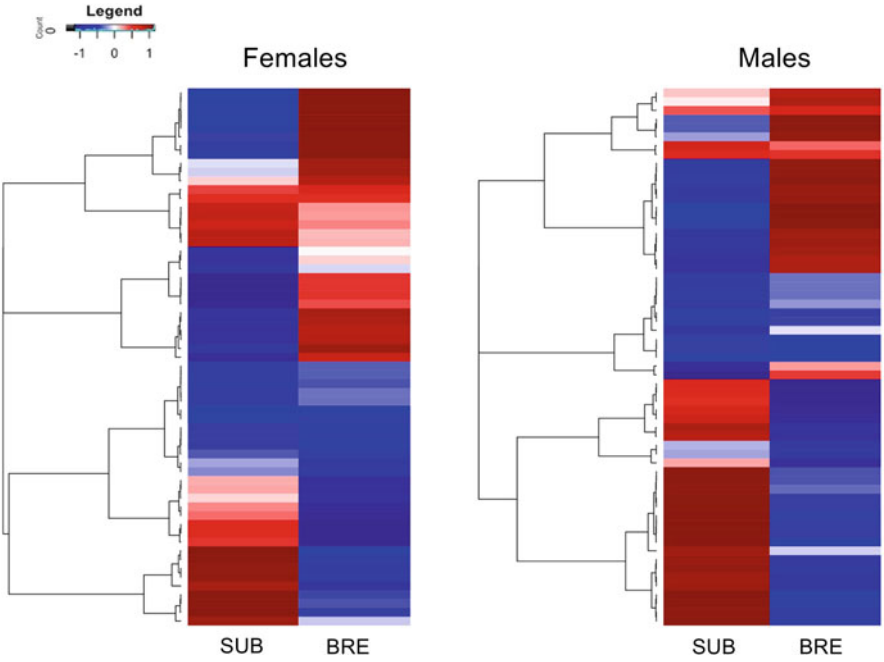

Fig. 3 Heatmap of mean gene expression (mRNA) in naked mole-rat brain regions, arranged using hierarchical clustering separated by sex. Each row represents a single gene in a specific brain region. Details can be found in source publication (Faykoo-Martinez et al. 2018). Subordinates (SUB) of each sex are on the left and breeders (BRE) of each sex are on the right. Means were adjusted to z-scores with blue indicating low relative expression and red indicating high relative expression. Modified from Faykoo-Martinez et al. (2018)

breeders (Holmes et al. 2008). However, subsequent gene expression results from the whole right hemisphere of the diencephalon displayed the opposite effect, with higher androgen receptor mRNA expression in breeding males than subordinate males, and no difference in androgen receptor expression between female breeders and subordinates (Swift-Gallant et al. 2015). This indicates that the status differences in androgen receptor expression may be region-specific and/or depend on methodology.

As for receptors and genes related to the female-biased sex hormones, estrogen receptor alpha (*Esr1*) and aromatase (*Cyp19a1*) are expressed at higher levels in the diencephalon in female naked mole-rats relative to males. There is a status difference in females for both genes, with higher levels of expression in breeding females relative to subordinate females (Swift-Gallant et al. 2015). Hence, even though subordinate females and males can have relatively high estradiol levels, they may not be as responsive to circulating estrogens as are the breeding females. Female and male naked mole-rats both also have status differences in progesterone receptor (*Pgr*) expression, with breeding females having higher progesterone receptor mRNA

levels than subordinate females, and breeding males having lower progesterone receptor mRNA levels than subordinate males (Swift-Gallant et al. 2015).

Additionally, several genes related to reproductive axis function and the onset of reproductive competence have higher levels of expression in male naked mole-rats relative to females. In the nucleus accumbens, males have higher expression of kisspeptin (*Kiss1*), neuropeptide VF precursor (*Npvf*), and tachykinin 3 receptor (*Tac3r*), and in the PVN, males have higher expression of RFRP receptor (*Gpr147*) and kappa opioid receptor (*Kor*) (Faykoo-Martinez et al. 2018). Though no main effect of status was detected on any of these reproductive genes, subordinate males in particular had the highest levels of *Npvf, Tac3r*, and *Gpr147* in the nucleus accumbens. These sex differences in neural gene expression may be due to sex differences in mechanisms and degrees of reproductive suppression in naked mole-rats. Reproductive suppression is less pronounced in subordinate males than subordinate females, as the majority of subordinate male naked mole-rats produce spermatozoa, whereas subordinate females lack mature gametes and do not ovulate (Faulkes et al. 1991; Coen et al. 2021; Faykoo-Martinez et al. 2021).

Other sex and status differences in naked mole-rat neural gene expression are in hypothalamic-pituitary-adrenal axis (HPA axis; "stress axis") related genes and receptors. The stress axis has pronounced sex differences in other rodent species, at multiple levels of regulation. In laboratory rats, females have higher circulating glucocorticoid levels than males and it takes longer for their glucocorticoid levels to return to baseline following an acute stressor (Oyola and Handa 2017; Heck and Handa 2019). This difference in stress axis regulation may be partially driven by sex differences in glucocorticoid receptors in the brain, which are involved in negative feedback of the stress axis. Female rats have less glucocorticoid receptors in the hypothalamus and pituitary than males, though there are likely additional differences in stress axis regulation beyond glucocorticoid receptor expression (Heck and Handa 2019). Further, corticotropin-releasing factor (CRF), which is synthesized in the PVN and initiates stress axis activity, is expressed at higher levels in female rats relative to male rats, and CRF expression is stimulated by estrogens and suppressed by testosterone (Heck and Handa 2019). CRF and other CRF family ligands bind to two receptor types in the brain: $CRFR_1$ and $CRFR_2$. In general, $CRFR_1$ activation is thought to stimulate the stress response, whereas $CRFR_2$ activation dampens it (Bale and Vale 2004). In several species of voles in genus *Microtus*, $CRFR_2$ density is higher in the BST of male voles than females voles, and estradiol treatment is known to alter $CRFR_2$ density in meadow voles (Beery et al. 2014, 2016). Male meadow voles additionally tend to have lower corticosterone levels than females, particularly in breeding individuals (Galea and McEwen 1999; Edwards et al. 2019).

In naked mole-rats, however, these sex differences in the stress axis are largely absent or reversed. Work comparing blood cortisol levels (the primary glucocorticoid in naked mole-rats; Ganem and Bennett 2004) in male and female naked mole-rats, collected during sacrifice, has shown that males have marginally higher circulating cortisol levels than females, regardless of status, though this difference appeared to be primarily driven by higher levels in reproductive males (Faykoo-Martinez et al. 2018). Additional research examining urinary or fecal

cortisol metabolites, as a measure of integrated baseline cortisol levels, has generally demonstrated no sex or status differences in cortisol levels in naked mole-rats (Clarke and Faulkes 1998, 2001; Edwards et al. 2020; c.f. Faulkes and Abbott 1997 – lower urinary cortisol in non-pregnant breeding females; Clarke and Faulkes 1997 – higher urinary cortisol levels in high ranking individuals in some, but not all, colonies). Although sex differences in CRF have not been quantified in naked mole-rats, this species has no sex differences in $CRFR_1$ levels, neither globally nor in specific brain regions (Beery et al. 2016; Faykoo-Martinez et al. 2018). Female naked mole-rats have higher $CRFR_2$ density than males globally and in the amygdala in particular, regardless of status, as demonstrated by receptor autoradiography (Beery et al. 2016). Subsequent work quantifying mRNA levels found no detectable sex difference in $CRFR_2$ expression, aside from a marginal increase in levels between breeding males relative to breeding females (but not subordinate males relative to subordinate females) in the PVN.

A few status differences in stress axis related genes in the brain have been found in naked mole-rats. Subordinates have higher $CRFR_1$ density than breeders, globally and in the piriform cortex and amygdala in particular (Beery et al. 2016), though this status difference was not found in subsequent $CRFR_1$ mRNA expression data from distinct brain regions. The mRNA expression data additionally indicates that breeders have higher $CRFR_2$ expression levels than subordinates in the MeA and arcuate nucleus, regardless of sex. No glucocorticoid receptor (*Nr3c1*) expression differences between breeders and subordinates were found for any region (Faykoo-Martinez et al. 2018).

Finally, status but not sex is associated with number of oxytocinergic neurons in the PVN with subordinates having more than breeders (Mooney and Holmes 2013). There is a sex difference in oxytocin receptor density in naked mole-rats, with males having higher levels in the MeA than females. This difference appears to be driven primarily by breeders, particularly by low oxytocin receptor levels in breeding females, though no significant main effect of status was detected in the analysis (Mooney et al. 2015b). No other sex differences in oxytocin receptor density were detected in any other region (Mooney et al. 2015b). Male-biased oxytocin receptor expression in naked mole-rats is consistent with sex differences found in some other rodent species, though less pronounced. Male singing mice (*Scotinomys xerampelinus*) and mandarin voles (*Lasiopodomys mandarinus*) both have higher levels of oxytocin receptor in the MeA than females of their respective species (Campbell et al. 2009; Smorkatcheva 2011). Male rats have higher densities of oxytocin receptors than females in several brain regions, including the MeA (Dumais et al. 2013). This male bias in rats was hypothesized to be due to a sex difference in social interest, with virgin males having higher levels of social investigation of conspecifics than virgin females (Dumais et al. 2013). This explanation perhaps does not apply to naked mole-rats, as there are minimal sex differences in social behavior, aside from the queen being more aggressive than other colony members. As such, sex differences in oxytocin receptor levels in the naked mole-rat may be related to a compensation effect (De Vries 2004) where increased oxytocin receptor levels are needed to promote baseline prosocial behavior in males.

4 Damaraland Mole-Rats as a Comparison

Naked mole-rats can be compared with another cooperatively breeding mole-rat, the Damaraland mole-rat (*Fukomys damarensis*). Damaraland mole-rats are also in family Bathyergidae, and similarly have a single breeding pair per colony supported by reproductively suppressed subordinates. Evidence suggests that eusociality evolved independently in naked mole-rats and Damaraland mole-rats, making them an interesting contrast to examine convergent characteristics of social structure (Allard and Honeycutt 1992; Jarvis and Bennett 1993; Faulkes and Bennet 2021). Damaraland mole-rats appear to be an intermediate between the extreme cooperative lifestyle of naked mole-rats and the solitary lifestyle of some other mole-rat species. The reproductive skew is lower in Damaraland than in naked mole-rats; Damaraland mole-rat colonies contain an average of 16 animals (maximum 41), as opposed to naked mole-rat colonies, which can have up to 300 individuals (Jarvis and Bennett 1993). It is estimated that 90% of Damaraland mole-rats will remain non-reproductive throughout their lives, whereas over 99% of naked mole-rats will remain non-reproductive (Jarvis and Bennett 1993; Jarvis et al. 1994).

The extent of the physiological reproductive suppression in Damaraland mole-rats is not quite as extreme as in naked mole-rats. In females, subordinate Damaraland mole-rats have greater follicular development in their ovaries than naked mole-rats. In males, subordinate Damaraland mole-rats have circulating testosterone levels comparable to breeding Damaraland mole-rats, whereas subordinate naked mole-rats tend to have much lower testosterone levels than breeding male naked mole-rats (Jarvis and Bennett 1993). Damaraland mole-rats additionally have more visible sexual dimorphism than naked mole-rats. Whereas subordinate male and female naked mole-rats have virtually no differences in their external genitalia, both subordinate and breeding Damaraland mole-rats do have sex differences (longer anogenital distance and phallus length in males), though these differences are less pronounced than in solitary silvery mole-rats (*Heliophobius argenteocinereus*) (Seney et al. 2009).

Like naked mole-rats, Damaraland mole-rats do not have sex differences in volume in the BST and PVN, but do have status differences in these regions, with breeders having larger regional volumes. Further, breeders have larger cells in the PVN and MeA, an additional status difference that has not been detected in naked mole-rats (Anyan et al. 2011). These differences reinforce the importance of reproductive status over sex in cooperative breeders. However, Damaraland mole-rats also have sex differences that were not detected in naked mole-rats: males have a larger MeA volume and more ON motoneurons than females. While the sex differences in MeA volume occurred regardless of breeding status, the difference in number of ON motoneurons appeared to be primarily driven by breeding males, with a marginal sex and status interaction effect (Anyan et al. 2011). This sex difference in ON motoneurons in Damaraland mole-rats is in contrast with naked mole-rats that have no sex differences in this measure, but is consistent with a variety of other mammals where males have more ON motoneurons (Peroulakis et al. 2002; Seney et al. 2006).

5 Reduced Sex Differences: Why and How?

The reduction of sex differences in naked mole-rats, relative to other rodent species, raises two major questions. The first is why naked mole-rats mostly lack sexual dimorphism in the brain. If we assume that sex differences in the brain are selected for to drive sex differences in reproduction, then the reduction in naked mole-rats would be associated with a lack of differences in reproductive behavior in subordinates of this species. Only after sexual maturation into a breeder do the female and male reproductive and social roles diverge. Supporting this are the observations of more pronounced reproductive status differences, not sex differences, in this species. The only major sex differences in subordinate naked mole-rats brains, discovered to date, are related to genes and receptors involved with puberty and the sex hormones, though there may be other sex differences in gene expression as of yet unstudied.

The second question is how, mechanistically, this lack of sexual dimorphism occurs in naked mole-rats. Many sex differences in mammals are known to be driven by androgen action during development, as opposed to genetic sex alone (Cooke et al. 1999; Forger et al. 2004). Androgens can act on tissues by interaction with androgen receptors, and by aromatization to estradiol and interaction with estrogen receptors, with the evidence of the latter being more important in inducing sex differences in the brain during development (McCarthy 2008). Sex differences in the BST and PVN in laboratory rodents can be reversed by manipulating androgen levels in the perinatal period (Forger et al. 2004). The same principle applies to the MeA in rats, where castrated males had posterodorsal regions of the MeA that were similar to females in volume (Cooke et al. 1999). This is also true of ON motoneurons: male rats with androgen insensitivity lack a developed spinal nucleus of the bulbocavernosus (ON homologous region) and in castrated Mongolian gerbils, size of the motoneurons was decreased (Breedlove and Arnold 1980; Ulibarri et al. 1995). Similarly, prenatally treating female dogs with androgens increased the number of ON motoneurons to the same amount as in males (Forger and Breedlove 1986).

Thus, Seney et al. (2009) proposed that naked mole-rats either have minimal sex differences in androgen exposure during development, or certain tissues are androgen-insensitive during development. Female naked mole-rats are not "masculinized" like female hyenas, which have masculinized external genitalia (pseudopenis); rather, Seney et al. (2009) suggested that it may be more appropriate to consider male naked mole-rats as "feminized." Similarly, Peroulakis et al. (2002) noted that while naked mole-rats have sexually differentiated internal sex organs, they do not have sexually differentiated external genitalia, perineal muscles, and motoneurons, which is consistent with differential androgen exposure earlier in gestation, when internal genitalia differentiate, but not later in gestation or early postnatal life when these other sex differences occur in rodents. Hence, timing of differential androgen exposure during sensitive periods could drive these lack of differences in male and female naked mole-rats.

While prenatal and early life androgens have been heavily studied in traditional laboratory rodents for their role in sexual differentiation, subsequent work has revealed other mechanisms that are important agents driving sex differences in the brain. For example, estrogen receptor activation in the brain, by testicular androgens aromatized into estradiol, triggers expression of the cyclooxygenase enzymes, which markedly elevate levels of prostaglandin E2. Prostaglandin E2, in turn, induces maturation of dendritic spines, thus altering brain morphology (McCarthy et al. 2009). Disrupting any step in this chain can alter sexual differentiation of the brain: blocking cyclooxygenase enzyme action leads to a female-like level of dendritic spines, and treatment with prostaglandin E2 leads to a male-like level of dendritic spines (McCarthy et al. 2009). Hence, there are potentially many places where natural selection could act to disrupt the development of neural sex differences in the brain of naked mole-rats.

Although naked mole-rats show few sex differences as subordinates, a number of status differences in sexually-relevant brain regions emerge following reproductive activation. These differences are likely induced by hormone and gene expression changes occurring during naked mole-rat "puberty," as individuals ascend to breeding status. Indeed, naked mole-rats that are removed from the colony and paired with an opposite sex conspecific – thus becoming pubescent but not yet established breeders – display a suite of hormone and gene expression changes. After 4 weeks of separation from the colony and pairing with an opposite sex conspecific, females and males have elevated estradiol and testosterone levels, respectively, relative to subordinates (Swift-Gallant et al. 2015; Faykoo-Martinez et al. 2018). The pubescent individuals additionally have altered expression of reproductive and stress related genes in the brain, in a unique signature that is distinct from both subordinates and breeders (Faykoo-Martinez et al. 2018). These changes during puberty likely drive many of the neurobiological differences observed between subordinate and breeder naked mole-rats. Additionally, gonadectomy of breeders does not reverse the differences in volume that develop in the PVN and BST in reproductively mature animals (Holmes et al. 2011), supporting the idea that irreversible changes in brain organization occur as naked mole-rats transition from subordinate to breeding status.

6 Conclusion

Our understanding of sex differences is inherently based on the species that have been studied in this respect. The majority of work to date focuses on laboratory rats and mice, which have different social and reproductive strategies between the sexes. This, in turn, presumably relates to the many observed neurobiological sex differences. However, investigation of diverse species indicates that the sex differences originally found in rats and mice cannot necessarily be generalized across species, especially to species lacking an analogous social structure. Naked mole-rats demonstrate that when considering the evolution of neurobiological sex differences, the

social organization of the species is critical in predicting and understanding the sex differences or lack thereof that may be observed.

Beyond research in social neurobiology, naked mole-rats are an exciting study system based on other aspects of their unique biology. They are very long-lived for a small mammal, and can live for over 30 years in captivity, with the oldest known naked mole-rat being 37 (Buffenstein and Craft 2021). They show resistance to many aspects of senescence, and there are no age effects on death rate, incidences of disease (including cancer), metabolic rate, bone integrity, or vascular function, until they approach their maximum lifespan where their condition finally deteriorates (Buffenstein 2008). Breeding females do not decrease reproduction with age and can breed until they die, and old males in their late 20s have been documented siring litters (Buffenstein 2005, 2008). The lack of age-related mortality risk, or "non-increasing mortality hazard," is present regardless of sex or status, though breeders have better survival rates than subordinates generally speaking (Ruby et al. 2018). Thus, in addition to foundational questions about sexual differentiation of the nervous system and behavior, naked mole-rats will also be an informative model for understanding aspects of aging and pathologies that show sex biases in humans and other species.

References

Ahmed EI, Zehr JI, Schulz KM, Lorenz BH, DonCarlos LL, Sisk CL (2008) Pubertal hormones modulate the addition of new cells to sexually dimorphic brain regions. Nat Neurosci 11:995–997. https://doi.org/10.1038/nn.2178

Allard MW, Honeycutt RL (1992) Nucleotide sequence variation in the mitochondrial 12S rRNA gene and the phylogeny of African mole-rats (Rodentia: Bathyergidae). Mol Biol Evol 9:27–40. https://doi.org/10.1093/oxfordjournals.molbev.a040706

Anyan JJ, Seney ML, Holley A, Bengston L, Goldman BD, Forger NG, Holmes MM (2011) Social status and sex effects on neural morphology in Damaraland mole-rats, Fukomys damarensis. BBE 77:291–298. https://doi.org/10.1159/000328640

Arnold AP (2009) The organizational-activational hypothesis as the foundation for a unified theory of sexual differentiation of all mammalian tissues. Horm Behav 55:570–578. https://doi.org/10.1016/j.yhbeh.2009.03.011

Bale TL, Vale WW (2004) CRF and CRF receptors: role in stress responsivity and other behaviors. Annu Rev Pharmacol Toxicol 44:525–557. https://doi.org/10.1146/annurev.pharmtox.44.101802.121410

Beery AK, Vahaba DM, Grunberg DM (2014) Corticotropin-releasing factor receptor densities vary with photoperiod and sociality. Horm Behav 66:779–786. https://doi.org/10.1016/j.yhbeh.2014.08.014

Beery AK, Bicks L, Mooney SJ, Goodwin NL, Holmes MM (2016) Sex, social status, and CRF receptor densities in naked mole-rats. J Comp Neurol 524:228–243. https://doi.org/10.1002/cne.23834

Bell MR, De Lorme KC, Figueira RJ, Kashy DA, Sisk CL (2013) Adolescent gain in positive valence of a socially relevant stimulus: engagement of the mesocorticolimbic reward circuitry. Eur J Neurosci 37:457–468. https://doi.org/10.1111/ejn.12058

Braude S (2000) Dispersal and new colony formation in wild naked mole-rats: evidence against inbreeding as the system of mating. Behav Ecol 11:7–12. https://doi.org/10.1093/beheco/11.1.7

Breedlove S, Arnold A (1980) Hormone accumulation in a sexually dimorphic motor nucleus of the rat spinal-cord. Science 210:564–566. https://doi.org/10.1126/science.7423210

Brett RA (1991) The population structure of naked mole-rat colonies. In: Sherman PW, Jarvis JUM, Alexander RD (eds) The biology of the naked mole-rat. Princeton University Press, pp 97–136

Brown AE, Mani S, Tobet SA (1999) The preoptic area/anterior hypothalamus of different strains of mice: sex differences and development. Dev Brain Res 115:171–182. https://doi.org/10.1016/S0165-3806(99)00061-9

Buffenstein R (2005) The naked mole-rat: a new long-living model for human aging research. J Gerontol Ser A 60:1369–1377. https://doi.org/10.1093/gerona/60.11.1369

Buffenstein R (2008) Negligible senescence in the longest living rodent, the naked mole-rat: insights from a successfully aging species. J Comp Physiol B 178:439–445. https://doi.org/10.1007/s00360-007-0237-5

Buffenstein R, Craft W (2021) The idiosyncratic physiological traits of the naked mole-rat; a resilient animal model of aging, longevity, and healthspan. In: Buffenstein R, Park TJ, Holmes MM (eds) The extraordinary biology of the naked mole-rat. Advances in experimental medicine and biology, vol 1319. Springer, Cham. https://doi.org/10.1007/978-3-030-65943-1_8

Campbell P, Ophir AG, Phelps SM (2009) Central vasopressin and oxytocin receptor distributions in two species of singing mice. J Comp Neurol 516:321–333. https://doi.org/10.1002/cne.22116

Clarke FM, Faulkes CG (1997) Dominance and queen succession in captive colonies of the eusocial naked mole-rat, *Heterocephalus glaber*. Proc R Soc Lond B Biol Sci 264:993–1000. https://doi.org/10.1098/rspb.1997.0137

Clarke FM, Faulkes CG (1998) Hormonal and behavioural correlates of male dominance and reproductive status in captive colonies of the naked mole–rat, Heterocephalus glaber. Proc R Soc Lond B Biol Sci 265:1391–1399. https://doi.org/10.1098/rspb.1998.0447

Clarke FM, Faulkes C (2001) Intracolony aggression in the eusocial naked mole-rat, Heterocephalus glaber. Anim Behav 61:311–324. https://doi.org/10.1006/anbe.2000.1573

Coen CW, Bennett NC, Holmes MM, Faulkes CG (2021) Neuropeptidergic and neuroendocrine systems underlying eusociality and the concomitant social regulation of reproduction in naked mole-rats: a comparative approach. In: Buffenstein R, Park T, Holmes M (eds) The extraordinary biology of the naked mole-rat. Springer

Cooke BM, Tabibnia G, Breedlove SM (1999) A brain sexual dimorphism controlled by adult circulating androgens. PNAS 96:7538–7540. https://doi.org/10.1073/pnas.96.13.7538

De Jonge FH, Louwerse AL, Ooms MO, Evers P, Endert E, Van De Poll NE (1989) Lesions of the SDN-POA inhibit sexual behavior of male Wistar rats. Brain Res Bull 23:483–492. https://doi.org/10.1016/0361-9230(89)90194-9

De Vries GJ (2004) Minireview: sex differences in adult and developing brains: compensation, compensation, compensation. Endocrinology 145:1063–1068. https://doi.org/10.1210/en.2003-1504

De Vries GJ, Simerly RB (2002) Anatomy, development, and function of sexually dimorphic neural circuits in the mammalian brain. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, Moss RL, Rubin RT (eds) Hormones, brain and behavior, volume IV. Academic Press, pp 137–191

Dumais KM, Bredewold R, Mayer TE, Veenema AH (2013) Sex differences in oxytocin receptor binding in forebrain regions: correlations with social interest in brain region- and sex- specific ways. Horm Behav 64:693–701. https://doi.org/10.1016/j.yhbeh.2013.08.012

Edwards PD, Dean EK, Palme R, Boonstra R (2019) Assessing space use in meadow voles: the relationship to reproduction and the stress axis. J Mammal 100:4–12. https://doi.org/10.1093/jmammal/gyy161

Edwards PD, Mooney SJ, Bosson CO, Toor I, Palme R, Holmes MM, Boonstra R (2020) The stress of being alone: removal from the colony, but not social subordination, increases fecal cortisol metabolite levels in eusocial naked mole-rats. Horm Behav 121:104720. https://doi.org/10.1016/j.yhbeh.2020.104720

Edwards PD, Arguelles DA, Mastromonaco GF, Holmes MM (2021) Queen pregnancy increases group estradiol levels in cooperatively breeding naked mole-rats. Integr Comp Biol. https://doi.org/10.1093/icb/icab106

Faulkes CG, Abbott DH (1993) Evidence that primer pheromones do not cause social suppression of reproduction in male and female naked mole-rats (*Heterocephalus glaber)*. Reproduction 99: 225–230. https://doi.org/10.1530/jrf.0.0990225

Faulkes CG, Bennet NC (2021) Social evolution in African mole-rats – a comparative overview. In: Buffenstein R, Park T, Holmes M (eds) The extraordinary biology of the naked mole-rat. Springer

Faulkes CG, Abbott DH, Jarvis JUM (1990) Social suppression of ovarian cyclicity in captive and wild colonies of naked mole-rats, *Heterocephalus glaber*. J Reprod Fertil 88:559–568

Faulkes CG, Abbott DH, Jarvis JUM (1991) Social suppression of reproduction in male naked mole-rats, Heterocephalus glaber. Reproduction 91:593–604. https://doi.org/10.1530/jrf.0.0910593

Faulkes CG, Trowell SN, Jarvis JUM, Bennett NC (1994) Investigation of numbers and motility of spermatozoa in reproductively active and socially supressed males of two eusocial African mole-rats, the naked mole-rat (*Hetercephalus glaber)* and the Damaraland mole-rat (*Crystomys damarensis)*. Reproduction 100:411–416

Faykoo-Martinez M, Holmes MM (2021) Spotlight feature: naked mole-rats. In: VanderLann D, Wong IW (eds) Gender and sexuality development: contemporary theory and research. Springer

Faykoo-Martinez M, Monks DA, Zovkic IB, Holmes MM (2018) Sex- and brain region-specific patterns of gene expression associated with socially-mediated puberty in a eusocial mammal. PLoS One 13. https://doi.org/10.1371/journal.pone.0193417

Faykoo-Martinez M, Kalinowski LM, Holmes MM (2021) Neuroendocrine regulation of pubertal suppression in the naked mole-rat: what we know and what comes next. Mol Cell Endocrinol 534:111360. https://doi.org/10.1016/j.mce.2021.111360

Forger NG, Breedlove SM (1986) Sexual dimorphism in human and canine spinal cord: role of early androgen. Proc Natl Acad Sci 83:7527–7531. https://doi.org/10.1073/pnas.83.19.7527

Forger NG, Rosen GJ, Waters EM, Jacob D, Simerly RB, de Vries GJ (2004) Deletion of Bax eliminates sex differences in the mouse forebrain. Proc Natl Acad Sci 101:13666–13671. https://doi.org/10.1073/pnas.0404644101

Galea LAM, McEwen BS (1999) Sex and seasonal changes in the rate of cell proliferation in the dentate gyrus of adult wild meadow voles. Neuroscience 89:955–964. https://doi.org/10.1016/S0306-4522(98)00345-5

Ganem G, Bennett NC (2004) Tolerance to unfamiliar conspecifics varies with social organization in female African mole-rats. Physiol Behav 82:555–562. https://doi.org/10.1016/j.physbeh.2004.05.002

Goldman SL, Forger NG, Goldman BD (2006) Influence of gonadal sex hormones on behavioral components of the reproductive hierarchy in naked mole-rats. Horm Behav 50:77–84. https://doi.org/10.1016/j.yhbeh.2006.01.013

Greenberg GD, Trainor BC (2016) Sex differences in the social behavior network and mesolimbic dopamine system. In: Shansky RM (ed) Sex differences in the central nervous system. Academic Press, pp 77–106. https://doi.org/10.1016/B978-0-12-802114-9.00004-4

Heck AL, Handa RJ (2019) Sex differences in the hypothalamic-pituitary-adrenal axis' response to stress: an important role for gonadal hormones. Neuropsychopharmacology 44:45–58. https://doi.org/10.1038/s41386-018-0167-9

Holekamp KE, Smale L, Szykman M (1996) Rank and reproduction in the female spotted hyaena. Reproduction 108:229–237. https://doi.org/10.1530/jrf.0.1080229

Holmes MM, Goldman (2021) Social behavior in naked mole-rats: individual differences in phenotype and proximate mechanisms of mammalian eusociality. In: Buffenstein R, Park TJ, Holmes MM (eds) The extraordinary biology of the naked mole-rat. Advances in experimental medicine and biology, vol 1319. Springer, Cham

Holmes MM, Rosen GJ, Jordan CL, de Vries GJ, Goldman BD, Forger NG (2007) Social control of brain morphology in a eusocial mammal. PNAS 104:10548–10552. https://doi.org/10.1073/pnas.0610344104

Holmes MM, Goldman BD, Forger NG (2008) Social status and sex independently influence androgen receptor expression in the eusocial naked mole-rat brain. Horm Behav 54:278–285. https://doi.org/10.1016/j.yhbeh.2008.03.010

Holmes MM, Goldman BD, Goldman SL, Seney ML, Forger NG (2009) Neuroendocrinology and sexual differentiation in eusocial mammals. Front Neuroendocrinol 30:519–533. https://doi.org/10.1016/j.yfrne.2009.04.010

Holmes MM, Seney ML, Goldman BD, Forger NG (2011) Social and hormonal triggers of neural plasticity in naked mole-rats. Behav Brain Res 218:234–239. https://doi.org/10.1016/j.bbr.2010.11.056

Jarvis JUM (1981) Eusociality in a mammal: cooperative breeding in naked mole-rat colonies. Science 212:571–573. https://doi.org/10.1126/science.7209555

Jarvis JUM (1991) Reproduction of naked mole-rats. In: Sherman PW, Jarvis JUM, Alexander RD (eds) The biology of the naked mole-rat. Princeton University Press, Princeton, pp 384–425

Jarvis JUM, Bennett NC (1993) Eusociality has evolved independently in two genera of bathyergid mole-rats — but occurs in no other subterranean mammal. Behav Ecol Sociobiol 33:253–260. https://doi.org/10.1007/BF02027122

Jarvis JUM, O'Riain MJ, Bennett NC, Sherman PW (1994) Mammalian eusociality: a family affair. Trends Ecol Evol 9:47–51. https://doi.org/10.1016/0169-5347(94)90267-4

Juraska JM, Sisk CL, DonCarlos LL (2013) Sexual differentuation of the adolescent rodent brain: hormonal influences and developmental mechanisms. Horm Behav 64:203–210. https://doi.org/10.1016/j.yhbeh.2013.05.010

Kelly AM, Goodson JL (2015) Functional interaction of dopamine cell groups relflect personality, sex, and social context in higly social finches. Behav Brain Res 280:101–112. https://doi.org/10.1016/j.bbr.2014.12.004

Lacey EA, Alexander RD, Braude SH, Sherman PW, Jarvis JUM (1991) An ethogram for the naked mole-rat: nonvocal behaviors. In: Sherman PW, Jarvis JUM, Alexander RD (eds) The biology of the naked mole-rat. Princeton University Press, Princeton, pp 209–242

Lázaro J, Hertel M, Sherwood CC, Muturi M, Dechmann DK (2018) Profound seasonal changes in brain size and architecture in the common shrew. Brain Struct Funct 223:2823–2840. https://doi.org/10.1007/s00429-018-1666-5

Maruska KP, Fernald RD (2010) Behavioral and physiological plasticity: rapid changes during social ascent in an African cichlid fish. Horm Behav 58:230–240. https://doi.org/10.1016/j.yhbeh.2010.03.011

McCarthy MM (2008) Estradiol and the developing brain. Physiol Rev 88:91–134. https://doi.org/10.1152/physrev.00010.2007

McCarthy MM, Wright CL, Schwarz JM (2009) New tricks by an old dogma: mechanisms of the organizational/activational hypothesis of steroid-mediated sexual differentiation of brain and behavior. Horm Behav 55:655–665. https://doi.org/10.1016/j.yhbeh.2009.02.012

Mooney SJ, Holmes MM (2013) Social condition and oxytocin neuron number in the hypothalamus of naked mole-rats (Heterocephalus glaber). Neurosci 230:56–61

Mooney SJ, Filice DCS, Douglas NR, Holmes MM (2015a) Task specialization and task switching in eusocial mammals. Anim Behav 109:227–233. https://doi.org/10.1016/j.anbehav.2015.08.019

Mooney SJ, Coen CW, Holmes MM, Beery AK (2015b) Region-specific associations between sex, social status, and oxytocin receptor density in the brains of eusocial rodents. Neuroscience 303:261–269. https://doi.org/10.1016/j.neuroscience.2015.06.043

Oyola MG, Handa RJ (2017) Hypothalamic–pituitary–adrenal and hypothalamic–pituitary–gonadal axes: sex differences in regulation of stress responsivity. Stress 20:476–494. https://doi.org/10.1080/10253890.2017.1369523

Peroulakis ME, Goldman B, Forger NG (2002) Perineal muscles and motoneurons are sexually monomorphic in the naked mole-rat (*Heterocephalus glaber*). J Neurobiol 51:33–42. https://doi.org/10.1002/neu.10039

Phoenix CH, Goy RW, Gerall AA, Young WC (1959) Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. Endocrinology 65:369–382

Polak K, Freeman LM (2010) Sex difference in Onuf's nucleus homologue in the Asian musk shrew. Brain Res 1346:62–68. https://doi.org/10.1016/j.brainres.2010.05.056

Reeve HK, Sherman PW (1991) Intracolonial aggression and nepotism by the breeding female naked mole-rat. In: Sherman PW, Jarvis JUM, Alexander RD (eds) The biology of the naked mole-rat. Princeton University Press, Princeton, pp 337–357

Ross AP, McCann KE, Larkin TE, Song Z, Grieb ZA, Huhman KL, Albers HE (2019) Sex-dependent effects of social isolation on the regulation of arginine-vasporessin (AVP) V1a, oxytocin (OT) and serotonin (5HT) 1a receptor binding and aggression. Horm Behav 116:1–9. https://doi.org/10.1016/j.yhbeh.2019.104578

Ruby JG, Smith M, Buffenstein R (2018) Naked mole-rat mortality rates defy Gompertzian laws by not increasing with age. eLife 7:e31157. https://doi.org/10.7554/eLife.31157

Sapolsky RM (2004) Social status and health in humans and other animals. Annu Rev Anthropol 33:393–418. https://doi.org/10.1146/annurev.anthro.33.070203.144000

Schulz KM, Molenda-Figueira HA, Sisk CL (2009) Back to the future: the organizational–activational hypothesis adapted to puberty and adolescence. Horm Behav 55:597–604. https://doi.org/10.1016/j.yhbeh.2009.03.010

Scott N, Prigge M, Yizhar O, Kimchi T (2015) A sexually dimorphic hypothalamic circuit controls maternal care and oxytocin secretion. Nature 525:519–522

Seney M, Goldman BD, Forger NG (2006) Breeding status affects motoneurson number and muscle size in naked mole-rats: recruitment of perineal motonerusons? J Neurobiol 66:1354–1364. https://doi.org/10.1002/neu.20314

Seney ML, Kelly DA, Goldman BD, Sumbera R, Forger NG (2009) Social structure predicts genital morphology in African mole-rats. PLoS One 4:1–10. https://doi.org/10.1371/journal.pone.0007477

Sherman PW, Jarvis JUM, Braude SH (1992) Naked mole rats. Sci Am 267:72–79. https://doi.org/10.1038/scientificamerican0892-72

Simerly RB (1989) Hormonal control of the development and regulation of tyrosine hydroxylase expression within a sexually dimorphic population of dopaminergic cells in the hypothalamus. Mol Brain Res 6:297–310. https://doi.org/10.1016/0169-328X(89)90075-2

Sisk CL, Zehr JL (2005) Pubertal hormones organize the adolescent brain and behavior. Front Neuroendocrinol 26:163–174. https://doi.org/10.1016/j.yfrne.2005.10.003

Smith TE, Faulkes CG, Abbott DH (1997) Combined olfactory contact with the parents colony and direct contact with non-breeding animals does not maintain suppression of ovulation in female naked mole-rats (*Heterocephalus glaber)*. Horm Behav 31:277–288. https://doi.org/10.1006/hbeh.1997.1384

Smorkatcheva AV (2011) Parental care in the captive mandarin vole, Lasiopodomys mandarinus. Can J Zool. https://doi.org/10.1139/z03-100

Swift-Gallant A, Mo K, Peragine DE, Monks DA, Holmes MM (2015) Removal of reproductive suppression reveals latent sex differences in brain steroid hormone receptors in naked mole-rats, Heterocephalus glaber. Biol Sex Differ 6:31. https://doi.org/10.1186/s13293-015-0050-x

Terranova JI, Song Z, Larkin TE II, Hardcastle N, Norvelle A, Riaz A, Albers HE (2016) Serotonin and arginine-vasporessin mediate sex differences int eh regulation of dominance and aggression by the social brain. PNAS 113:13233–13238. https://doi.org/10.1073/pnas.1610446113

Toor I, Edwards PD, Kaka N, Whitney R, Ziolkowski J, Monks DA, Holmes MM (2020) Aggression and motivation to disperser in eusocial naked mole-rats, Hetercephalus glaber. Anim Behav 168:45–58. https://doi.org/10.1016/j.anbehav.2020.07.022

Ulibarri C, Popper P, Micevych PE (1995) Motoneurons dorsolateral to the central canal innervate perineal muscles in the mongolian gerbil. J Comp Neurol 356:225–237. https://doi.org/10.1002/cne.903560207

Viau V, Bingham B, Davis J, Lee P, Wong M (2005) Gender and puberty interact on the stress-induced activation of parvocellular neurosecretory neurons and corticotropin-releasing hormone messenger ribonucleic acid expression in the rat. Endocrinology 146:137–146. https://doi.org/10.1210/en.2004-0846

Voight C, Gahr M (2011) Social status affects the degree of sex difference in the songbird brain. PLoS One 6:1–7. https://doi.org/10.1371/journal.pone.0020723

Watarai A, Arai N, Myyawaki S, Okano H, Miura K, Mogi K, Kikusui T (2018) Responses to pup vocalizations in subordinate naked mole-rats are induced by estradiol ingested through coprophagy of queen's feces. Proc Natl Acad Sci 115:9264–9269. https://doi.org/10.1073/pnas.1720530115

Zhou S, Holmes MM, Forger NG, Goldman BD, Lovern MB, Caraty A, Kalló I, Faulkes CG, Coen CW (2013) Socially regulated reproductive development: analysis of GnRH-1 and kisspeptin neuronal systems in cooperatively breeding naked mole-rats (Heterocephalus glaber). J Comp Neurol 521:3003–3029. https://doi.org/10.1002/cne.23327