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Short communication

Androgen receptor distribution in the social decision-making network of eusocial naked mole-rats

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HIGHLIGHTS

- Naked mole-rats are eusocial rodents, living in large social groups with strict hierarchies.
- Subordinate naked mole-rats have more androgen receptor protein in the social decision-making network than breeders.
- Naked mole-rats exhibit some unique features in neural androgen receptor distribution.
- Social status controls androgen receptor protein in the brain of eusocial rodents.

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ABSTRACT

Naked mole-rats are highly social rodents that live in large groups and exhibit a strict reproductive and social hierarchy. Only a few animals in each colony breed; the remainder are non-reproductive and are socially subordinate to breeders. We have examined androgen receptor immunoreactive (AR+) cells in brain regions comprising the recently described social decision-making network in subordinate and breeder naked mole-rats of both sexes. We find that subordinates have a significantly higher percentage of AR+ cells in all brain regions expressing this protein. By contrast, there were no significant effects of sex and no sex-by-status interactions on the percentage of AR+ cells. Taken together with previous findings, the present data complete a systematic assessment of the distribution of AR protein in the social decision-making network of the eusocial mammalian brain and demonstrate a significant role for social status in the regulation of this protein throughout many nodes of this network.

Social behaviors are broadly controlled by a complex neural network and, in turn, social interactions feed back to sculpt the brain throughout life. O'Connell and Hofmann [1] recently described those brain regions involved in the expression of motivated behaviors and their conservation across species, ultimately proposing the social decision-making (SDM) network. The SDM network essentially marries the social behavior network [2] and mesolimbic reward system to form an integrated network underlying the expression of adaptive motivated behaviors in vertebrates. It is clear that to better understand general mechanisms associated with the neural control of social interactions and concomitant socially controlled neural plasticity, we need a more systematic and comprehensive characterization of these brain regions in diverse species. Indeed, species that exhibit striking adaptations in their social interactions are particularly valuable for such comparisons as they better illuminate those features that are the exception versus those that are the rule.

Naked mole-rats are a powerful animal model for understanding the reciprocal relationship between social interactions and brain plasticity. They are eusocial and display the most rigid social hierarchy and reproductive skew among mammals [3]. These animals live in large subterranean colonies of up to 300 animals and reproduction is restricted to a single breeding female and her 1–3 male consorts [4,5]. Breeders are socially dominant over all other members of the colony, which are non-reproductive subordinates [6]. We have previously shown various neural changes associated with transitions in social status in this species (reviewed in [7]). For example, the paraventricular nucleus, bed nucleus of the stria

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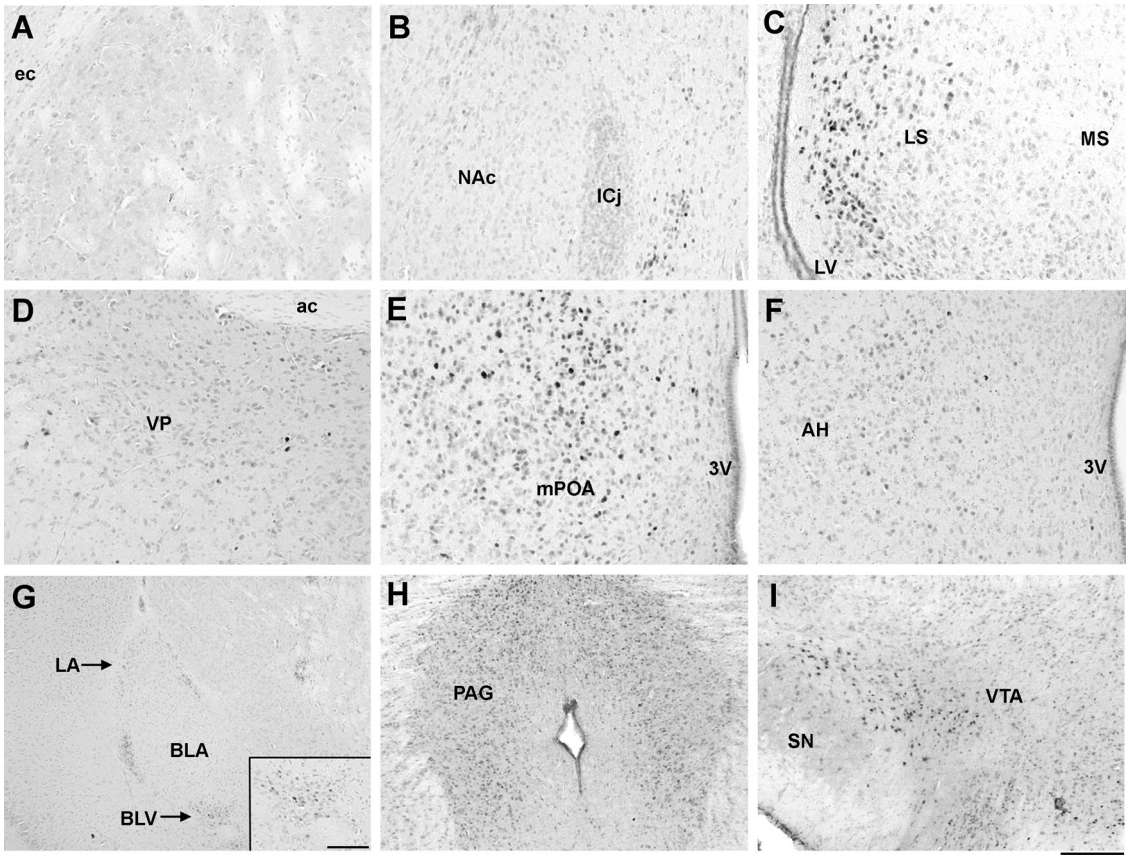

Fig. 1. Photomicrographs of AR immunoreactivity in the (A) dorsal striatum, (B) nucleus accumbens (NAc), (C) lateral septum (LS), (D) ventral pallidum (VP), (E) medial preoptic area (mPOA), (F) anterior hypothalamus (AH), (G) basolateral amygdala, ventral portion (BLV); inset is a higher magnification view of the BLV, (H) periaqueductal gray (PAG), and (I) ventral tegmental area (VTA) of a subordinate male naked mole-rat. Midline is on the right side of each image (except H). Other abbreviations: ac = anterior commissure; BLA = basolateral amygdala; ec = external capsule; ICj = Island of Calleja major; LA = lateral amygdala; LV = lateral ventricle; MS = medial septum; SN = substantia nigra; 3V = third ventricle. Scale bar at lower right: 100 μm for A–F and 200 μm for H and I. Scale bar in G: 200 μm for larger view and 100 μm for inset.

terminalis and medial amygdala, all brain regions important for reproduction in other species, are larger in breeder than subordinate naked mole-rats [8,9].

To begin to understand the putative endocrine mechanisms associated with changes in status and corresponding changes in brain morphology and social behavior, we previously evaluated the expression of androgen receptor (AR) protein in the same brain areas in which we saw morphological plasticity [10]. Social status significantly altered AR immunoreactivity in regions that increased in size in breeders, however, an inverse relationship was found whereby subordinates had *more* AR+ cells than breeders.

The present report describes our efforts to more fully characterize the neuroendocrinology and neurobiology of these unique animals. Using the same tissue in our initial report [10], we have analyzed AR immunoreactivity in the remaining regions of the SDM network of breeding and subordinate naked mole-rats of both sexes. Brains from 5 breeding females, 5 breeding males, 4 subordinate females, and 4 subordinate males were collected; all animals were gonadally intact. Brains were removed, immersion fixed in 5% acrolein for 4 h, transferred to 30% sucrose in 0.1 M phosphate buffer for cryoprotection and sectioned into 4 series in the coronal plane using a sliding microtome. One series was processed for AR immunohistochemistry using the polyclonal AR antiserum, PG21, and counter-stained with methyl green, as fully described in [10]. In addition, one series from each of three gonadally intact adult male C57BL/6 mice was processed concurrently as a positive control and for between-species comparisons.

Qualitative analyses indicated that not all brain regions involved in the SDM network contained high levels of AR+ cells. The dorsal striatum, nucleus accumbens (NAc) and ventral pallidum (VP) had little to no AR immunoreactivity (Fig. 1A, B and D). Similarly, while there was consistent albeit sparse AR in the lateral amygdala, the basolateral amygdala (BLA) was unlabeled with the exception of the ventral portion (Fig. 1G). AR immunoreactivity was consistently present in the periaqueductal gray (PAG) and ventral tegmental area (VTA) though quantification of these regions was not possible due to insufficient tissue for all animals in all groups. Quantitative stereological analyses of the percentage of cells positive for AR immunoreactivity in the lateral septum (LS; equivalent to plates 25–30 in the mouse brain atlas of [11]; Figs. 1C and 2A), medial preoptic area (mPOA; plates 30–33; Figs. 1E and 2B), anterior hypothalamus (AH; plates 35–38; Figs. 1F and 2C), and basolateral amygdala, ventral portion (BLV; plates 40–42; Figs. 1G and 2D) were performed using Stereologer software (Stereology Resource Center, Inc.). Outlines of each region were traced in each section, and unbiased estimates of the number of darkly labeled, lightly labeled, and unlabeled cells were obtained using the optical disector method. Counting frames were 20 μm × 20 μm and frame size was held constant across animals. Sampling grid size varied from 60 μm × 60 μm to 200 μm × 200 μm depending on brain region, and was held constant for all animals. All brain regions were analyzed bilaterally unless tearing or tissue artifacts were present in the region of interest. Because the pattern of results was the same whether only darkly labeled or all labeled cells were considered, we have combined dark plus light cell counts in the analyses presented here, collectively referred to as AR+.

To account for possible group differences in total cell number in some brain regions [8], the percentage of AR+ cells was calculated