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Subcaste differences in neural activation suggest a prosocial role for oxytocin in eusocial naked mole-rats

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ABSTRACT

The neuropeptide oxytocin (OT) influences prosocial behavior(s), aggression, and stress responsiveness, and these diverse effects are regulated in a species- and context-specific manner. The naked mole-rat (*Heterocephalus glaber*) is a unique species with which to study context-dependent effects of OT, exhibiting a strict social hierarchy with behavioral specialization within the subordinate caste: soldiers are aggressive and defend colonies against unfamiliar conspecifics while workers are prosocial and contribute to in-colony behaviors such as pup care. To determine if OT is involved in subcaste-specific behaviors, we compared behavioral responses between workers and soldiers of both sexes during a modified resident/intruder paradigm, and quantified activation of OT neurons in the hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus (SON) using the immediate-early-gene marker c-fos co-localized with OT neurons. Resident workers and soldiers were age-matched with unfamiliar worker stimulus animals as intruders, and encounters were videorecorded and scored for aggressive behaviors. Colony-matched controls were left in their home colony for the duration of the encounters. Brains were extracted and cell counts were conducted for OT immunoreactive (ir), c-fos-ir, and percentage of OT-c-fos double-labeled cells. Results indicate that resident workers were less aggressive but showed greater OT neural activity than soldiers. Furthermore, a linear model including social treatment, cortisol, and subcaste revealed that subcaste was the only significant predictor of OT-c-fos double-labeled cells in the PVN. These data suggest that in naked mole-rats OT promotes prosocial behaviors rather than aggression and that even within subordinates status exerts robust effects on brain and behavior.

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Introduction

A wealth of research has demonstrated the important role of neuropeptides in mammalian social behavior. One such neuropeptide, oxytocin (OT), plays a particularly prominent role in diverse social behavior both within and between species. OT promotes pair bonding and/or affiliation in a variety of mammals, including prairie voles (Insel and Hulihan, 1995; Williams et al., 1994), meadow voles (Beery and Zucker, 2010), tamarins (Snowdon et al., 2010), chimpanzees (Crockford et al., 2013) and humans (Kosfeld et al., 2005). However, in addition to its role in affiliation, OT has also been linked to social dominance and aggression, and is associated with inter-male aggression in rats (Ebner et al., 2000) and mate-guarding in male and female prairie voles (Bales and Carter, 2003; Winslow et al., 1993). Importantly, the effects of OT on behavior are context-dependent, as OT may promote affiliation in one context but aggression in another.

Across species, differing levels of sociality and social organization correspond to differences in OT levels and OT receptor (OTR) distribution. For example, monogamous prairie voles, which exhibit greater social attachments to both mates and young than polygamous meadow voles, have greater OT receptor binding in the nucleus accumbens (NAcc) and the bed nucleus of the stria terminalis (BST) (Insel and Shapiro, 1992). Furthermore, in comparison with the solitary Cape mole-rat, eusocial naked mole-rats exhibit greater OT receptor binding in these same areas (Kalamatianos et al., 2010). In regards to context, for pairs of hamsters that produce stable dominant-subordinate relationships, infusions of OT into the medial preoptic anterior hypothalamic continuum (MPOA-AH) increase flank-marking in dominant hamsters in a dose-dependent way (Harmon et al., 2002). In dominant male squirrel monkeys, intracerebroventricular (i.c.v.) injections of OT increase sexual advances and aggressive behavior toward female conspecifics (Winslow and Insel, 1991). The same manipulation performed in subordinates increases affiliation, having no effect on other behaviors.

One mammalian species in which social roles are highly relevant for survival and fitness is the eusocial naked mole-rat (*Heterocephalus glaber*). Naked mole-rats live in large subterranean groups numbering

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up to approximately 300 individuals (Brett, 1991; Lacey and Sherman, 1991). Each colony has a single breeding female (the queen) and 1–3 breeding males. All other animals, called subordinates, are reproductively suppressed, do not generally exhibit sex differences (Holmes et al., 2007), and participate in foraging, nest and tunnel maintenance, pup care, and defense against intruders. Within the subordinate caste, further social stratification and divisions of labor exist: 'soldiers' or larger, older mole-rats are observed to be more aggressive and defend the colony, whereas 'workers' or younger, smaller individuals participate in cooperative behaviors such as pup care and colony maintenance (Jarvis et al., 1991; Lacey and Sherman, 1991). Recent work from our lab (Mooney et al., 2015) supports the classification of these 'subcastes' and further indicates that such behavioral specializations remain stable in the long term if colony demands are consistent.

The present study sought to determine whether differences in OT activity are associated with the distinct behavioral roles found in workers and soldiers. Specifically, we hypothesized that soldiers would be more aggressive and exhibit greater OT activation than workers in a context where aggression serves the needs of the group. Therefore, we employed a modified resident/intruder paradigm and compared the behavioral responses to unfamiliar intruders in worker and soldier residents of both sexes, and assessed associated activation of OT neurons using double-label immunohistochemistry for OT and the immediate early gene marker c-fos (Flanagan et al., 1993). We also measured circulating cortisol to evaluate the putative relationship between level of arousal and activation of OT neurons in the different subcastes.

Methods

Animals and housing

We used a total of 60 adult subordinate naked mole-rats from 3 different colonies: 20 experimental animals were tested as residents in a modified resident/intruder (RI) paradigm, paired with 20 stimulus intruder animals, and 20 un-manipulated animals served as in-colony controls. Representatives from each group came from each of the 3 colonies. RI and in-colony groups contained both workers and soldiers (N = 10 each per group) while stimulus intruder animals were all age-matched workers from a different colony than the respective resident. Naked mole-rat colonies were housed in large (65 cm L × 45 cm W × 23 cm H), medium (46 cm L × 24 cm W × 15 cm H), and small (30 cm L × 18 cm W × 13 cm H) polycarbonate cages connected by tubes (25 cm L × 18 cm D) and kept on a 12:12 light/dark cycle at 28–30 °C. Animals were fed ad libitum with a diet of sweet potato and wet 19% protein mash (Harlan Laboratories, Inc.). The age of the animals ranged from 13 months to 3 years of age and the animals weighed between 23 and 69 g (Table 1). All experimental procedures followed federal and institutional guidelines and were approved by the University Animal Care Committee.

Table 1
Breakdown of experimental groups: stimulus animals, residents, and controls.

	Stimulus animals	Residents		Controls	
Sub-caste	–	S	W	S	W
N	20	10	10	10	10
Sex, N female	9 F	3 F	5 F	2 F	8 F
Age, years (SD)	1.59 (0.47)	2.51 (0.45)	1.87 (0.53)	2.15 (0.47)	1.47 (0.46)
Weight, g (SD)[a]	30.6 (3.28)	53.10 (11.26)	31.20 (3.22)	47.90 (9.01)	26.70 (3.89)

[a] Prescreened resident soldiers weighed significantly more than prescreened workers ($t(21) = -6.27$, $p < 0.0001$, Cohen's $d = -2.62$). W = workers, S = soldiers. F = number of females (number of males calculated by subtracting from N in each group). g = grams. SD = standard deviation.

In this study, we selected soldiers and workers that had been prescreened for aggressive and non-aggressive behavior (respectively) toward unfamiliar conspecifics. We note here that labeling animals as "workers" may require additional confirmation, since we did not select animals based on in-colony working behavior but instead confirmed them as "non-soldiers". In the case where an appropriate prescreened RI animal could not be age-matched with a stimulus animal from another colony (N = 1), we pseudo-randomly selected animals on the basis of weight: animals over 40 g were categorized as soldiers and those under 35 g as workers, a classification based on earlier findings (Mongillo et al., 2014). This same classification was used for the majority of in-colony animals (N = 16). All stimulus intruder animals were classified as workers based on weight (<35 g). To further confirm that weight was a suitable method for subcaste classification in the current sample, we performed a t-test comparing workers and soldiers across treatment (RI versus in-colony) for animals that were prescreened for aggressive behavior. This demonstrated that subcaste differences in weight, favoring soldiers, were present in those animals classified using behavior alone (Table 1). While every effort was made to have equal representation of males and females in our groups, including sex-matching RI animals and stimulus intruders, it is often difficult to determine naked mole-rat sex on the basis of external observation alone (Peroulakis et al., 2002; Holmes et al., 2009). We confirmed sex at the time of dissection and discovered that our groups were not balanced across sex (e.g., only 2 males were present in the soldier in-colony group). Due to the lack of statistical power in our resulting sex groups and the fact that status effects outweigh the effects of sex in this species (Holmes et al., 2007) we did not include sex as a variable in our t-test and ANOVA analyses (see below).

Behavioral testing and scoring

Each RI worker or soldier was habituated in isolation for 5 min in one medium-sized cage of their home colony by sealing the connecting tube. Following habituation, the stimulus intruder animal was introduced to the same chamber as the RI animal and pairs were video-recorded for 30 min. Animals remained in the chamber for an additional 1.5 h to provide the time required for c-fos accumulation. In-colony control animals were marked for identification and placed back in their home colony for 2 h. We used behavioral scoring software (Observer, Noldus Information Technology) to quantify the frequency and duration of behaviors for the first 10 min of resident/intruder videos, scoring one animal at a time from each pair. Behaviors were broadly categorized as interactive (including non-aggressive and aggressive acts) or non-interactive (such as climbing and digging). A list of all behaviors scored is provided in Table 2. When quantifying behavior, scorers were blind to the sex and subcaste of the animal and inter-rater reliability was 97%. We did not collect behavioral data for in-colony animals because no direct behavioral comparisons could be made between animals interacting freely with multiple colony members and those interacting with a single intruder.

Tissue extraction and immunohistochemistry

Following the 2 h experimental window, all animals were deeply anesthetized with isoflurane and rapidly decapitated. Brains were then extracted, post-fixed in 4% paraformaldehyde for 4 h, and transferred to a solution of 20% sucrose kept at 4 °C. Using a sliding microtome, brains were blocked at the olfactory bulbs and the cerebellum and sliced into four series of coronal sections 30 μm thick. These were then stored at −20 °C until processing. Trunk blood was collected and kept on ice until centrifugation. Serum was stored at −20 °C until processing.

In order to identify active OT neurons, we performed double-label immunohistochemistry for OT and the immediate-early gene product c-fos (see Fig. 1). The specificity of the OT antibody used in this study

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