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Research Report

Differential effects of chronic fluoxetine on the behavior of dominant and subordinate naked mole-rats

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HIGHLIGHTS

- Naked mole-rats are eusocial rodents, living in large social groups with strict hierarchies.
- Fluoxetine, a selective serotonin reuptake inhibitor, affects behavior in naked mole-rats in a status- and context-dependent manner.
- Fluoxetine decreases aggression in dominant naked mole-rats when paired with unfamiliar animals.
- Fluoxetine decreases digging behavior in subordinate naked mole-rats when in their home colony.

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ABSTRACT

Naked mole-rats are eusocial rodents that live in large subterranean colonies with a strict reproductive and social hierarchy. The breeding female (referred to as the queen) and 1 to 3 breeding males are the only reproductive members of the colony. Breeders are socially dominant and all other colony members are non-reproductive subordinates. The effects of manipulating the serotonergic neurotransmitter system on aggression and dominance behaviors are well studied in many species, but not in eusocial rodents like the naked mole-rat. The current study investigated how the serotonergic system influences aggressive/dominant behaviors in this species. To do this, two separate but related experiments were conducted: the effects of fluoxetine hydrochloride (FLX) on status-specific behaviors of subordinates (Experiment 1) and dominant queens (Experiment 2) were evaluated both in-colony and in a social-pairing paradigm. In accordance with our main hypothesis, chronic treatment of FLX attenuated the frequency and duration of aggression in queens, but not subordinates, when paired with an unfamiliar conspecific. Further exploration of pharmacological manipulation on status-specific behaviors of this eusocial species may elucidate the neurobiological mechanisms underlying their unique and rigid social hierarchy.

1. Introduction

Serotonin (5-hydroxytryptamine; 5-HT) is a highly-conserved neurotransmitter that regulates social interactions in both invertebrates [1,2] and vertebrates [3–9]. Specifically, 5-HT is an important mediator of social status and aggression. Numerous studies have demonstrated that higher levels of aggression are associated with lower 5-HT activity in many species [5,10,11]. For example, elevating serotonergic activity decreases aggression and, in some instances, reverses dominance relationships [3,4,8,12]. However, although this inverse relationship is well-established, it is not universal. For example, artificially increasing 5-HT levels in crustaceans can temporarily reverse social status, turning subordinates into aggressive and territorial dominant males [1], and repeated exposure to a low-dose selective 5-HT reuptake inhibitor (SSRI) in adolescent hamsters increases nearly all measures of offensive aggression [13].

The administration of SSRIs has been used to investigate the role of 5-HT in social status and aggression. Fluoxetine hydrochloride (FLX), also known as Prozac™, is an example of a SSRI that prevents 5-HT reabsorption at the presynaptic terminal. Studies examining effects of chronic FLX treatment have demonstrated a robust decrease in territorial aggression [14] and change in social status [4,15]. In rodents, FLX was found to inhibit muricidal behavior in spontaneously muricidal rats [16] and attenuate footshock-induced aggression [17]. Moreover, FLX was found to decrease alcohol-potentiated aggression in a shock-induced aggression model in mice [18], and more recently in humans, reduce measures of anger and physical aggression in alcoholic perpetrators of intimate partner violence [19].

Eusociality refers to a strict form of social organization based on reproductive castes, where animals live in large groups that consist

Abbreviations: FLX, fluoxetine hydrochloride; 5-HT, 5-hydroxytryptamine/serotonin; SSRI, selective serotonin reuptake inhibitor; ED, experimental day.

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of multiple adult generations [20]. The naked mole-rat (*Heterocephalus glaber*), native to Africa, exhibits the closest mammalian equivalent to eusociality [21,22]. These nearly hairless rodents live in large subterranean colonies with a rigidly organized reproductive hierarchy [21]. Each colony, averaging 60 to 80 individuals (with as many as 300), consists of a breeding female (referred to as the queen) and one to three breeding males; all other members of the colony are sexually quiescent and referred to as subordinates [21–23]. Queens are socially dominant over the other colony members and this social dominance is currently thought to suppress reproduction in subordinates [22,24–26]. Consequently, naked mole-rats are a powerful animal model for studying the effects of FLX, a well-established low-risk manipulation of the 5-HT system, on status-specific differences in aggression and other behaviors.

Numerous status-specific behavioral specializations are seen in this species. The most conspicuous are reproductive behaviors: only breeders exhibit direct reproductive behaviors including copulation and nursing [22,23,27,28], and mutual genital nuzzling [27,29]. Subordinates assist with pup care and are predominantly responsible for foraging, colony defense and maintenance of the tunnel system [21–23,28]. In addition, subtle and overt agonistic interactions (e.g., shoving) by breeders inhibits gonadal function in subordinates [25]. Specifically, subordinates, irrespective of sex, shove at lower rates relative to breeders [26,30–32]. Lastly, there is a correlation between body size and shoving among subordinates: higher-ranking, larger subordinates shove more than lower-ranking, smaller subordinates [32].

In light of (a) how aggression is altered by varying levels of 5-HT in other species, and (b) how behaviors that, to a certain extent, define the social role or status in a naked mole-rat colony may be altered by FLX, the current study explored a role for 5-HT in maintenance of the naked mole-rat social hierarchy. To do this, two separate but related experiments were conducted: the effects of FLX on status-specific behaviors of subordinates (Experiment 1) and queens (Experiment 2) were evaluated both in-colony and in a social-pairing paradigm. We hypothesized that FLX would differentially affect behavior in dominant and subordinate animals. Specifically, we predicted that chronic treatment of FLX would attenuate aggression in queens, but not subordinates.

2. Material and methods

2.1. Animals and housing

Naked mole-rats were maintained at the University of Toronto Mississauga. Colonies were housed in polypropylene tubs, containing corncob bedding and connected by lengths of acrylic tubing. Animals were fed sweet potato and wet 19% protein mash (Harlan Laboratories, Inc.) ad libitum and housed in temperature-controlled rooms (28 to 30 °C) on a 12 h light-dark cycle.

2.2. Ethics

Experimental procedures were approved by the University of Toronto Animal Care Committee, and adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals [65], 8th ed., and the Canadian Council on Animal Care Guidelines.

2.3. Experimental procedure

2.3.1. Experiment 1: Subordinates

In each of four separate colonies, eight subordinates (four experimental and four control animals) were randomly selected, identified and distinguished by markings made with a marker. To explore potential sex differences, each group consisted of an equal number of males and females. In total, 32 subordinates were used: 16 experimental animals (8 male; 8 female) and 16 control animals (8 male; 8 female). All subordinates were 1 to 3 years of age and weighed between 29 and 71 g on experimental day (ED) 1. There were no significant differences in age or body weights recorded on ED 1 between experimental and control groups. In addition, naked mole-rats reach adult body size anywhere between 4 and 24 months of age and they can survive for over 20 years in captivity [33,34]. Therefore, all subordinates used in this experiment were young adults.

On ED 1, subordinates were videotaped for 30 min in their home colony to establish baseline in-colony behaviors. On ED 2, to establish baseline paired behaviors, subordinates were videotaped for 30 min while paired with a non-colony [unfamiliar] member of the opposite sex in a novel polycarbonate cage filled with clean bedding. Paired subordinates were matched based on body weight (measured at the start of each ED). Beginning on ED 3, 16 (8 male; 8 female) subordinates were weighed and injected intraperitoneally with 10 mg/kg of FLX dissolved in 10 ml/kg of 0.9% sterile saline and another 16 (8 male; 8 female) age-matched subordinates received 10 ml/kg of 0.9% sterile saline (CTL). Injections were repeated once daily at the same time every day for 4 weeks (ED 3 to 30). After injection, subordinates were immediately returned to their respective colony. Beginning 30 min after injection, in-colony behaviors were recorded for 30 min on every seventh day of injection (i.e., on ED 9, 16, 23 and 30). On ED 31, 30 min after injection, subordinates were paired as above with a new unfamiliar, size-matched subordinate animal and videotaped for 30 min (see Fig. 1A for experimental design and timing of procedures).

2.3.2. Experiment 2: Dominant queens

Due to their scarcity, and the fact that established breeders are crucial to the continuation of our colonies, we employed a within-animal design for this experiment. A total of 13 queens, 2 to 14 years of age, weighing between 51 and 72 g on ED 1, were used (pregnant queens were excluded).

On ED 1 to 3, all queens received an intraperitoneal injection of 0.9% sterile saline (10 ml/kg) 30 min prior to the start of behavioral testing. On ED 1, queens were videotaped for 30 min in their home colony. On ED 2 and 3, queens were videotaped for 30 min while paired with a non-colony [unfamiliar] subordinate. On ED 2, half of the queens were paired with presumed soldiers (i.e., born from first litter and larger phenotype; see below) and the other half were paired with presumed workers (i.e., born from later litters and smaller phenotype; see below). The pairing with presumed soldiers vs. workers was counterbalanced on ED 3. Seven queens were paired with male workers/soldiers and the remaining 6 queens were paired with female workers/soldiers. Starting on ED 4, queens were injected intraperitoneally with 10 mg/kg of FLX dissolved in 10 ml/kg of 0.9% sterile saline at the same time every day for 4 weeks (ED 4 to 33). As with Experiment 1, beginning 30 min after injection, in-colony behaviors were recorded on every seventh day of FLX injection (i.e., ED 10, 17, 24 and 31). On ED 32 and 33, queens were again paired with unfamiliar subordinates 30 min following injection. The experimental procedure on ED 32 and 33 paralleled the experimental procedure on ED 2 and 3, respectively (see Fig. 1B for experimental design and timing of procedures).

2.4. Data collection and analysis

All behaviors (see Table 1 for operational definitions) were scored using The Observer® XT 10 by Noldus Information Technology Inc. (Leesburg, VA), by an individual who was blind to experimental condition. In addition, high-ranked naked mole-rats typically pass over a lower ranked animal, while lower