Differential Effects of Chronic Fluoxetine on the Behaviour of Dominant and Subordinate Naked Mole-Rats

by

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A thesis submitted in conformity with the requirements for the degree of Master of Arts
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Abstract

Naked mole-rats are eusocial rodents that live in subterranean colonies with a strict reproductive and social hierarchy. Breeders are socially dominant and other colony members are non-reproductive subordinates. The effects of manipulating the serotonergic system on aggression are well studied in many species, but not in eusocial rodents like the naked mole-rat. For the current study, the effects of fluoxetine hydrochloride (FLX) on status-specific behaviours of subordinates (Experiment 1) and queens (Experiment 2) were evaluated both in-colony and in a social-pairing paradigm to investigate how the serotonergic system influences aggression in this species. 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 behaviours of this eusocial species may elucidate the neurobiological mechanisms underlying their unique and rigid social hierarchy.
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1 Introduction

For the past several decades, sexual differentiation of the mammalian nervous system has been studied extensively. Most of this research, however, has focused on relatively non-social species. While the endocrine correlates of cooperative breeding and social hierarchies of eusocial species have been extensively studied, less is known of their neurobiological mechanisms. Consequently, the main objective of the current study, as well as other related research projects in our laboratory, is to further our understanding of the bidirectional relationship between complex social behaviours like cooperative breeding and the central nervous system (Holmes, Seney, Goldman, & Forger, 2011). In particular, the naked mole-rat (*Heterocephalus glaber*), one of only two eusocial mammals, is used in our laboratory as an animal model for studying interactions between sociality and sexually differentiated neural circuits and behaviours.

In light of findings supporting that neuroanatomy may be influenced more by an individual’s social status than by its sex (Holmes *et al.*, 2007), the current study investigated how the serotonergic system influences aggressive/dominant behaviours in this eusocial mammalian species. To do this, two separate but related experiments were conducted both in-colony and in a social-pairing paradigm; specifically, the effects of fluoxetine hydrochloride (FLX; also known as Prozac™) on status-specific behaviours of subordinates (Experiment 1) and queens (Experiment 2) were evaluated. The following will describe the serotonergic system, including relevant information regarding the role of selective serotonin reuptake inhibitors (SSRIs) and in particular, FLX, in mediating dominant and aggressive behaviours in a variety of species.

1.1 Serotonin (5-HT)

Serotonin (5-hydroxytryptamine; 5-HT) is a biogenic amine that functions as both a neurotransmitter and a hormone in the mammalian central nervous system (CNS) and periphery. In the brain, 5-HT neurons project to most areas of the CNS, regulating a wide variety of sensory, motor and cognitive processes (Osborne, 1982; Siever *et al.*, 1991). In the periphery, serotonin is involved in many functions, including the regulation of enteric reflexes, modulation of vascular smooth muscle contraction and regulation of lymphocyte phagocytosis (Richardson & Engel, 1986).
In mammals, the biosynthesis of 5-HT includes two enzymatic steps: (1) ring hydroxylation of the essential amino acid tryptophan by tryptophan hydroxylase and (2) side chain decarboxylation of 5-hydroxytryptophan by aromatic amino acid decarboxylase (reviewed in Nichols & Nichols, 2008; Figure 1). Within the brain, serotonergic neurons originate primarily in a group of cell bodies known as the raphe nuclei of the brainstem reticular formation responsible for the production and, in response to an action potential, release of 5-HT across synapses to receptors on dendrites and cell bodies of their targets (Jacobs & Fornal, 1995; Figure 2). Specifically, after 5-HT release at an axon terminal, some 5-HT molecules migrate across the synapses and bind to receptors on the postsynaptic neurons to elicit downstream responses. 5-HT action is moderated by 5-HT reuptake transporters (SERT) on the presynaptic axon terminal that removes 5-HT from the synapse to prevent further binding to target receptors until the next action potential arrives. These 5-HT molecules are recycled into the presynaptic axon terminal by endocytosis (see Figure 3 for a schematic representation of a serotonin synapse).

Figure 2. Schematic representation of serotonin pathways in the central nervous system, including projections from the raphe nuclei to the (1) prefrontal cortex, (2) basal ganglia, (3) hippocampus, (4) hypothalamus and (5) spinal cord. Reprinted with permission from “Mechanism of Action of Serotonin Selective Reuptake Inhibitors: Serotonin Receptors and Pathways Mediate Therapeutic Effects and Side Effects,” by S. M. Stahl, 1998, Journal of Affective Disorders, 51, p. 217. Copyright 1998 by Elsevier Science B.V.

Figure 3. Schematic representation of a serotonin synapse. Reprinted with permission from “Prozac (Fluoxetine, Lilly 110140), the First Selective Serotonin Uptake Inhibitor and an Antidepressant Drug: Twenty Years since its First Publication,” by D. T. Wong, F. P. Bymaster, and E. A. Engleman, 1995, Life Sciences, 57, p. 415. Copyright 1995 by Elsevier Science Ltd.
1.2 Brain Areas Underlying Aggression

In socially organized and cohesive species, dominance hierarchies that are established and maintained by aggressive behaviours determine which animals receive preferential access to available resources (reviewed in Miczek, Faccidomo, Fish, & DeBold, 2007). According to Newman (1999), several brain areas are known to control aggression including the medial preoptic area (MPOA), lateral septum (LS), anterior hypothalamus (AH), ventromedial (VMH) hypothalamus, periaqueductal gray (PAG), medial amygdala (MA) and bed nucleus of the stria terminalis (BNST). Functional or structural abnormalities in one or more of these regions can increase the susceptibility for impulsive aggression and violence (Newman, 1999).

Although brain areas mediating aggression are fairly conserved among mammals, the details of the regulatory pathways may be species-specific. For example, in male rats, lesions of the LS, AH, MA and BNST reduce aggression (reviewed in Kruk, 1992). Similarly, in hamsters, c-fos immunoreactivity is exhibited in areas that are interconnected with the AH, including the MA, BNST, and dorsolateral part of the PAG, after displays of offensive aggression towards an intruder (Delville, De Vries, & Ferris, 2000). Thirdly, defensive rage behaviour in cats involves the medial hypothalamus (MH) and PAG (Gregg & Siegel, 2001). Furthermore, projections from other brain areas including the prefrontal cortex (PFC) to the MH and PAG may modulate the intensity of attack and rage (Gregg & Siegel, 2001).

1.3 Aggression & Serotonergic Activity

The expression of social behaviour often results in an alteration of monoaminergic activity (Winberg & Nilsson, 1993). 5-HT regulates social interactions in both invertebrates (Huber, Smith, Delago, Isaksson, & Kravitz, 1997; Kravitz, 2000) and vertebrates (Ferris et al., 1997; Larson & Summers, 2001; Raleigh, McGuire, Brammer, Pollack, & Yuwiler, 1991; Summers, Larson, Summers, Renner, & Greenberg, 1998; Summers & Winberg, 2006; Villalba, Boyle, Caliguri, & De Vries, 1997; Winberg, Winberg, & Fernald, 1997). 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 (Kohlert et al., 2012; Raleigh et al., 1991; Winberg et al., 1997). For example, elevating serotonergic activity decreases aggression and, in some instances, reverses dominance relationships (Deckel,
Also noteworthy, the ratio of 5-HT metabolite (5-hydroxyindoleacetic acid; 5-HIAA) to 5-HT is often used as a measure of the activity of serotonergic neurons (Sloman et al., 2005; Winberg & Lepage, 1998; Winberg & Nilsson, 1993). For example, in humans, impulsivity and high aggressiveness are correlated with low cerebrospinal fluid concentrations of 5-HIAA (reviewed in Lesch & Merschdorf, 2000). A similar negative association between 5-HT metabolites and aggressive behaviour has been reported in many species, including macaques (Mehlman et al., 1994), mice (Caramaschi, de Boer, & Koolhaas, 2007) and fish (Clotfelter et al., 2007). Similarly, pharmacological studies also support this relationship such that aggression is increased by depletion of tryptophan (Bjork, Dougherty, Moeller, & Swann, 1999; Chamberlain, Ervin, Pihl, & Young, 1987).

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 (Kravitz, 1988; Huber et al., 1997), chronic antidepressant treatments increase aggression in rats (Mitchell, 2005) and repeated exposure to a low-dose selective 5-HT reuptake inhibitor (SSRI) in adolescent hamsters increases nearly all measures of offensive aggression (Ricci & Melloni, 2012).

Previous research has also reported interaction of the serotonergic system with other molecules that mediate aggression in a variety of species (e.g., steroid hormones, arginine vasopressin (AVP) and histamine; reviewed in Nelson & Chiavegatto, 2001). Firstly, androgens alone, either acting directly or via estrogenic metabolites, tend to facilitate aggression. However, such hormones interact with 5-HT to influence the likelihood of aggression. For example, both androgens and estrogens modulate 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor agonist effects on murine aggression (Cologer-Clifford, Simon, Richter, & Smoluk, 1998). Secondly, the effects of AVP on aggression, centered in the AH, appear to be mediated by 5-HT. For example, according to Ferris, Stolberg, and Delville (1999), 5-HT appears to inhibit AVP-facilitated offensive aggression by activating 5-HT$_{1A}$ receptors. Lastly, other neurotransmitters, like histamine, influence aggression via the 5-HT system. For example, mice lacking histamine (H1) receptors exhibit less aggression and increased 5-HT in several brain areas (Yanai et al., 1998).
1.3.1 Serotonergic Receptors

The specific role of the various 5-HT receptors and the interactions among 5-HT receptor subtypes that underlie aggression has been extensively studied. 5-HT receptors are the largest family of G-protein coupled receptors, with the exception of the 5-HT\textsubscript{3} receptor, which is the only ligand-gated ion channel receptor (reviewed in Nichols & Nichols, 2008). Drugs that target 5-HT\textsubscript{1A}, 5-HT\textsubscript{1B}, and to a lesser extent, 5-HT\textsubscript{2} sites have been implicated in the modulation of many behaviours including aggression (Lee and Simansky, 1997; Olivier, Mos, & Van Oorschot, 1995; Parsons, Weiss, & Koob, 1998; Sari, 2004). A high concentration of 5-HT\textsubscript{1A} receptors is located in the raphe nuclei (Zifa & Fillion, 1992). The 5-HT\textsubscript{1B} receptor is expressed in a variety of brain regions, including the basal ganglia, PAG, hippocampus, LS and raphe nuclei, either presynaptically inhibiting 5-HT release or modulating the release of other neurotransmitters (reviewed in Nelson & Chiavegatto, 2001).

Behaviourally, administration of a 5-HT\textsubscript{1A} agonist dose-dependently decreases aggression in mice and rats (de Boer, Lesourd, Mocaer, & Koolhaas, 2000; Fish, Faccidomo, & Miczek, 1999; McKenzie-Quirk, Girasa, Allan, & Miczek, 2005; Olivier & Mos, 1992; Sánchez & Meier, 1997). However, other drug effects, including sedation, slowed motor routines and stereotyped movements also manifest (de Boer & Koolhaas, 2005; McKenzie-Quirk et al., 2005). In contrast, 5-HT\textsubscript{1B} receptor agonists typically reduce aggressive behaviour without sedation (de Almeida & Miczek, 2002; de Boer & Koolhaas, 2005). Neuroleptics that act as 5-HT\textsubscript{2A} receptor antagonists have been shown effective in the clinical management of aggressive behaviour in patients with diagnoses that include disruptive behaviour disorder, autism and schizophrenia (Le Blanc et al., 2005; McCracken et al., 2002; Swanson, Swartz, Elbogen, & Van Dorn, 2004). In addition to 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} receptor agonists or 5-HT\textsubscript{2A} antagonists, other pharmacological strategies, such as the administration of SSRIs, have been used to investigate the role of 5-HT in social status and aggression.

1.3.2 Selective Serotonin Reuptake Inhibitors (SSRIs)

SSRIs increase synaptic 5-HT levels by selectively inhibiting the SERT that functions to remove 5-HT from the synaptic cleft back into the presynaptic neuron (Barker & Blakely, 1995; Leonard, 1996). The SERT is localized on presynaptic axon terminals and cell bodies of 5-HT neurons (Barker & Blakely, 1995). The SERT is organized as a component of a molecular complex which includes the enzyme sodium-potassium adenosine triphosphatase, and several
binding sites that serve either to increase or to decrease 5-HT binding (Barker & Blakely, 1995; Leonard, 1996). Specifically, the sodium binding site increases transporter affinity for 5-HT (i.e., positive allosteric modulation), which allows 5-HT to bind to the transporter. However, the SSRI binding site decreases transporter affinity for 5-HT (i.e., negative allosteric modulation), which inhibits 5-HT binding to the transporter. In other words, when 5-HT is released, the transporter is used to remove 5-HT from the synapse to either (a) terminate the actions of 5-HT in the synaptic cleft and/or (b) allow the captured 5-HT to be stored for subsequent reuse. When an SSRI binds to a SERT, 5-HT is no longer transported to the presynaptic terminal, and it therefore, accumulates in the synapse to prolong and increase its interactions at all the various 5-HT receptor subtypes.

Numerous studies employing SSRIs, including in humans, have demonstrated attenuated aggression following increased serotonergic activity (Ferris et al., 1997; Kavoussi, Liu, & Coccaro, 1994; Muehlenkamp, Lucion, & Vogel, 1995; Sánchez & Hyttel, 1994; White, Kucharik, & Moyer, 1991). For example, rats that typically do not engage in fighting have become moderately more aggressive after chronic treatment with an SSRI (Mitchell, 2005). Aside from the elevation in extracellular 5-HT, the anti-aggressive effects of SSRIs may result from increasing the γ-aminobutyric-acid-A (GABA_A) receptor agonist allopregnanolone (Pinna, Dong, Matsumoto, Costa, & Guidotti, 2003). Interestingly, depending on the individual’s prior experience, allopregnanolone can inhibit or activate aggression (Pinna et al., 2003) and therefore, increase the probability of instances when SSRIs can increase aggression.

1.3.2.1 Fluoxetine Hydrochloride (Prozac™)

The most widely used SSRI among humans for selective psychiatric conditions is fluoxetine hydrochloride (FLX), also known as Prozac™. Studies examining effects of chronic FLX treatment have demonstrated a robust decrease in territorial aggression (Lynn, Egar, Walker, Sperry, & Ramenofsky, 2007; Perreault, Semsar, & Godwin, 2003) and change in social status (Larson & Summers, 2001; Olivier, Mos, Vanderheyden, & Hartog, 1989). In rodents, FLX was found to inhibit muricidal behaviour in spontaneously muricidal rats (Molina, Ciesielski, Gobaille, Isle, & Mandel, 1987) and attenuate footshock-induced aggression (Datla, Mitra & Bhattacharya, 1984). Moreover, FLX was found to decrease alcohol-potentiated aggression in a shock-induced aggression model in mice (Wagner, Fisher, Pole, Borge, & Johnson, 1993), and
more recently in humans, reduce measures of anger and physical aggression in alcoholic perpetrators of intimate partner violence (George et al., 2011). Lastly, although FLX and its main metabolite norfluoxetine (Figure 4) have low affinities to 5-HT, muscarinic acetylcholine or dopamine receptors, some effects of this drug might be partially attributable to effects on these receptor sites under conditions of high FLX or norfluoxetine concentrations (reviewed in Hiemke & Härtter, 2000).


1.4 Naked Mole-Rats

The effects of manipulating the serotonergic system on aggression are well studied in many species, but not in eusocial rodents like the naked mole-rat. In the following sections, eusociality and its relevance to the naked mole-rat as an animal model for the current study will be discussed. Secondly, the unique and very rigid reproductive hierarchy that naked mole-rats exhibit will be described with respect to its role in development of their nervous system. And lastly, status-specific behaviours of the naked mole-rat will be discussed with focus on the
current study’s investigation of aggression and dominance via manipulation of the serotonergic system.

1.4.1 Cooperative Breeding & Eusociality

Social interactions are important regulators of reproduction in highly social mammals (e.g., Asa, 1997; Creel & Waser, 1997; French, 1997). In cooperatively breeding species, lower-ranking members of the social hierarchy forego direct reproductive efforts to facilitate reproduction by socially dominant conspecifics (Lacey & Sherman, 1991). In other words, an individual’s inclusive fitness may include contributions to the gene pool through the support of reproductive efforts of close relatives (Michener, 1969). Moreover, cooperative breeding may be associated with a relative lack of sex differences and a reduced influence of gonadal hormones on some functions to which these hormones are closely related (Alexander, Noonan, & Cespi, 1991). Therefore, research that focuses on cooperative breeding not only bolsters current understanding of the evolution of sex differences, but also, the neuroendocrinology of reproductive suppression.

Eusociality refers to the highest level of social organization based on reproductive castes, where species live in large groups that consist of multiple adult generations (Michener, 1969). Eusociality is when reproductive skew is very high and very stable, thereby representing an extreme form of cooperative breeding (Alexander et al., 1991). While eusociality has been a highly successful strategy for insects, with eusocial species constituting 75% of the insect biomass in some ecosystems, few mammalian species have similarly complex social structures (Fittkau & Klinge, 1973).

The social and reproductive system evident in naked mole-rats was the first claim of eusociality in a mammal (Jarvis, 1981). Naked mole-rats, native to Africa, exhibit the closest mammalian equivalent to eusociality (Brett, 1991; Jarvis, 1981). These nearly hairless rodents live in large subterranean colonies with a rigidly organized reproductive hierarchy (Brett, 1991). 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 non-reproductive subordinates (Brett, 1991; Jarvis, 1981; Lacey & Sherman, 1991).
1.4.2 Rigid Reproductive Hierarchy

As previously mentioned, each naked mole-rat colony consists of a breeding female and one to three breeding males; all other members of the colony are termed “non-breeders” or “subordinates”. The term “breeder” refers to an animal that at some point produced a litter but is not necessarily actively breeding to maintain their status (Jarvis, 1991). The terms “non-breeder” or “subordinate” are synonymous, and refer to the other members of the colony that have never reproduced (Faulkes & Abbott, 1997). Some researchers also argue that subordinates can be further divided into three groups: (1) frequent workers, (2) infrequent workers and (3) non-workers or “soldiers”, who instead, are involved in colony defence against predators (Jarvis, 1981; Lacey & Sherman, 1991). These social roles will play an important role in classifying unique behaviours that are characteristic of their status. At the root of these behavioural differences that will be discussed in detail later, are neural and hormonal differences that are also based on their reproductive hierarchy.

Physiological suppression in subordinates is more profound in females compared to males. Female subordinates have small uteri as well as small ovaries without corpora lutea, remaining in a pre-pubertal state throughout life (reviewed in Holmes et al., 2009). Male subordinates have lower levels of urinary testosterone (Clarke, Miethe, & Bennett, 2001), and smaller mean testis weights and numbers of spermatozoa (Faulkes, Trowell, Jarvis, & Bennett, 1994) compared to male breeders. As mentioned above, the reproductive hierarchy of naked mole-rats is very rigid. The queen and male breeders are socially dominant over the other colony members and this social dominance is currently thought to suppress reproduction in subordinates (Clarke & Faulkes, 1997; Faulkes & Abbott, 1993; Jarvis, 1981; Reeve, 1992). Specifically, it has been suggested that frequent and repetitive “shoving”, as well as other dominant behaviours by the queen, albeit without direct evidence, suppress reproduction in colony subordinates (Clarke & Faulkes, 1997; Faulkes & Abbott, 1993; Jarvis, 1981; Reeve, 1992).

Subordinate naked-moles rats may become breeders within their natal colony if a breeding member of the colony dies or if they are removed from the colony and housed with a mate (Margulis, Saltzman, & Abbott, 1995). In the case that a queen dies or is removed from the colony, a subset of genetically-related subordinate females fight (to the death in some cases) for the single breeding position (Clarke & Faulkes, 1997; Margulis et al., 1995). For the new queen,
morphological changes ensue (e.g., elongated body associated with lengthening of individual vertebrae) that distinguish her from subordinates (O’Riain, Jarvis, Alexander, Buffenstein, & Peeters, 2000).

Similarly, when an animal is removed from the colony and paired, it experiences neural and endocrine changes (Holmes et al., 2011). As a result, subordinates never exhibit sexual behaviours within the colony, but do so after pairing (Holmes et al., 2011). An important cue triggering changes in brain morphology in new breeders may therefore be the removal of such suppressive signals from the queen (Holmes et al., 2007). Alternatively, or in addition, the introduction of “positive” social cues arising from the new mate may also play a role (Holmes et al., 2007). Once established, however, the breeders are rarely overthrown, and it is estimated that in nature less than 1% of all naked mole-rats attain reproductive status (Jarvis, O’Riain, Bennett, & Sherman, 1994). Therefore, their reproductive hierarchy is very stable; once an animal becomes a breeder, it generally remains so for life, which can exceed 20 years, making them exceptionally long-lived for their size (Buffenstein, 2005).

There is an impressive lack of sex differences in physical and behavioural parameters in subordinate naked mole-rats. First, subordinate males and females participate equally in grooming, resting, thermoregulation, feeding, elimination, coprophagy, locomotion, orientation, transport of food and nesting material, digging, pup tending, interactive behaviours, agonistic behaviours, and alarm reactions (Lacey, Alexander, Braude, Sherman, & Jarvis, 1991; Lacey & Sherman, 1991). Second, there is no sex difference in overall body size or weight or anogenital distance (Lacey & Sherman, 1997; Peroulakis, Goldman, & Forger, 2002). Lastly, naked mole-rats lack many of the sexual dimorphisms in the brain and spinal cord that are seen in other mammals (Holmes et al., 2007; Peroulakis et al., 2002; Seney, Goldman, & Forger, 2006).

1.4.3 Effects of Sex and Breeding Status on the Brain

The reduction of morphological and behavioural sex differences in subordinate naked mole-rats, despite sex-typical gonadal differentiation, may be a feature that has evolved in relation to the reproductive hierarchy and associated reproductive skew in this species (Holmes et al., 2009). On the other hand, status differences have been found in the nervous system. In a study examining the brains of breeding and subordinate naked mole-rats of both sexes, stereological analyses revealed that neural morphology depends on status, such that breeders have increased
volume of several brain regions linked to neuroendocrine function and reproductive behaviours in other rodents (de Vries & Simerly, 2002): the paraventricular nucleus of the hypothalamus (PVN), the principal nucleus of the bed nucleus of the stria terminalis (BSTp), and the MA (Holmes et al., 2007). It has been postulated that these changes contribute to the differences in behaviour and neuroendocrine function that exist between subordinates and breeders (Holmes et al., 2011).

In a study investigating social and hormonal triggers of neural plasticity in naked mole-rats, Holmes et al. (2011) found that some neural differences between subordinates and breeders are triggered by removing animals from the natal colony and pairing them, whereas other variables seem to require reproductive experience. For example, pairing alone was sufficient to cause breeder-like changes in volume of the PVN (Holmes et al., 2011). However, increases in BSTp volume were evident only in animals that reproduced (Holmes et al., 2011). It was also found that long-term gonadectomy did not reverse the breeder-like neural changes in the PVN or BSTp (Holmes et al., 2011). Therefore, neural changes associated with breeding status in naked mole-rats may be triggered by different aspects of the social and reproductive environment and once the changes occur, they are largely independent of gonadal hormones (Holmes et al., 2011). These findings confirm that reproductive or social status, rather than sex, is predictive of neural morphology and may therefore be responsible for specific behavioural differences between subordinates and breeders (Holmes et al., 2011). In sum, naked mole-rats are at one extreme of the spectrum of sociality displayed by mammals, with larger colony sizes, greater genetic similarity shared between colony members and more marked reproductive skew than seen in any other social mammals (Holmes et al., 2009). Consequently, naked mole-rats are a powerful animal model for studying the effects of social status on development and plasticity of the mammalian nervous system. For the current study, further analysis of behavioural differences that are contingent upon the social status of the naked mole-rat will be discussed with focus on differences in aggression.

1.4.4 Behavioural Specializations

Numerous status-specific behavioural specializations are seen in this species. The most conspicuous are reproductive behaviours; only breeders exhibit direct reproductive behaviours including copulation and nursing (Jarvis, 1981; Jarvis, 1991; Lacey, Alexander, Braude,
Sherman, & Jarvis, 1991; Lacey & Sherman, 1991), and mutual genital nuzzling (Jarvis, 1991; Goldman, Forger, & Goldman, 2006). Subordinates assist with pup care and are predominantly responsible for foraging, colony defense and maintenance of the tunnel system (Brett, 1991; Jarvis, 1981; Lacey et al., 1991; Lacey & Sherman, 1991). In addition, subtle and overt agonistic interactions (e.g., shoving) by breeders inhibits gonadal function in subordinates (Faulkes & Abbott, 1997). Specifically, subordinates, irrespective of sex, shove at lower rates relative to breeders (Clarke & Faulkes, 2001; Jacobs & Jarvis, 1996; Reeve, 1992; Reeve & Sherman, 1991). Lastly, there is a correlation between body size and shoving among subordinates: higher-ranking, larger subordinates shove more than lower-ranking, smaller subordinates (Reeve & Sherman, 1991). 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 behaviours.

1.5 Current Study

In light of (a) how aggression is altered by varying levels of 5-HT in other species, and (b) how behaviours 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 behaviours 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 behaviour in dominant and subordinate animals. Specifically, we predicted that chronic treatment of FLX would attenuate aggression in queens, but not in subordinates.

2 Method

2.1 Animals & 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 hr 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, 8th ed., (2011) and the Canadian Council on Animal Care guidelines.

2.3 Experimental Procedure

2.3.1 Experiment 1: Subordinates

In each of 4 separate colonies, 8 subordinates (4 experimental and 4 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 (Buffenstein, 2005; O’Riain & Jarvis, 1998). 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 behaviours. On ED 2, to establish baseline paired behaviours, 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 adult 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 minutes after injection, in-colony behaviours were recorded for 30 minutes 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 Figure 5A for experimental design and timing of procedures).

2.3.2 Experiment 2: 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 behavioural 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 versus 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 minutes after injection, in-colony behaviours were recorded on every seventh day of FLX injection (ED 10, 17, 24 and 31). On ED 32 and 33, queens were again paired with unfamiliar subordinates 30 minutes following injection. The experimental procedure on ED 32 and 33 paralleled the experimental procedure on ED 2 and 3, respectively (see Figure 5B for experimental design and timing of procedures).
Figure 5. Experimental design and timing of (A) Experiment 1 and (B) Experiment 2.

2.4 Data Collection & Analysis

All behaviours (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, Clarke and Faulkes (1997) established that high-ranked naked mole-rats typically pass over a lower ranked animal, while lower ranked animals pass under. Due to the fact that these state behaviours are not mutually-exclusive, pass-over and pass-
under behaviours were expressed as a percentage (i.e., the total number of overpasses divided by the sum of overpasses and underpasses).

Table 1. Operationally defined naked mole-rat behaviours scored during in-colony and paired paradigms.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Operational Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td>In transit; default behaviour</td>
</tr>
<tr>
<td>Dig/Climb</td>
<td>Displace bedding using fore/hind limbs or equivalent movement against chamber wall</td>
</tr>
<tr>
<td>Gnaw</td>
<td>Repeated opening/closing of mouth; teeth scrap against a surface of the chamber</td>
</tr>
<tr>
<td>Groom</td>
<td>Repeated forward/backward motion of fore or hind limbs to clean/scratch body area</td>
</tr>
<tr>
<td>Self Nuzzle</td>
<td>Coprophagy</td>
</tr>
<tr>
<td>Maintain Environment²</td>
<td>Displacement/movement of nesting material</td>
</tr>
<tr>
<td>Food Interaction²</td>
<td>Eating or moving food</td>
</tr>
<tr>
<td>Inactive</td>
<td>Not in transit, isolated from other NMRs</td>
</tr>
<tr>
<td>Sniff Appraisal</td>
<td>Olfactory investigation of genital, facial or other body area of another NMR</td>
</tr>
<tr>
<td>Huddle</td>
<td>Not in transit while making contact with one or more inactive NMRs</td>
</tr>
<tr>
<td>Genital Nuzzle¹</td>
<td>Mutual (or not) sniffing/licking appraisal of genital area among breeders in a colony</td>
</tr>
<tr>
<td>Pass Over³</td>
<td>Entire body actively crosses over top of another NMR</td>
</tr>
<tr>
<td>Pass Under³</td>
<td>Entire body actively crosses underneath another NMR</td>
</tr>
<tr>
<td>Incisor Fencing⁴</td>
<td>Locking teeth with another NMR to aggressively control/maneuver them</td>
</tr>
<tr>
<td>Bite &amp; Drag⁴</td>
<td>Clasping the skin/body of another NMR and pulling them a given distance</td>
</tr>
<tr>
<td>Open-Mouth Gape⁴</td>
<td>Territorial head-to-head action marked by the stretching open the mouth</td>
</tr>
<tr>
<td>Tetany</td>
<td>Submission to a dominant NMR by laying on its reverse side</td>
</tr>
</tbody>
</table>

¹Queen-specific behaviour. ²Not scored for paired paradigm. ³Point behaviour (measures frequency); other [state] behaviours measure duration. ⁴Combined for overall measure of aggression.

Statistical analyses were conducted via SPSS Statistics Desktop V21.0.0 by IBM Corporation (Armonk, NY). To demonstrate a successful drug manipulation (i.e., physiological effect), changes in body weight for FLX animals were compared to body weights of CTLs before, during and after the chronic dosing regimen. A 2X5 mixed-design analysis of variance (ANOVA) was conducted for subordinate body weight with group (FLX vs. CTL) as a between-subject factor
and ED (1 vs. 9 vs. 16 vs. 23 vs. 30) as a within-subject factor. For queens, a repeated measures ANOVA was conducted to compare body weight on ED 1, 10, 17, 24 and 31.

For Experiment 1, the subordinate in-colony behaviours were analyzed using 2X2X5 mixed-design ANOVAs for each behaviour of interest with group (FLX vs. CTL) and sex as between-subject factors, and ED (1 vs. 9 vs. 16 vs. 23 vs. 30) as a within-subject factor. For the paired testing, 2X2X2 mixed-design ANOVAs were conducted for the behaviours of interest with group (FLX vs. CTL) and sex as between-subject factors, and ED (2 vs. 31) as a within-subject factor.

For Experiment 2, the queen in-colony behaviours were analyzed using repeated measures ANOVAs to compare the behaviours of interest on ED 1, 10, 17, 24 and 31. For the paired testing, 2X2 mixed-design ANOVAs were conducted for the behaviours of interest with caste of subordinate pairing partner (worker vs. soldier) as a between-subject factor and ED (2-3 vs. 32-33) as a within-subject factor. One queen was removed from these particular analyses due to injury by a presumed soldier and consequent withdrawal from the second half of the paired behaviour tests, resulting in a new sample size of 12.

Also, a consistent and accurate classification of the caste of subordinate conspecifics (i.e., worker vs. soldier) in the queen out-pairing paradigm was necessary to validate/interpret any behavioural effects of FLX treatment that might be dependent on the caste of the stimulus subordinate. Soldiers are typically (1) older and (2) heavier than other subordinates within a colony (Reeve & Sherman, 1991). First, to ensure soldiers were older than workers, first-born subordinates were classified as soldiers and subsequent generations were classified as workers. Second, weights of stimulus animals classified as workers were compared to weights of stimulus animals classified as soldiers. A 2X2 mixed-design ANOVA, with presumed subordinate caste (worker vs. soldier) as a between-subject factor and ED (2-3 vs. 32-33) as a within-subject factor, revealed a main effect of subordinate caste \( F(1, 18) = 30.94, p < 0.001 \). Specifically, presumed soldiers \( (M = 51.96, SEM = 2.92) \) weighed (g) more than presumed workers \( (M = 35.44, SEM = 1.90) \), satisfying the second condition above.

Lastly, post hoc comparisons consisted of independent and paired t-tests where appropriate. All means are reported with SEMs and \( p < 0.05 \) was considered statistically significant.
3 Results

3.1 Experiment 1: Subordinates

Consistent with previous reports for this species (Sherman, Jarvis, & Alexander, 1991), no significant effects of sex were detected for any of the behaviours examined. Thus, data from males and females were combined in all subsequent analyses.

3.1.1 Body Weight

A 2x5 mixed-design ANOVA revealed a significant Group x Day interaction (F(4, 120) = 29.30, p < 0.001), such that subordinates who received FLX lost a significant amount of weight during the dosing regimen compared to the relative increase in body weight of CTLs (Figure 6A).

3.1.2 In-Colony Behaviour

For the subordinate in-colony behaviour tests, 2x5 mixed-design ANOVAs revealed a significant Group x Day interaction (F(4, 120) = 2.65, p = 0.037) for the time subordinates spent digging/climbing (Figure 7). Specifically, FLX animals spend less time (s) digging/climbing compared to CTLs on ED 30, after four weeks of FLX treatment (t(30) = 2.52, p = 0.01). No other in-colony behaviours were affected by FLX treatment; all Group x Day interactions for these behaviours were non-significant (data not shown). Importantly, virtually no aggression was seen with in-colony subordinates, which is consistent with previous reports (Lacey et al., 1991). Therefore, we were unable to perform statistical analyses on the expression of aggression; any effect of FLX on the reduction of aggression would be masked by a floor effect.

3.1.3 Paired Behaviour

For the duration of aggression displayed by the subordinates during the out-pairing paradigm, a 2x5 mixed-design ANOVA revealed a main effect of Day (F(1, 30) = 4.08, p = .052) that approached statistical significance (Figure 8A). The frequency of aggression differed such that a main effect of Group (F(1, 30) = 4.80, p = 0.036) reached statistical significance (Figure 8B). Specifically, the frequency of aggression in FLX animals was higher compared to CTLs. However, this difference was not only present after (ED31), but also before the start of the chronic drug regimen (ED 2). Importantly, the Group x Day interactions for duration (F(1, 30) < 0.01, p = 0.96) and frequency (F(1, 30) = 0.24, p = 0.63) of out-paired aggression displayed by
subordinates was not affected by FLX treatment. Lastly, all other Group x Day interactions for other behaviours displayed in this paradigm were not significant (data not shown).

3.2 Experiment 2: Queens

3.2.1 Body Weight

A repeated measures ANOVA revealed a main effect of Day \( (F(4, 48) = 6.39, p < 0.001) \) on the body weight of queens (Figure 6B). A significant decrease in body weight after the chronic dosing regimen confirms a physiological effect of FLX for the queens similar to that seen in subordinates.

3.2.2 In-Colony Behaviour

For the queen in-colony behaviour tests, FLX significantly affected sniffing behaviour, whereby a repeated measures ANOVA revealed a significant main effect of Day for time spent sniffing other members of the colony \( (F(4, 44) = 3.02, p = 0.03; \) Figure 9A). Specifically, post hoc paired \( t \)-tests revealed that queens spend less time (s) sniffing on ED 24 compared to baseline on ED 1 \( (t(11) = 3.01, p = 0.01) \). Additionally, FLX significantly affected pass-over percentage, (main effect of Day: \( F(4, 44) = 4.14, p = 0.006; \) Figure 9B), where post hoc paired \( t \)-tests revealed that queens had a greater pass-over percentage compared to baseline on ED 24 \( (t(12) = 2.44, p = 0.03) \) and ED 31 \( (t(11) = 2.36, p = 0.04) \).
Figure 6. Body weight before, during and after chronic dosing regimen. (A) Mean (±SEM) body weight (g) of subordinates at baseline [Experimental Day (ED) 1] and after peripheral administration of 0.9% sterile saline or 10 mg/kg of fluoxetine hydrochloride (FLX) on ED 9, 16, 23 and 30. FLX-treated animals weighed significantly less than control animals on ED 30. (B) Mean (±SEM) body weight (g) of queens at baseline on ED 1 and after peripheral administration of 10 mg/kg of FLX on ED 10, 17, 24 and 31. Body weight was significantly reduced after 4 weeks of FLX treatment compared to ED 1.
Figure 7. Subordinate in-colony digging/climbing. Mean (±SEM) duration (s) subordinates spent digging/climbing in-colony at baseline [Experimental Day (ED) 1] and on ED 9, 16, 23 and 30. Fluoxetine hydrochloride treatment reduced digging/climbing behaviour on ED 30.

Duration (main effect of Day: $F(4, 44) = 0.94, p = 0.45$; Figure 9C) and frequency (main effect of Day: $F(4, 44) = 1.09, p = 0.37$; Figure 9D) of in-colony aggression was not significantly affected by FLX treatment. Although queens did exhibit aggression in-colony, in contrast to subordinates, it was at much lower levels compared to the paired paradigm (see below). Therefore, as with subordinates, any potential effect of FLX might be masked by floor effects. Similarly, no effect of FLX was found on digging/climbing behaviour in queens (main effect of Day: $F(4, 44) = 0.74, p = 0.57$; Figure 9E). Queens exhibit this behaviour at a much lower duration compared to subordinates at baseline (compare Figure 7 and Figure 9E), which is in accordance with the naked mole-rat ethogram (Brett, 1991; Jarvis, 1981; Lacey et al., 1991; Lacey & Sherman, 1991). No other in-colony behaviours were affected by FLX treatment; all main effects of Day for these behaviours were not significant (data not shown).
Figure 8. Subordinate paired aggression. (A) Mean (±SEM) duration (s) of subordinate, out-paired aggression at baseline [Experimental Day (ED) 2] and after the final injection on ED 31. No significant difference in aggression was found between groups pre- or post-treatment. (B) Mean (±SEM) frequency of subordinate, out-paired aggression at baseline [Experimental Day (ED) 2] and after the final injection on ED 31. Fluoxetine-treated animals had decreased aggression compared to control animals both pre- and post-treatment.
Figure 9. Queen in-colony behaviour. In-colony behaviours for queens at baseline (Experimental Day (ED) 1) and after chronic fluoxetine hydrochloride (FLX) treatment (ED 10, 17, 24, and 31). (A) Mean (±SEM) duration (s) for sniffing other colony members and (B) mean (±SEM) pass-over %. FLX significantly decreased sniffing behaviour and increased pass-over percentage but had no significant effects on in-colony aggression or digging/climbing.
Figure 9. Queen in-colony behaviour. In-colony behaviours for queens at baseline [Experimental Day (ED) 1] and after chronic fluoxetine hydrochloride (FLX) treatment (ED 10, 17, 24, and 31). (C) Mean (±SEM) duration (s) of queen-initiated aggression and (D) mean (±SEM) frequency of queen-initiated aggression. FLX significantly decreased sniffing behaviour and increased pass-over percentage but had no significant effects on in-colony aggression or digging/climbing.
3.2.3 Paired Behaviour

FLX treatment significantly reduced the expression of queen-initiated aggression in the out-pairing paradigm. Both duration (main effect of Day: $F(1,22) = 4.39, p = 0.048$; Figure 10A) and frequency (main effect of Day: $F(1,22) = 6.32, p = 0.02$; Figure 10B) were reduced on ED 32-33 compared to baseline (ED 2-3). While the Day x Subordinate Caste interactions were not significant (duration: $F(1,22) = 2.73, p = 0.11$; frequency: $F(1,22) = 2.26, p = 0.15$), visual inspection of the graphs (Figure 10A and B) suggests that the main effects of Day on duration and frequency of queen-initiated aggression are heavily influenced by the caste of the subordinate. To explore this possibility, paired $t$-tests for queen-soldier and queen-worker pairings for aggression were conducted. First, the duration of queen-initiated aggression directed at soldiers appeared to decrease after FLX treatment (ED 32-33 compared to ED 2-3), but this only approached statistical significance ($t(11) = 2.17, p = 0.053$). The duration of queen-initiated aggression directed at workers was not affect by FLX treatment ($t(11) = 0.44, p = 0.67$). Second, the frequency of queen-initiated aggression directed at soldiers decreased significantly
after FLX treatment ($t(11) = 2.38$, $p = 0.036$); this effect was not seen in queen-initiated aggression directed at workers ($t(11) = 0.94$, $p = 0.37$).

No other behaviours were affected by FLX treatment in queens in the paired paradigm; all main effects and Day x Subordinate Caste interactions for these behaviours were non-significant (data not shown).

Figure 10. Queen paired aggression. Mean (±SEM) duration (s) (A) and frequency (B) of queen-initiated aggression at baseline [Experimental Day (ED) 2 or 3] and after the final injection on ED 31 between queen-soldier or queen-worker out-pairings. Fluoxetine significantly decreased both measures.
4 Discussion

Results of the current study demonstrate status-dependent (i.e., subordinate vs. queen) behavioural effects of FLX in the naked mole-rat that are also contingent upon the social environment (i.e., in-colony vs. out-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. Particularly, decreased aggression following FLX treatment in queens appears to be contingent on the caste of the subordinate such that attenuated queen-initiated aggression is largely driven by out-pairings with unfamiliar soldiers compared to workers. Also, FLX treatment increased the pass-over percentage of queens in their colony and decreased the time a queen sniffed other colony members. Lastly, FLX had no effects on aggression in subordinates either in-colony or during pairing. It did, however, decrease in-colony digging/climbing in subordinates.

Studies examining the effects of chronic FLX treatment have demonstrated a robust decrease in aggression (Kohlert et al., 2012; Lynn et al., 2007; Olivier et al., 1989; Perreault et al., 2003) and reversal of dominant social status (Larson & Summers, 2001). Similarly, the current study demonstrated decreased frequency of queen-initiated aggression against unfamiliar animals after chronic treatment with FLX. Particularly, attenuated queen-initiated aggression is largely driven by out-pairings with soldiers compared to workers.

Aggression was not significantly attenuated by FLX in subordinates, either in-colony or during pairing. However, specific interactions between workers and soldiers were not investigated in the current study (i.e., subordinates were not classified as workers or soldiers in Experiment 1). Therefore, it is possible that FLX influences aggression in subordinate naked mole-rats via a mechanism that differs from breeders (i.e., status-specific), or that differs between different sub-castes of subordinates. Assessing worker-soldier interactions, similar to the queen-subordinate interactions in the current study, will enhance existing knowledge of the differences between naked mole-rat castes.

With regards to status-specific differences, during agonistic interactions in several species, serotonergic activity increases consistently in subordinates (Summers et al., 2005; Yodyingyuad, De La Riva, Abbott, Herbert, & Keverne, 1985) and decreases or remains unchanged in dominants (Ferrari, Van Erp, Tornatzky, & Miczek, 2003; Summers et al., 2003; van Erp &
Miczek, 2000). Moreover, many studies have shown that subordinate animals have higher serotonergic activity than dominant, aggressive individuals (Blanchard et al., 1991; Matter et al., 1998; Raleigh et al., 1991; Winberg and Nilsson, 1993). Therefore, subordinate naked mole-rats may already have very high levels of endogenous 5-HT compared to breeders prior to FLX treatment.

McDonald, Gonzalez, and Sloman (2011) found that FLX affected aggressive behaviour in dominant, but not subordinate fish, despite having the same elevation in circulating 5-HT concentrations. According to Summers et al. (2005), it is the timing and magnitude of the cortisol response of dominant versus subordinate fish that differs. Specifically, the cortisol response in dominant fish is very brief and levels return to baseline within hours of establishing a stable hierarchy, while less aggressive subordinate fish are characterized by a chronic elevation in plasma cortisol (Sloman et al., 2005). Therefore, if this relationship can be generalized to a eusocial mammalian species like the naked mole-rat, it is possible that subordinates treated with FLX do not differ in their behaviour compared with controls because the elevation in cortisol would interfere with the action of 5-HT. Indeed, in mammals, many 5-HT receptors respond to stress by reducing mRNA expression (e.g., Chalmers, Kwak, Mansour, Akil, & Watson, 1993). Consequently, future investigation of interacting molecules, such as cortisol, with the serotonergic system may help elucidate underlying status-specific neural mechanisms that differentiate the behavioural responses of these eusocial animals.

Neural morphology in naked mole-rats has been shown to be status dependant. Breeders show more cells in the VMH, and a larger volume in the BNST and MA (Holmes et al., 2007), brain areas that are involved in aggression (Nelson & Trainor, 2007; Newman, 1999). Thus, breeders may be differentially responsive to FLX treatment compared to subordinates based on differences in neural morphology of brain regions known to control aggression.

The behavioural effects of elevated 5-HT can be affected by the social environment (i.e., in-colony vs. unfamiliar conspecific out-pairing paradigm). According to Nesher et al. (2012) dominant behaviour may be expressed differentially according to the environment and social situation. For naked mole-rats, aggression within a colony is rare and often limited to situations of social instability (i.e., between high-ranked female subordinates in the absence of a queen; Clarke & Faulkes, 1997). Consequently, no FLX effect on displays of in-colony aggression by
any member of a naked mole-rat colony may be attributed to a floor effect in that low levels of aggression were already present prior to drug treatment.

Although FLX did not affect in-colony aggression in queens or subordinates, it did increase the pass-over percentage of queens in their colony. Clarke and Faulkes (1997) established that high-ranked naked mole-rats typically pass over a lower ranked animal, while lower ranked animals pass under. Furthermore, dominance rank based on the number of passes an animal makes over another is highly correlated with dominance rank based on agonistic behaviours (Clarke & Faulkes, 1997). In addition, dominant behaviours are often reflections of self-perceived social rank, and high 5-HT levels have been reported to enhance the perception of dominance in others (reviewed in Kiser, Steemers, Branchi, & Homberg, 2012). Taken with the fact that queens also exhibit a significant decrease in sniffing of other colony members following FLX treatment, and that olfactory cues appear important for individual recognition and maintaining social roles in this species (Kutsukake, Inada, Sakamoto, & Okanoya, 2012), we propose that an increased pass-over percentage by a queen reflects a compensatory mechanism in light of the possibility that her perceived social status may have been compromised.

In subordinates, in-colony digging/climbing decreased following FLX treatment. Many species subject to barren or unnatural environments tend to exhibit stereotypies, which represent a wide range of frequently repeated patterns of movements that may not serve the intended function under the conditions in which they appear (Crowell-Davis, 2007). In the laboratory, naked mole-rats robustly exhibit digging behaviours despite no goal acquisition (i.e., they do not actually dig “out” of the caging). Thus, the FLX-induced reduction of digging agrees with a growing literature on similar stereotypical behaviours in animal models of human psychiatric disorders that have been linked to a disruption in the brain’s 5-HT system, including Obsessive-Compulsive Disorder (OCD). In particular, FLX has been found to be effective in decreasing stereotypies in animal models of OCD including food-restriction-induced running (Altemus, Glowa & Murphy, 1993), canine acral lick dermatitis (Stein, Shoulberg, Helton, & Hollander, 1992), schedule-induced polydipsia (Woods et al., 1993) and spontaneous alternation behaviour (Yadin, Friedman, & Bridger, 1991). Relatively recently, prosocial effects were seen in individually housed Mongolian gerbils treated with FLX, but only sedative effects were evident in gerbils maintained in groups (Hendrie et al., 2003). Similarly, differential effects of FLX
were found in the current study such that digging/climbing decreased in colony, but not during subordinate-subordinate out-pairings.

It is important to note that this is the first study involving chronic treatment of FLX using naked mole-rats. Consequently, dose levels and the time between injection and behavioural testing were established based on other animal models. According to Hendrie et al. (2003) 10 mg/kg of FLX for the Mongolian gerbil is considered a “high” dose and an uptake period of 30 min was sufficient to maximize the likelihood of a physiological effect. In addition, the aforementioned dose level was used in a related study using female mice (Haug, Wallian, & Brain, 1990) that found significant changes in behaviour following FLX administration. It is likely that the drug dosage and uptake period of the current study was appropriate given a significant decrease in body weight for (a) subordinates after chronic treatment of FLX compared to controls and (b) in queens after similar FLX treatment relative to baseline. Furthermore, variability associated with in-colony behaviours, including aggression, could be attributed to differences in the composition of the colonies studied, such as the colony’s age, size and worker-to-soldier ratio. According to Clarke and Faulkes (2001), social context at the time of observation may also be important, for example, periods of reproductive quiescence or activity. With that said, discrepancies between the results of Experiment 1 and 2 may be partially attributed to seasonal variations in behaviour at the time of testing.

Several avenues of future research are warranted given the results of the current study. First, 5-HT has an inhibitory effect on aggression, and this seems to be well-conserved in vertebrates (Edwards & Kravitz, 1997; Popova, 2006; Summers & Winberg, 2006; Winberg & Nilsson, 1993). Indeed, decreasing 5-HT concentration using p-chlorophenylalanine (PCPA, an inhibitor of 5-HT synthesis) increases aggression in domestic chickens (Buchanan, Shrier, & Hill, 1994) and fish (Adams, Liley, & Gorzalka, 1996). Replicating the current study using a suitable 5-HT antagonist to assess potential reversal of our behavioural observations would further confirm a role for 5-HT in aggression in naked mole-rats. Second, comparing the social hierarchy profile of a colony (via observation of pass-over behaviours) before and after FLX administration would provide more insight into subtle concomitant disruptions in social status that may have gone undetected among sub-castes of subordinates. Third, it is possible that other social mechanisms may be involved in establishing social status and formation of a rigid reproductive hierarchy in a naked mole-rat colony. For example, vocal cues may be important in naked mole-rats (Yosida &
Okanoya, 2009). According to Pepper et al. (1991) naked mole-rats show several vocal agonistic behaviours which may be performed in conjunction with more subtle behavioural cues not detected in the current study. Antiphonal vocalizations are associated with affiliation in squirrel monkeys (Biben 1993; Biben & Symmes 1991; Soltis et al., 2002) and stump-tailed macaques (Bauers & De Waal 1991), social dominance in white-faced capuchins (Digweed et al., 2007), and affiliation and dominance behaviours in African elephants (Leighty et al., 2008). Assessing changes in antiphonal vocalizations as a result of chronic FLX treatment may provide further insight into status-specific behaviours that maintain the rigid naked mole-rat reproductive hierarchy.

Collectively, these data raise interesting questions about status- and environment-specific behaviours that are differentially affected by FLX in the naked mole-rat. Further exploration of pharmacological manipulation on status-specific behaviours in this eusocial species may elucidate the neurobiological mechanisms underlying their unique and rigid social hierarchy.
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