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AGONISTIC ENCOUNTERS AND BRAIN ACTIVATION IN DOMINANT AND SUBORDINATE MALE GREATER LONG-TAILED HAMSTERS

Yongliang Pan^a, Linxi Xu^a, Kimberly A. Young^b, Zuoxin Wang^b, and Zhibin Zhang^a

^aState Key Laboratory of Integrated Management of Pest Insects and Rodents in Agriculture, Institute of Zoology, Chinese Academy of Sciences, Datun Road, Chaoyang District, Beijing 100101, PR China

^bDepartment of Psychology and Program in Neuroscience, Florida State University, Tallahassee, FL 32306, USA

Abstract

During an agonistic encounter test, dominant male greater long-tailed hamsters (*Tscheskia triton*) initiated attacks sooner and displayed higher levels of aggression and flank marking behavior than their subordinate counterparts. Accordingly, subordinate males exhibited more defensive behavior than dominant ones. Specific patterns of neuronal activation, measured by Fos-immunoreactive staining (Fos-ir), were found in the hamster brain following agonistic interactions. Increased Fos-ir was observed in the bed nucleus of the stria terminalis (BST), ventromedial hypothalamus (VMH), and medial (MeA) and anterior cortical (ACo) nuclei of the amygdala (AMYG) in both dominant and subordinate males. In contrast, dominant males had significantly higher Fos-ir densities in the medial preoptic area (MPOA) than subordinate males, whereas subordinate males expressed higher densities of Fos-ir in the anterior hypothalamus (AH) and central nucleus of the amygdala (CeA). Additionally, Fos-ir levels in the MPOA were significantly correlated with aggression and Fos-ir levels in the AH and CeA were correlated with defensive behavior. Together, our data indicate distinct patterns of neuronal activation associated with agonistic encounters in a behavior-specific manner in male greater long-tailed hamsters.

Keywords

aggression; defense; c-fos; medial preoptic area; anterior hypothalamus; amygdala

Introduction

Agonistic behavior has been defined as adaptive aggressive and defensive actions that occur during a conflict between members of the same species (Scott and Fredericson, 1951). Agonistic encounters usually lead to the establishment of a dominant-subordinate hierarchy, which, in turn, plays an important role in socialization, resource acquisition, reproductive

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Correspondence to: Zhi-Bin Zhang, Ph.D., Phone: 86 10 62554027, Fax: 86 10 62565689, zhangzb@ioz.ac.cn.

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success, and even survival. Agonistic behavior has been examined in a variety of rodent species, including rats, mice, hamsters and voles, under different social contexts (Davis and Marler, 2004; Delville et al., 2000; Gammie and Nelson, 2001; Gobrogge et al., 2007; Haller et al., 2006; Veening et al., 2005). Although these studies have provided important information about agonistic behavior and its underlying neural mechanisms, the majority have focused exclusively on aggressive behavior (Delville et al., 2000; Gobrogge et al., 2007; Haller et al., 2006; Veening et al., 2005), while little attention has been paid to defensive behavior. Given that an agonistic encounter is a dynamic behavioral interaction involving both aggressive and defensive behaviors, each of which depend upon the other, it is essential to examine the entire repertoire of behaviors displayed during agonistic interactions. Such information could offer insight into whether aggressive and defensive behaviors represent opposite ends of a single behavioral continuum regulated by a complex neural circuit, or alternatively, represent two independent behavioral patterns that could be regulated by distinct, but well integrated, neural circuits (Adams, 1979; Adams, 2006; Ramirez et al., 1988).

The greater long-tailed hamster (*Tscheskia triton*) is a solitary, polygamous rodent species that lives in the farmlands of Northern China (Yang et al., 1996; Zhang et al., 2001a; Zhang et al., 2001b). Previous studies have shown that males of this species display intense agonistic interactions during same-sex encounters, and such interactions typically result in a clearly identifiable dominant versus subordinate status, with the dominant animal displaying more aggression and the subordinate animal displaying a higher level of defensive behavior (Wang et al., 2009; Zhang et al., 2001b). Although circulating levels of hormones (e.g., testosterone and corticosterone) have been implicated in such agonistic encounters (Wang et al., 2009; Zhang et al., 2001a; Zhang et al., 2001b), the underlying neural substrates are still unknown. In the present study, we tested the hypothesis that agonistic behavior correlates with patterns of neuronal activation in the brain in a behavior-specific manner. We compared behavioral patterns of dominant versus subordinate male hamsters during agonistic encounters, used the protein product (Fos) of an immediate early gene, *c-fos*, to map related neuronal activation in the brain, and correlated agonistic behaviors with regional neuronal activation in male greater long-tailed hamsters.

Materials and methods

Subjects

Subjects were sexually naïve adult male greater long-tailed hamsters (*Tscheskia triton*) that were F1 offspring of a laboratory breeding colony originating from field captured animals. The hamsters were weaned at 25 days of age and housed individually in plastic cages (40×25×15 cm) that contained wood shavings as bedding. Food and water were provided *ad libitum*. All cages were maintained under a reversed light/dark cycle (16L:8D with lights on at 1700). Temperature was maintained at 20±2°C. All experimental procedures complied with the guidelines for animal use and care as stipulated by the Institute of Zoology at the Chinese Academy of Sciences.

Behavioral procedures

Twenty adult males were randomly assigned into one of two groups, control (n=8) or encounter (n=12). The males in the encounter group were then assigned into 6 pairs with matching body weights. The dominant-subordinate relationship within each pair was determined using an established behavioral test (Wang et al., 2009; Wang et al., 2006). Briefly, the test took place in a neutral arena (60×40×100 cm plexiglas box), in which two screens were placed parallel with the lateral wall to reduce the intensity of aggression and to provide a buffer for subordinate males to avoid further attack by dominant ones (Figure 1A).

The arena was divided into two equal compartments using a removable opaque partition. Subjects were placed into each compartment for a 3-min acclimation period. The partition was then removed and subjects were allowed to interact freely for 10 min during which behavior was recorded using a digital camera. Control males were exposed to the same neutral arena in the absence of another animal. All behavioral tests were conducted under dim red illumination during the first 6 hrs of the dark phase.

Frequency and duration of the following behavioral patterns, as defined previously (Wang et al., 2009; Zhang et al., 2001a; Zhang et al., 2001b), were quantified using the OBSERVER (V5.0; Noldus, NL). These behavioral patterns included aggression (attack, sideways posture, biting, and chasing), defense (fleeing, upright posture, cowering, threatening, and lying on the back on the ground), flank marking (arching back and rubbing toward the wall), locomotion, self-grooming, and sniffing. Latency of the initial attack displayed by each animal was quantified. Within each pair, the male that initiated attack and had higher aggression scores was defined as dominant, and the other was subordinate. Fleeing latency was also recorded which referred to the duration from the beginning of the behavioral test to the first time that the subordinate fled from agonistic interactions. The arena was thoroughly cleaned between trials with water and 75% ethanol.

c-fos immunocytochemistry

After the 10-min behavioral test, subjects were returned to their own cages and left without further disturbance. One hr later, all subjects were anesthetized with sodium pentobarbital (0.1 mg/10 g body weight) and then perfused through the ascending aorta with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M phosphate-buffered solution (PBS; pH 7.4). Brains were harvested, postfixed for 2 hrs in 4% paraformaldehyde, stored in 30% sucrose in PBS, and then cut into 40- μ m coronal sections on a microtome. Floating sections at 240- μ m intervals were processed for Fos immunocytochemistry using an established procedure (Curtis and Wang, 2003; Stowe et al., 2005). Briefly, sections were blocked in 10% normal goat serum (NGS) in 0.05 M Tris-NaCl and then incubated in rabbit polyclonal IgG antibody for *c-fos* (1:10,000, Santa Cruz Biotechnology, Santa Cruz, CA) overnight, biotinylated goat anti-rabbit secondary antibody (1:300, Jackson Immuno, West Grove, PA) for 2 hrs, and Vector Elite ABC complex (Vector Laboratories, Inc. Burlingame, CA) for 90 min. Sections were stained with Nickel-DAB, mounted on slides, and then cover slipped. To control for variability, all sections were processed simultaneously.

Data quantification and analysis

The frequency and duration of each behavior as well as latency to attack was compared between dominant and subordinate animals using two-tailed paired t-tests (if data were normally distributed) or the non-parametric Wilcoxon matched pairs test (if data were not normally distributed). All slides were coded to conceal group identity. The Fos-ir labeled cells were examined in the cingulate cortex (Cg), lateral septum (LS), bed nucleus of the stria terminalis (BST; the anterior dorsal part), medial preoptic area (MPOA), paraventricular nucleus (PVN), anterior hypothalamus (AH); ventromedial hypothalamus (VMH); and medial (MeA), anterior cortical (ACo), and central (CeA) subnuclei of the amygdala (AMYG) (Figure 2). These brain areas were chosen because they have been implicated in agonistic and other social behaviors in several rodent species (Gobrogge et al., 2007; Kollack-Walker et al., 1999; Kollack-Walker et al., 1997; Veening et al., 2005; Wang et al., 1997). Brain sections were matched between animals with 2–3 sections per brain area being examined. Fos-ir cells within each brain area were quantified bilaterally. The size of each brain area was measured to convert data to cell density. Group differences in the density of Fos-ir cells in each brain area were analyzed by a one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls (SNK) posthoc test. In addition,

correlations between aggressive or defensive behavior and the density of Fos-ir cells in selected brain areas were analyzed using a Spearman Correlation (because of nonhomogeneity of variance). The level of significance was set at $p < 0.05$ for all tests.

Results

Behaviors

The dominant-subordinate status was determined rapidly (fleeing latency: 68.4 ± 29.1 sec.) after pairing the two males, and once established, the relationship remained consistent throughout the entire 10-min behavioral test. Dominant males displayed higher levels of aggression, compared to their subordinate counterparts, as indicated by a shorter latency to initiate attacks ($Z = 1.99$, $p < 0.05$; Figure 1B) and a higher frequency ($Z = 2.21$, $p < 0.05$) and duration ($Z = 2.20$, $p < 0.05$) of aggression, whereas subordinate males showed a higher frequency ($Z = 2.20$, $p < 0.05$) and duration ($Z = 2.20$, $p < 0.05$) of defensive behavior (Figure 1C & D). Flank marking ($Z = 2.20$, $p < 0.05$ for frequency; $Z = 2.20$, $p < 0.05$ for duration), self-grooming ($Z = 2.20$, $p < 0.05$ for frequency; $Z = 2.20$, $p < 0.05$ for duration), and frequency of locomotion ($t = 3.14$, $p < 0.05$) were also higher in dominant males compared to subordinates (Figure 1C & D).

Neural activation associated with behavioral interaction

Agonistic encounters induced increases in the density of Fos-ir cells in the hamster brain in a region- and stimuli-specific manner. Among the brain regions examined, three patterns of Fos-ir expression were found. In the BST ($F_{(2,19)} = 7.40$, $p < 0.01$), VMH ($F_{(2,19)} = 18.39$, $p < 0.01$), and AMYG ($F_{(2,19)} = 13.60$, $p < 0.01$), both dominant and subordinate males had similar levels of Fos-ir expression, which were significantly higher than that of the control males (Figure 3A). Group differences in the density of Fos-ir cells were also found in the MPOA ($F_{(2,19)} = 23.10$, $p < 0.01$) and AH ($F_{(2,19)} = 11.15$, $p < 0.01$) (Figure 3A & 4). In the MPOA, posthoc analysis revealed that dominant males had a higher density of Fos-ir cells than the control and subordinate males, while the latter two groups did not differ from each other (Figure 3A & Figure 4A–C). In the AH, however, subordinate males showed a greater density of Fos-ir cells than control and dominant males (Figure 3A & Figure 4D–F). Finally, in other brain areas, including the Cg, LS, and PVN, no group differences were found in the density of Fos-ir cells (Figure 3A).

Because the LS and AMYG are large heterogeneous areas, we further analyzed the density of Fos-ir cells in each of the subnuclei. In the dorsal (LSd), intermediate (LSi), and ventral (LSv) subnuclei of the LS, no group differences were found in the density of Fos-ir cells (Figure 3B, Figure 4J–L). In the medial (MeA; $F_{(2,19)} = 14.93$, $p < 0.01$; Figure 4G–I) and anterior cortical (ACo; $F_{(2,19)} = 5.81$, $p < 0.05$) subnuclei of the AMYG, dominant and subordinate males showed similar densities of Fos-ir cells and these densities were significantly higher than that of the control males (Figure 3C). In contrast, in the central subnucleus of the AMYG (CeA), subordinate males had more Fos-ir cells than the other two groups ($F_{(2,19)} = 5.23$, $p < 0.05$; Figure 3C).

Correlations between aggression and Fos-ir

The density of Fos-ir cells in the MPOA, AH, and CeA differed significantly between dominant and subordinate males, indicating the potential involvement of regional neuronal activation in different patterns of agonistic behaviors. Therefore, Spearman Correlation tests were performed to reveal correlations between the level of aggression and the density of Fos-ir cells in those brain areas. The density of Fos-ir cells in the MPOA was correlated positively with the frequency ($r = 0.72$, $p < 0.01$) and duration ($r = 0.71$, $p < 0.01$) of aggression, but negatively with the frequency ($r = -0.70$, $p < 0.05$) and duration ($r = -0.72$, $p < 0.01$; Figure

5A–D) of defense. The density of Fos-ir cells in the AH was correlated negatively with the duration of aggression ($r=-0.65$, $p<0.05$) but positively with the frequency ($r=0.77$, $p<0.01$) and duration ($r=0.69$, $p<0.05$; Figure 5E–H) of defense. Finally, the density of Fos-ir cells in the AH was also correlated positively with the latency to attack ($r=0.62$, $p<0.05$), and the density of Fos-ir cells in the CeA was correlated positively with the frequency of defense ($r=0.67$, $p<0.05$).

Discussion

In the present study, we found that agonistic encounters between male greater long-tailed hamsters resulted in the establishment of a dominant-subordinate relationship with dominant males displaying flank marking, initiating attack sooner, and showing more aggression and subordinates displaying more defensive behavior and fleeing. Such behavioral interactions resulted in significant neuronal activation, as indicated by Fos-ir in selected brain areas. Most interestingly, discrete patterns of neuronal activation were found between dominant and subordinate males, and such neuronal activation correlated with distinct patterns of behavior.

A region- and behavior-specific pattern of Fos-ir expression was found in the brain of greater long-tailed hamsters. Dominant males had a higher density of Fos-ir in the MPOA than their subordinate counterparts. Further, a positive correlation was shown between MPOA Fos-ir and aggression, indicating a potential role of the MPOA in aggression. This finding is supported by previous studies. In rats and Syrian hamsters, for example, lesions of the MPOA suppressed aggression toward conspecifics (Albert et al., 1986; Hammond and Rowe, 1976). In rats, aggression significantly increased the expression of Fos-ir in the MPOA (Veening et al., 2005). Furthermore, in rhesus monkeys, electrical stimulation of the preoptic area (including the MPOA) induced an increase in aggression towards subordinate males (Alexander and Perachio, 1973). Therefore, increased neuronal activation in the MPOA may be related to the display of aggressive behavior in male greater long-tailed hamsters.

During agonistic encounters, dominant male hamsters usually flank mark more than their subordinates (Ferris et al., 1987; Ferris et al., 1986) and the MPOA-AH has been shown to play an important role in regulating flank marking behavior (Albers and Bamshad, 1998; Ferris et al., 1990). In the present study, dominant, but not subordinate, greater long-tailed hamsters displayed flank marking behavior. Therefore, a high level of Fos-ir expression in the MPOA may also indicate potential involvement of this brain area in flank marking behavior in dominant males.

A distinct pattern of Fos-ir expression was also found in subordinate males, in which an agonistic interaction resulted in an increase in Fos-ir in the AH and CeA compared to dominant males. This increase in Fos-ir was correlated with defensive behavior. The AH has been implicated in defensive behavior in a variety of rodent species (Matsuda et al., 1996; Olivier et al., 1983). In male Syrian hamsters, in particular, neuronal activation in the AH was associated with the display of defensive behavior (Kollack-Walker et al., 1999; Kollack-Walker et al., 1997). Further, acute social defeat has been shown to increase Fos activation in the CeA in Syrian hamsters (Kollack-Walker et al., 1999; Kollack-Walker et al., 1997) and rats (Martinez et al., 1998). Given the reciprocal connections between the CeA and AH (Delville et al., 2000), these two areas may act together in mediating defensive behavior. It should be mentioned that the CeA also regulates the activity of the hypothalamic-pituitary-adrenal (HPA) axis (Herman and Cullinan, 1997) and particularly, mediates glucocorticoid secretion in response to stress (Cullinan et al., 1995; Herman et al., 1996). Therefore, our finding that the CeA is activated in subordinate, as compared to

dominant, males may indicate the involvement of the CeA in the regulation of endocrine responses to stress from social defeat. This idea is supported by a study demonstrating that social defeat resulted in a high level of fecal corticosterone in male greater long-tailed hamsters (Wang et al., 2009).

Finally, in several brain areas including the BST, VMH, MeA and ACo, an agonistic interaction resulted in equally elevated levels of Fos-ir expression in both dominant and subordinate males relative to controls. The lack of differences in Fos-ir expression between dominant and subordinate males indicates that such increased neuronal activation might be due to olfactory stimulation and/or arousal associated with an agonistic encounter in general, rather than to a particular subtype of agonistic behavior (*e.g.*, aggression or defense). This is supported by a previous study in Syrian hamsters in which, despite their differences in agonistic behaviors, both dominant and subordinate males showed identical levels of Fos-ir expression in the above-mentioned brain areas (Kollack-Walker and Newman, 1995). Further, a broad range of social behaviors including mating, parental care, affiliation, and agonistic behaviors has been shown to induce Fos-ir expression in those brain areas in a variety of rodent species (Gobrogge et al., 2007; Kirkpatrick et al., 1994; Kollack-Walker and Newman, 1995; Wang et al., 1997).

To date, a limited number of studies have directly compared the pattern of brain neuronal activation with different agonistic behaviors. In Syrian hamsters, dominant and subordinate males did not differ in the number of Fos-ir cells in any brain areas measured (Kollack-Walker and Newman, 1995) but showed some group differences in the density of regional Fos mRNA expression (Kollack-Walker et al., 1997). Although consistency was found between these studies and our own, as discussed above, some discrepancies are worth mentioning. For example, subordinate Syrian hamsters showed a higher level of Fos mRNA expression in the MPOA than their dominant counterparts (Kollack-Walker et al., 1997), whereas the opposite pattern of Fos-ir staining was found in the MPOA in greater long-tailed hamsters. In addition, the LS has been implicated in aggression in several rodent species including rats, mice and voles (Duncan et al., 2009; Halasz et al., 2002; Trainor et al., 2006; Wang et al., 1997). In Syrian hamsters, subordinates had a higher level of Fos mRNA expression in the LS compared to their dominant counterparts (Kollack-Walker et al., 1997), but no group differences were found in the LS Fos-ir staining in greater long-tailed hamsters. There are no ready explanations for such discrepancies. In addition to species differences, variations in experimental procedures and paradigms (*e.g.*, resident-intruder paradigm vs. neutral environment paradigm) make comparisons across studies difficult (Albers et al., 2002). In the case of the LS, for example, no differences were found in the density of Fos-ir staining between control and aggressive males in greater long-tailed hamsters (present study) or golden hamsters (Delville et al., 2000). This may reflect the insensitivity of Fos-ir in illustrating neuronal activation in the LS. Alternatively, these hamsters, unlike Syrian hamsters (Kollack-Walker et al., 1997), may have a species-specific neural circuit that does not include the LS for the regulation of agonistic behaviors.

In summary, greater long-tailed hamsters are solitary and display high levels of agonistic actions during social encounters (Wang et al., 2009; Yang et al., 1996; Zhang et al., 2001b). Our data illustrate specific patterns of agonistic behaviors distinguishing dominant from subordinate animals. Furthermore, for the first time in this species, our data reveal anatomically integrated yet discrete neural circuits correlating with aggressive and defensive behaviors. While several brain areas are involved in general arousal and responses related to exposure to a conspecific, MPOA activation appears more specific for aggression whereas the AH and CeA are more involved in defensive behavior and/or in stress responses to social defeat. Together, our data from this newly studied species provide evidence to further support the notion that complex social behaviors are regulated by distinct yet integrated

neural circuits in a behavior-specific manner (Adams, 1979; Adams, 2006; Ramirez et al., 1988). Further studies are needed to identify the specific aspects of agonistic interactions, such as chemosensory cues and/or the display of particular behaviors, which are responsible for aggression- or defense-induced region-specific neuronal activation and to reveal the functional significance of regional neuronal activation on agonistic behaviors.

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Figure 1.

Agonistic behaviors displayed by dominant and subordinate male greater long-tailed hamsters. (A) A photograph showing the testing apparatus for agonistic behaviors. (B) Dominant males attacked the opponent sooner than subordinates. (C) Frequency and (D) duration of agonistic behaviors displayed by male greater long-tailed hamsters. Dominant males displayed a higher frequency and longer duration of aggression, flank marking, and self-grooming, than subordinate males. Dominant males also showed a higher frequency of locomotion than subordinates. On the other hand, the subordinates were more frequently involved in and spent more time in defensive behavior than their dominant counterparts. *: $p < 0.05$.

Figure 2.

Diagrams showing the location of the brain areas selected (shaded areas) for quantification of Fos-ir cells. Diagrams were modified from the atlas of Paxinos and Watson (1997). ac, anterior commissure; ACo, anterior cortical nucleus of the amygdala; AH, anterior hypothalamus; BST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; Cg, cingulate cortex; CPu, caudate putamen; f, fornix; LH, lateral hypothalamus; LPO, lateral preoptic area; LS, lateral septum; LSD, anterior dorsal subnucleus of LS; LSI, intermediate subnucleus of LS; LSV, ventral subnucleus of LS; MeA, medial nucleus of the amygdala; MPOA, medial preoptic area; opt, optic tract; ox, optic chiasm; PVN, paraventricular nucleus of the hypothalamus; VMH, ventromedial hypothalamus.

Figure 3.

Density of Fos-ir cells displayed in various brain regions one hour after an agonistic interaction. (A) Group differences in the density of Fos-ir cells in the cingulate cortex (Cg), lateral septum (LS), bed nucleus of the stria terminalis (BST), medial preoptic area (MPOA), paraventricular nucleus (PVN), anterior hypothalamus (AH), ventromedial hypothalamus (VMH) and amygdala (AMYG) of greater long-tailed hamsters. Group differences in the density of Fos-ir cells were also analyzed in (B) the dorsal (LSd), intermediate (LSi), and ventral (LSv) subnuclei of the LS, as well as in (C) the medial (MeA), anterior cortical (ACo), and central (CeA) subnuclei of the AMYG. Alphabetic letters indicate the results of SNK posthoc tests following the determination of significant

group differences by a one-way ANOVA. Bars with different letters differ significantly from each other.

Figure 4.

Photomicrographs displaying Fos-ir stained cells in the medial preoptic area (MPOA; A–C), anterior hypothalamus (AH; D–F), medial nucleus of the amygdala (MeA; G–I), and lateral septum (LS; J–L) in male greater long-tailed hamsters that were without agonistic test (control; A, D, G & G), or that were dominants (B, E, H & K) or subordinates (C, F, I & L) determined in an agonistic test. 3v: third ventricle, f: fornix, LV: lateral ventricle, opt: optic tract; Scale bar = 100 μ m.

Figure 5.

Correlations between the density of Fos-ir cells and agonistic behaviors. (A–D) In the medial preoptic area (MPOA), the density of Fos-ir cells was correlated positively with aggression and negatively with defensive behavior. (E–H) In the anterior hypothalamus (AH), the density of Fos-ir cells was correlated negatively with the duration of aggression but positively with defensive behavior.