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Agonistic encounters and brain activation in dominant and subordinate male greater long-tailed hamsters

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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.

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 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, 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,b). 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,b), 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.

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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 (27×16×13 cm) that contained wood shavings as bedding. Food and water were provided *ad libitum*. All cages were maintained under a reversed light/dark cycle (16 L: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, 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 (Fig. 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 h of the dark phase.

Frequency and duration of the following behavioral patterns, as defined previously (Wang et al., 2009; Zhang et al., 2001a,b), 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 hour 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 h in 4% paraformaldehyde, stored in 30% sucrose in PBS, and then cut into 40-μm coronal sections on a microtome. Floating sections at 240-μm 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) 36 h, biotinylated goat anti-rabbit secondary antibody (1:300, Vector Laboratories, Inc. Burlingtone, CA) for 2 h, and Vector Elite ABC complex (Vector Laboratories, Inc. Burlingtone, 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.

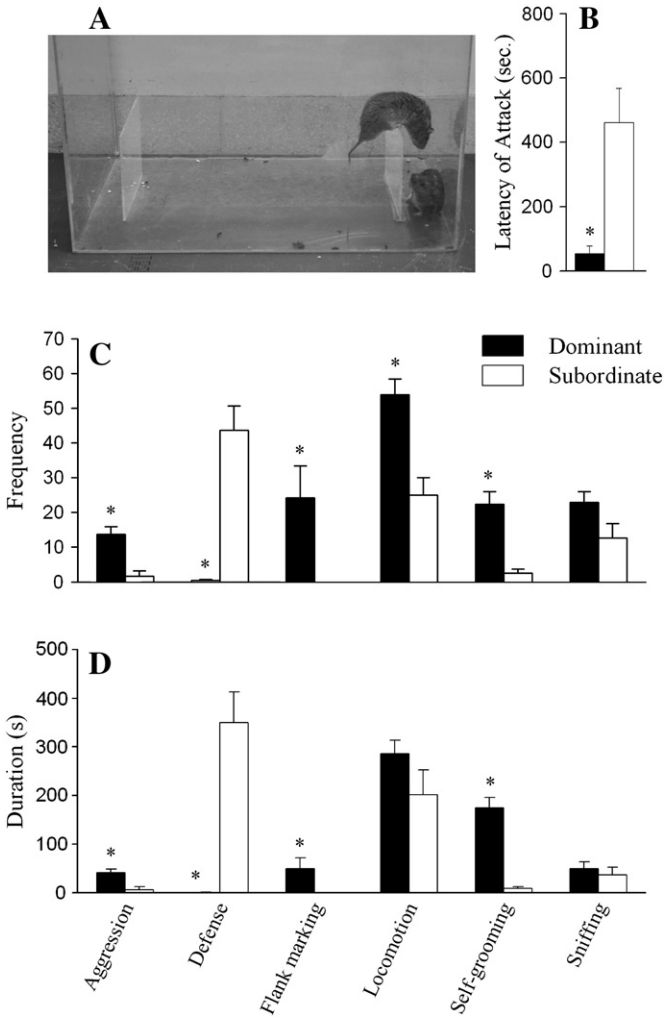

Fig. 1. Agonistic behaviors displayed by dominant and subordinate male greater long-tailed hamsters. (A) A photographic showing the testing apparatus for agonistic behaviors. (B) Dominant males attacked the opponent sooner than subordinates. (C) Frequency and (D) duration of agonistic behaviors displayed 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. $^{*}p<0.05$.

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) (Fig. 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, 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