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NEURAL CIRCUITRY OF PLAY FIGHTING IN GOLDEN HAMSTERS

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Abstract—In hamsters, play fighting matures gradually into adult aggression. As these two behaviors share many similarities in this species, we predicted that a single neural circuitry controls their offensive component. The goal of the present study was to identify neural systems associated with offensive play fighting in male juvenile golden hamsters. The neural circuitry related to this behavior was identified through quantification of *c*-Fos immunolabeling. We also looked for vasopressin cells possibly associated with play fighting. We found that areas previously associated with offensive aggression in adult hamsters, including the ventrolateral hypothalamus, the medial amygdala, and the bed nucleus of the stria terminalis, also showed enhanced *c*-Fos expression after play fighting. In addition, vasopressin neurons in the nucleus circularis and the medial division of the supraoptic nucleus expressed enhanced *c*-Fos immunolabeling in juveniles after play fighting, as previously reported in adult hamsters after aggression. Finally, enhanced *c*-Fos expression associated with play fighting was also found in areas previously unexplored in adult hamsters, such as the prefrontal cortex. Together, our results support the hypothesis of a single core neural circuitry controlling the offensive components of play fighting and adult aggression throughout puberty in hamsters.

Key words: aggression, vasopressin, *c*-Fos-immunoreactivity, hypothalamus, cortex.

Play fighting, a form of agonistic behavior, is common in juvenile mammals and typically performed around puberty before adult aggressive behavior (Vanderschuren et al., 1997; Blanchard et al., 2003; Delville et al., 2005; Pellis, 2002). Play fighting also differs greatly between species, first, in levels of complexity, from complex in rats, including sophisticated attack and defense repertoires between players, to simple in mice, limited to only chasing and evasion (Pellis et al., 1989; Pellis and Pasztor, 1999). In addition, play fighting is not a unitary behavior. As in aggression, play fighting includes offensive and defensive components as adult animals (Pellis, 2002; Delville et al., 2005). Second, two main types of play fighting have been described during puberty: "playful" and "serious" (Pellis and Pellis, 1998; Pellis, 2002). The former is a rewarding behavior for both participants whereas the latter is used to establish dominant/subordinate hierarchies (Pellis, 1988; Panksepp and Burgdorf, 2003; Delville et al., 2005). Both behaviors are easily observed in a laboratory setting, as rats engage constantly in "playful" play fighting in early puberty around postnatal day (P-35) (Bolles and Woods, 1964; Panksepp, 1981; Pellis and Pellis, 1987, 1997). Later, their behavior is replaced by "serious" play fighting before maturing to adult aggression (Pellis and Pellis, 1997; Pellis, 2002; Foroud and Pellis, 2003). The relative importance of these different forms of play fighting may also differ between species during specific developmental period. In golden hamsters, play fighting is limited to "serious" interactions during puberty (Delville et al., 2003). In this species, play fighting peaks in early puberty around P-35 and gradually matures into adult aggression in late puberty (Goldman and Swanson, 1975; Wommack et al., 2003). The "serious" play fighting of hamsters differs substantially from adult aggression quantitatively and qualitatively. Qualitatively, these two behaviors have different target areas. Play fighting attacks of juvenile hamsters are targeted at the face of the protagonist, while attacks by adults are focused on the lower belly and rump (Pellis and Pellis, 1988a,b; Wommack et al., 2003). Quantitatively, juvenile hamsters are more active and perform many more attacks during agonistic contacts than adults (Wommack et al., 2003; Taravosh-Lahn and Delville, 2004). In particular, attacks and bouts of contact are much more repetitive in play fighting than in adult aggression (Cervantes et al., 2006).

Few studies have attempted to identify neural sites associated with play fighting behavior in animals. For instance, in rats, one study showed brain areas activated during "playful" play fighting through changes in *c*-Fos expression in the sub-nuclei of the striatum and the tectum, the inferior colliculus, the dorsal midbrain central gray, and the parietal zone of the somatosensory cortex (Gordon et al., 2002). However, the neural structures associated with "serious" play fighting remain unknown. Based on behavioral similarities between the offensive components of "serious" play fighting and adult offensive aggression, we predicted that a single neural circuitry mediates the activation of both behaviors (Delville et al., 2003). In adult hamsters, brain areas associated with offensive aggression include the posterodorsal part of the medial amygdala (MePD), the bed nucleus of the stria terminalis (BST), the

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Abbreviations: AH, anterior hypothalamus; APC, anterior parietal cortex; AVP, vasopressin; BST, bed nucleus of the stria terminalis; CeA, central amygdala; CGdl, dorsolateral part of the midbrain central gray; Cg1, cingulate cortex; IL, infralimbic cortex; ir, immunoreactive; KPBS, potassium phosphate–buffered saline; LS, lateral septum; lSON, lateral division of the supraoptic nucleus; MePD, the posterodorsal part of the medial amygdala; mSON, medial division of the supraoptic nucleus; NC, nucleus circularis; NDS, normal donkey serum; NGS, normal goat serum; P, postnatal day; PBS, phosphate-buffered saline; PFC, prefrontal cortex; POA, preoptic area; PrL, prelimbic cortex; PVN, paraventricular hypothalamic nucleus; VLH, ventrolateral hypothalamus.

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ventrolateral hypothalamus (VLH), and the dorsolateral part of the midbrain central gray (CGdl) (Delville et al., 2000). These areas show enhanced *c*-Fos expression after the performance of offensive aggression. It is hypothesized that similar brain areas will be activated in juvenile hamsters after consummation of offensive play fighting.

These limbic areas associated with offensive aggression have reciprocal connections with the anterior hypothalamus (AH), a key area in the control of this behavior in hamsters, thus, forming a neural circuitry (Delville et al., 2000). The AH and most of this circuitry have vasopressin (AVP) receptors (Ferris et al., 1993). Micro-injections of AVP into the AH or other elements of this circuitry facilitate offensive aggression or other aspects of agonistic behavior in hamsters (Ferris et al., 1984, 1997; Hennessey et al., 1992; Delville et al., 1996). The consummation of offensive aggression in hamsters is associated with enhanced activity within AVP neurons near the AH, within the medial division of the supraoptic nucleus (mSON) and the nucleus circularis (NC) (Delville et al., 2000). This finding suggests that these cells are the likely source of AVP to the AH in the control of offensive aggression. As this area and this circuitry are likely to be central to the control of "serious" play fighting, it is possible that these two populations of AVP neurons are also associated with this juvenile agonistic behavior.

The main goal of this study was to test the possibility that a single neural circuitry modulated by AVP is associated with the control of the offensive components of "serious" play fighting and adult aggression. This possibility was tested through immunolabeling of *c*-Fos expression, as a marker of neuronal activity (Morgan and Curran, 1989; Kovács, 1998) both within the neural circuitry centered of the AH and within AVP cells.

EXPERIMENTAL PROCEDURES

Animals and treatment

The present studies were carried out with male golden hamsters (*Mesocricetus auratus*) bred in the laboratory from a colony originating from Harlan Sprague Dawley (Indianapolis, IN, USA). All litters were culled to six pups (four males, two females) by P-7. All males were weaned on P-25 and housed individually in Plexiglas cages (20×33×13 cm). The hamsters were housed under a reversed light cycle (14:10-h light/dark cycle and lights off at 10:00 h) and received food and water *ad libitum*. Their body weights were measured weekly and recorded to monitor their development. The studies were conducted in early puberty (P-35) around the time of peak play fighting activity in this species (Goldman and Swanson, 1975; Taravosh-Lahn and Delville, 2004; Cervantes et al., 2006). All procedures were performed according to National Institutes of Health guidelines approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin and conducted in an AALAC-accredited facility. Procedures were optimized for minimizing the number of animals used and their suffering.

Experimental design

On P-30, male hamsters (n=20) were pre-tested for agonistic behavior for 10 min individually in the presence of a 10–20% lighter, younger and unfamiliar male intruder. This resident/intruder procedure favors offensive responses by residents (Delville et al., 2003). All animals attacked intruders at least once in this test so no animal was excluded. Then, animals were divided into two homogeneous groups (n=10 in each) based on the body weight and the agonistic behaviors observed in this test. On P-35, animals in the experimental group were observed for agonistic behaviors during a 10-min encounter with another unfamiliar intruder. Animals in the control group were remained in the presence of a block of wood (5×9×1.5 cm), which had been left overnight in the cage of unfamiliar intruders and carried their odor, for 10 min. This control was used to activate neural activity associated with social arousal and to make sure the enhanced neural activity observed in experimental animals was specific to the consummation of play fighting. In previous studies with adults, exposure to the woodblock elicited flank-marking behavior (Delville et al., 2000), an element of the ethogram of agonistic behavior in hamsters, but short of its consummation (Siegel, 1985). Agonistic encounters of animals were videotaped in both groups for later review. After 50 min, all animals were deeply anesthetized with an injection of sodium pentobarbital (Nembutal, Abbott Laboratories, North Chicago, IL, USA; 100 mg/kg, i.p.) and perfused transcardially. The perfusion procedure started with 0.9% saline containing 0.2% sodium nitrite for 2 min to dilate the blood vessels, and then was followed by a fixative solution containing 4% paraformaldehyde and 2.5% acrolein in 0.1 M potassium phosphate–buffered saline (KPBS, pH 7.4) for 18 min, and then followed by saline for another 2 min to wash out residual fixative solution. Afterward, brains were removed from the skulls and placed in 20% sucrose in KPBS at 4 °C for at least 24 h. Then, brains were sliced into 40 μm-thick coronal sections with a freezing rotatory microtome. Brain sections were saved in a cryoprotectant (Watson et al., 1986) at −20 °C until labeled by immunocytochemistry.

Behavior observations

During the 10-min resident/intruder encounters, a number of agonistic behaviors performed by residents were observed, including attack, pin, and flank marking. Attacks were defined as a combination of an approach immediately followed by an attempt to bite (Wommack et al., 2003). Pins were defined as one animal lying on its back with the other animal on top. Flank marking was identified when hamsters rubbed their flank glands on the walls of cages (Johnston, 1985), which was usually performed by residents after successful attacks.

c-Fos immunocytochemistry

Brain sections were processed for immunocytochemistry to *c*-Fos as described in a previous study (Delville et al., 2000). First, brain sections were washed in 0.05 M KPBS buffer to remove the cryoprotectant, and the sections were treated with 1% sodium borohydrate in KPBS for 10 min to remove the residual aldehydes. After several washes, brain sections were pre-incubated in a KPBS solution with 20% normal goat serum (NGS) to prevent non-specific labeling, 1% hydrogen peroxide to eliminate unreacted peroxidase in the blood vessels, and 0.3% Triton X-100 to make the sections permeable. Then, the sections were incubated for 48 h at 4 °C with a rabbit polyclonal primary antibody to *c*-Fos (0.05 μg/ml, sc-52, Lot H024, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) recognizing an sequence (residues 1–16) at the N-terminus of *c*-Fos of human origin (Finkel et al., 1966; Nishizawa et al., 1987) in "KPBS wash" solution (0.05 M KPBS with the presence of 2% NGS and 0.3% Triton X-100). After several washes, the sections were successively incubated for 45 min at room temperature in a secondary antibody (biotinylated goat anti-rabbit IgG, 2.5 μg/ml, Lot T0411, Vector Laboratories, Inc., Burlingame, CA, USA) in "KPBS wash" solution. After several rinses, the sections were placed for 45 min at room temperature in a tertiary incubation with an avidin-peroxidase complex (Vec-