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# Testicular hormone exposure during adolescence organizes flank-marking behavior and vasopressin receptor binding in the lateral septum

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**Abstract**

Adolescence is a period during which many social behaviors emerge. One such behavior, flank marking, is a testosterone-modulated scent marking behavior that communicates dominance status between adult male Syrian hamsters. Testosterone modulates flank-marking behavior by altering neural transmission of vasopressin within a forebrain circuit. This study tested whether testicular hormones secreted during adolescence play purely a transient activational role in the display of flank-marking behavior, or whether adolescent steroid hormone secretions also cause long-term organizational changes in vasopressin binding within brain regions underlying flank-marking behavior. We tested this hypothesis by manipulating whether testicular secretions were present during adolescent development and then tested for flank-marking behavior and vasopressin receptor binding within the flank-marking neural circuit in young adulthood. Specifically, males were gonadectomized immediately before or after adolescence, replaced with testosterone 6 weeks following gonadectomy in young adulthood, and behavior tested 1 week later. Adult testosterone treatment activated flank-marking behavior only in males that were exposed to testicular hormones during adolescence. In addition, males exposed to testicular hormones during adolescence exhibited significantly less vasopressin receptor binding within the lateral septum than males deprived of adolescent hormones, suggesting that hormone-dependent remodeling of synapses normally occurs in the lateral septum during adolescence. These data highlight the importance of gonadal steroid hormone exposure during adolescence for the organization of neural circuits and social behavior.


*Keywords:* Puberty; Steroid hormones; Testosterone; Vasopressin; Organizational effects; V1a receptors; Scent marking; Adolescence; Agonistic

## Introduction

Scent marking is an important form of social communication for many mammalian species (Johnston, 1973). The Syrian hamster exhibits a stereotyped form of scent marking behavior called flank marking (Johnston, 1975). Flank marking occurs when hamsters rub pigmented sebaceous glands located on their dorsal flank region against objects in their environment (Johnston, 1975). This behavior can be stimulated by the odors of conspecifics alone but is most often displayed during social encounters (Johnston, 1975). Importantly, flank-marking behavior serves to communicate dominance status between males and is essential for the maintenance of these dominance relationships (Ferris et al., 1987).

Flank-marking behavior is influenced by testosterone (T) in adult male Syrian hamsters (Johnston, 1981). Castration significantly reduces and T replacement restores flank-marking behavior (Johnston, 1981). T modulates flank-marking behavior by altering arginine vasopressin (AVP) neural transmission within a zone that extends from the posterior medial and lateral preoptic area to the posterior medial and lateral anterior hypothalamus (MPOA-AH, reviewed in Albers et al., 2002). Microinjections of AVP within this region cause dose-dependent increases in flank-marking behavior (Albers and Ferris, 1986; Ferris et al., 1988), and the presence of T further

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enhances these effects (Albers et al., 1988). These data suggest that T influences flank marking by altering the sensitivity or response of the MPOA-AH to AVP, possibly by increasing AVP binding. Indeed, castration reduces and T replacement restores V1a binding within the MPOA-AH continuum (Johnson et al., 1995; Young et al., 2000).

The MPOA-AH is reciprocally connected to the lateral septum (LS), bed nucleus of the stria terminalis (BST), and the periaqueductal gray (PAG). AVP microinjection into these areas also induces flank-marking behavior (Irvin et al., 1990; Hennessey et al., 1992), suggesting they are part of a flank-marking circuit in which AVP is a neurotransmitter at multiple levels. Just as T facilitates the effects of AVP injection into the MPOA-AH on flank marking, T also enhances the effects of AVP injection into the LS-BST and PAG on flank-marking behavior, although to a much lesser extent (Albers and Cooper, 1995). Thus, while these brain regions contribute to the display of flank-marking behavior, the MPOA-AH may be the primary site mediating the activational effects of T on behavior.

The factors responsible for the development of flank-marking behavior are largely unexplored. Preadolescent hamsters are capable of flank marking in response to male odors around day 22 (Ferris et al., 1996). However, levels of flank marking at this age are much lower than what is typically observed in adults (Johnston, 1981), suggesting that this behavior continues to develop during adolescence. Although increased gonadal secretions are a hallmark of adolescent development, what role testicular secretions play in the development of flank-marking behavior is not known. One possibility is that the rise in gonadal hormones during adolescence simply activates adult levels of flank-marking behavior. Alternatively, the rise in gonadal hormones during adolescence may permanently *organize* neural circuits to permit activation of behavior by T in adulthood. For example, gonadal hormones during adolescence organize reproductive behavior in male Syrian hamsters (Schulz et al., 2004). Males gonadectomized prior to adolescence, and therefore not exposed to gonadal hormones during this time, show long lasting deficits in adult reproductive behavior that are not reversed by prolonged T treatment and repeated sexual experience. Thus, full hormonal activation of reproductive behavior in this species requires the presence of testicular hormones during adolescence, and the effects of adolescent testicular hormones may generalize to other hormone-modulated social behaviors such as flank marking.

The current study tested the hypothesis that exposure to gonadal hormones during adolescence is necessary for the activation of flank-marking behavior by T in adulthood. We further hypothesized that exposure to gonadal hormones during adolescence influences the degree of V1a receptor binding in the brain regions regulating flank-marking behavior.

## Methods

### *Animals*

All animals were housed in a 14-h light–10-h dark schedule (lights off at 1300 h EST) and had ad libitum access to food (Teklad Rodent Diet No. 8640, Harlan) and water. Animals were treated in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and all protocols were approved by the Michigan State University All-University Committee for Animal Use and Care.

#### *Experimental animals (resident)*

Fifty-two 18-day-old male Syrian hamsters (*Mesocricetus auratus*) were obtained from Harlan Sprague-Dawley (Madison, WI) laboratories and arrived with their mothers and littermates. Experimental males remained with their mothers and littermates until weaning at 21 days of age. At weaning, males were housed individually in 30.5 × 10.2 × 20.3-cm clear polycarbonate cages. Approximately 1 week prior to behavior testing, animals were transferred to larger home cages measuring 37.5 × 33 × 17 cm, and these cages were not cleaned or disturbed before behavior testing occurred.

#### *Partner animals (intruders)*

Fifty two adult male Syrian hamsters were obtained from Harlan Sprague-Dawley approximately 1 week prior to behavior testing. All intruders were group housed (4–5/cage) in clear polycarbonate cages (30.5 × 10.2 × 20.3 cm).

### *Experimental design*

#### *Castrate groups*

Two groups of males were gonadectomized (GDX) before adolescence at 21 days of age ($n$ = 8–9/group) and therefore were deprived of testicular hormones during adolescence (NoT@P). Two additional groups were GDX immediately after adolescence at 62 days of age ($n$ = 8–9/group) and therefore were exposed to testicular hormones throughout adolescent development (T@P). All males were behavior tested 7 weeks following GDX in young adulthood (10 and 16 weeks old, respectively). To determine the activational effects of T on flank-marking behavior, one of the NoT@P and T@P groups were administered 3.0 mg of T (0.5 mg and 2.5 mg T pellets; Innovative Research of America, Sarasota, FL) 1 week prior to testing. One animal in each of these four groups died prior to behavior testing, and the data for two animals were not collected due to a brief video camera failure. Therefore, final sample sizes were 6–8/group.

#### *Sham groups*

Two groups of sham-gonadectomized males were included in this study. One group of males received a sham GDX immediately before adolescence (Shm-NoT@P; $n$ = 9), and the other received a sham GDX immediately after adolescence (Shm-T@P; $n$ = 8). The sham surgeries and behavior tests were conducted at the same time as their respective castrate group (NoT@P or T@P). The sham groups served two purposes: (1) to assess whether chronological age (10 vs. 16 weeks old) at the time of behavior testing influences V1a receptor binding or flank-marking behavior in adulthood, and (2) to assess whether the one week of adult T replacement experienced by the castrate groups is sufficient to activate adult-typical levels of flank-marking behavior. If 1 week is sufficient, then the T-treated T@P group should display levels of flank-marking behavior similar to that of sham males.

### *Behavior testing*

#### *Testing paradigm*

The resident–intruder paradigm was employed in which an age (10 or 16 weeks old)- and weight-matched (within 10 grams) gonad-intact intruder was placed into the home cage of the resident male for a 10-min test. Prior to testing, the resident's cage lid was removed and clear plexiglass wall extensions were fitted inside the cage (extended to the floor) to prevent animals from escaping during testing (increased total wall height to 32.2 cm). Five minutes following the insertion of cage wall extensions, the intruder was placed into the cage with the resident. Each intruder was only tested once during the experiment. All behavior tests began 1 h into the dark phase of the light–dark cycle and were videotaped under dim red light illumination for later behavioral analysis. A flank mark was recorded by an observer blind to experimental condition each time a resident or intruder rubbed his dorsolateral flank gland against the walls of the test arena. An attack was recorded if the resident or intruder moved quickly toward their partner in an attempt to bite.

#### *Prescreening of intruders*

In order to increase the likelihood of the resident male displaying dominance behavior toward the intruder and also to minimize individual differences in