Dopamine Mediates Testosterone-Induced Social Reward in Male Syrian Hamsters

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Adolescent maturation of responses to social stimuli is essential for adult-typical sociosexual behavior. Naturally occurring developmental changes in male Syrian hamster responses to a salient social cue, female hamster vaginal secretions (VS), provide a good model system for investigating neuroendocrine mechanisms of adolescent change in social reward. Sexually naïve adult, but not juvenile, males show a conditioned place preference (CPP) to VS, indicating that VS is not rewarding before puberty. In this series of experiments, the authors examined the roles of testosterone and dopamine receptor activation in mediating the adolescent gain in positive valence of VS. Experiment 1 showed that testosterone replacement is necessary for gonadectomized adult hamsters to form a CPP to VS. Experiment 2 showed that testosterone treatment is sufficient for juvenile hamsters to form a CPP to VS, and that the dopamine receptor antagonist haloperidol blocks formation of a CPP to VS in these animals. Experiments 3 and 4 demonstrated that the disruption of VS CPP with low doses of haloperidol is the result of a reduction in the attractive properties of VS and not attributable to aversive properties of haloperidol. Together, these studies demonstrate that the unconditioned rewarding properties of a social cue necessary for successful adult sociosexual interactions come about as the result of the pubertal increase in circulating testosterone in male hamsters. Furthermore, this social reward can be prevented by dopamine receptor antagonism, indicating that hypothalamic and/or mesocorticolimbic dopaminergic circuits are targets for hormonal activation of social reward. **(*Endocrinology* 154: 1225–1234, 2013)**

Given the necessity of appropriately interpreting social stimuli in successful adult social interactions and reproductive fitness, a fundamental problem for developmental psychobiology is the identification of the neuroendocrine mechanisms underlying adolescent maturation of social information processing. Male Syrian hamsters provide a useful model with which to study developmental change in perception of and responses to social cues because their sexual behavior is dependent on neural processing of female hamster vaginal secretions (VS) (1, 2), and their endocrine, neural, and behavioral responses to VS mature during the second month of postnatal life, which corresponds to puberty and adolescence in this species (3, 4). Juvenile male hamsters do not show adult-typical attraction to VS (5). Moreover, VS are an unconditioned reward only after puberty because sexually naïve adult, but not juvenile, male hamsters will form a conditioned place preference (CPP) for them (6, 7). Attraction to VS, like the performance of male sexual behavior, is dependent on activational effects of testosterone in adults (8, 9), and attraction to VS can be induced by testosterone treatment of juvenile males (5). However, it is unknown whether the reinforcing value of VS is similarly testosterone-dependent in either adult or juvenile hamsters.

An important neural response to chemosensory stimuli and copulation in rodents is the release of dopamine in the medial preoptic area (MPOA) and nucleus accumbens (Acb) (10–20). Specifically, dopamine has been implicated in multiple aspects of sexual reward. For example, systemic administration of haloperidol, a predominantly D2

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Abbreviations: Acb, nucleus accumbens; CPA, conditioned place avoidance; CPP, conditioned place preference; GDX, gonadectomized; MPOA, medial preoptic area; VS, vaginal secretions.

dopamine receptor antagonist (NIMH Psychoactive Drug Screening Program, http://pdsp.med.unc.edu), decreases unconditioned motivation for primary female visual, auditory, and chemosensory cues in sexually naïve male rats and conditioned motivation for olfactory cues previously associated with sexual behavior (21, 22). In addition, formation of CPP for sexual behavior in female hamsters is blocked by administration of a D2 receptor antagonist (23). However, other studies have found that dopamine receptor activation is not required for CPP for sexual rewards in male rats and mice (24–26). It remains to be determined whether dopamine receptor activation is necessary for CPP to VS in male hamsters. However, we do know that behavioral differences between gonad-intact juvenile and adult hamsters are mirrored by their dopaminergic responses to VS. Adult, but not juvenile, hamsters show an increase in dopamine release and metabolism in response to VS in the MPOA (18). Similarly, adult, but not juvenile, hamsters express Fos in response to VS in the Acb, ventral tegmental area, and medial prefrontal cortex (7). Thus, gain of dopaminergic function across adolescence may be necessary for VS reward and attraction.

Dopaminergic involvement in sexual reward is regulated by testosterone in rodents. Castration causes a decrease in sexual behavior after 2 to 8 wk, which coincides with decreases in basal dopamine levels and turnover in the Acb and MPOA (27). The absence or presence of a precopulatory MPOA dopaminergic response to a stimulus female is predictive of the extinction or recovery, respectively, of copulatory behavior after gonadectomy and subsequent testosterone-replacement (11, 28). Moreover, sexual behavior can be partially restored in long-term castrated male rats by systemic and intra-MPOA injections of apomorphine, a dopamine agonist (29). Finally, testosterone concentrations and dopamine circuitry change during puberty (30, 31). Therefore, this series of studies tested the hypothesis that testosterone activates social reward via influences on dopaminergic reward circuitry, using the formation of CPP to VS in adult and juvenile male hamsters as a model system.

Materials and Methods

Animals

Syrian hamsters (*Mesocricetus auratus*) were obtained from Harlan Laboratories (Madison, Wisconsin) and housed in temperature- and humidity-controlled vivaria with a light:dark cycle of 14 hr light:10 hr dark and ad libitum access to food (Teklad Rodent diet 8640; Harlan Laboratories) and water. Upon arrival (see specific experiments for ages), juvenile males were housed with their male littermates and biological mothers until weaning at P18. Weanling and adult males were singly housed in clear polycarbonate cages (30.5 × 10.2 × 20.3 cm). All males were sexually naïve at the time of study and used in only one experiment. Sixty adult female hamsters, approximately 12 mo old, were housed under similar conditions in separate vivaria and used as the source of VS. Female hamsters were ovariectomized several weeks before hormone administration for experimental control of day of hormone-induced estrus, when VS secretion is maximal. They were injected subcutaneously with 10 μg estradiol benzoate and 500 μg progesterone in sesame oil, 52 and 4 hours, respectively, before collection of VS by gentle vaginal palpation. All experiments were conducted under $<$ 4 lux red light 1 to 5 hours into the dark phase. Hamsters were treated in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and protocols were approved by the Michigan State University Institutional Animal Care and Use Committee.

Surgery and hormone implantation

Hamsters in gonadectomized (GDX) experimental groups underwent surgery with isoflurane anesthesia. Bilateral longitudinal scrotal incisions were made, and the testes were removed with a cut distal to ligature (adults) or cauterization (juveniles). GDX+0 and GDX+T groups were also subcutaneously implanted with 2 blank or testosterone-containing silastic capsules, respectively (one 5 mm and one 13 mm of testosterone [Sigma-Aldrich, St. Louis, Missouri], sealed on each end with 4 mm silastic adhesive; inner diameter 1.98 mm; outer diameter 3.18 mm). These capsules produce adult physiological levels of circulating testosterone (~2–7 ng/ml, Table 1). Subjects received a subcutaneous injection of ketoprofen analgesic at time of surgery and again 24 hours after.

Plasma testosterone measures

One hour after completion of the CPP test or last olfactory test, hamsters were euthanized with an overdose of sodium pentobarbital (150 mg/kg, intraperitoneal), and a terminal blood sample was collected via cardiac puncture for radioimmunoassay of circulating plasma testosterone. Duplicate 50–μl samples of plasma testosterone were analyzed within a single assay using the Coat-A-Count Total Testosterone Kit (Diagnostic Products, Los Angeles, California). The minimum detectable concentration and the intra-assay coefficient of variation were 0.08 ng/ml and 7.9% in experiments 1 and 2, and 0.12 ng/ml and 5.8% in experiments 3 and 4, respectively. Five (experiment 2) and 2 (experiment 3) hamsters removed their testosterone capsules midexperiment and were excluded from behavioral or testosterone analyses. Final group sizes are given in Table 1.

CPP tests

Place preference conditioning occurred as described previously (6, 7) in an apparatus with 1 middle compartment and 2 outer compartments (Med Associates, St. Albans, Vermont). These outer compartments were designed to allow for compartment-specific associations, with distinct visual, tactile, and olfactory cues. Animals were acclimated to handling and novel chambers 2 d before the CPP regimen was begun. The CPP regimen included a pretest, 10 conditioning sessions, and test, all of which occurred at the same time of day (±1 h) for each hamster. To reduce the number of cohorts required and prevent exposing

Table 1. Final Group Size, Body Weight, and Plasma Testosterone Concentration at Time of Sacrifice

Age	Hormone	Stimulus	N	Body Weight (g)		Plasma T (ng/ml)	
				Mean	SD	Mean	SD
Experiment 1: Adult CPP to VS							
Adult	Intact	No VS	12	132.50	13.67	1.41	0.53
Adult	GDX+0	VS	10	150.72	11.95	<0.08	-
Adult	GDX+T	VS	10	116.77	12.09	2.60	0.87
Experiment 2: Juvenile CPP to VS							
Juvenile	Intact	No VS + 0.00	11	58.76	3.90	0.89	0.50
Juvenile	GDX+0	VS + 0.00	7	54.19	4.48	<0.08	-
Juvenile	GDX+T	VS + 0.00	6	56.05	5.69	3.87	1.21
Juvenile	GDX+T	VS + 0.05	8	56.01	6.77	2.70	1.47
Juvenile	GDX+T	VS + 0.15	7	56.51	6.91	4.09	0.65
Juvenile	GDX+T	VS + 0.45	8	52.34	4.59	4.09	1.15
Experiment 3: Juvenile CPA to haloperidol							
Juvenile	GDX+T	VS + 0.00	9	53.91	4.46	6.19	1.85
Juvenile	GDX+T	VS + 0.05	8	52.79	3.68	5.90	0.88
Juvenile	GDX+T	VS + 0.15	9	53.98	5.32	5.13	1.83
Juvenile	GDX+T	VS + 0.45	8	55.79	7.02	6.07	1.01
Experiment 4: Juvenile attraction to VS							
Juvenile	GDX+T	Repeated testing	9	57.46	3.31	5.27	0.85

Note: Adults were consistently larger than juveniles, but groups within an age generally did not differ in body weight. Testosterone capsules produced circulating testosterone concentrations within adult-typical physiological ranges.

Abbreviations: CPP, conditioned place preference; GDX, gonadectomized; VS, vaginal secretions.

control animals to the smell of the stimuli, control animals were housed in a separate room in which the dark phase began at 8:00 AM and testing at 9:00 AM. Experimental animals were housed in rooms in which the dark phase began at 2:00 PM and testing at 3:00 PM.

A pretest (2 min in the middle compartment followed by 15 min access to all compartments) was used to determine each hamster's initial compartment preference without any stimulus present. The outer compartment in which the hamster spent more time was defined as the initially preferred compartment. A preference score, defined as [time in the initially nonpreferred compartment/(time in the initially preferred compartment + time in the initially nonpreferred compartment)], and a difference score, defined as [time in the initially preferred compartment − time in the initially nonpreferred compartment] were calculated for each animal (6). To ensure that each hamster had the opportunity to make an informed preference, hamsters that did not enter each compartment at least 5 times were excluded from further training. Animals were assigned to experimental and control groups so as to equate groups for initial chamber preferences and preference scores and litter representation in the different groups.

After the pretest, the hamsters received a total of 10 30-minute conditioning sessions in the side compartments, 1 session per day on consecutive days, alternating 5 no-stimulus and 5 stimulus-paired sessions. During the no-stimulus conditioning sessions, hamsters in both the experimental and the control groups were placed in their initially preferred compartments, where they remained alone. During stimulus-paired conditioning sessions, hamsters in the experimental group were placed in the initially nonpreferred compartments with the stimulus. The hamsters in the control groups were also placed in their initially nonpreferred compartments but were not given the stimulus. This group served to quantify any changes in preference or difference score across tests that were attributable to habituation during conditioning. The CPP apparatus was cleaned thoroughly with 25% ethanol between each animal, and with 75% ethanol at the end of each conditioning day.

In experiments 1 and 2, VS were used as the stimulus in conditioning sessions. An hour before use, approximately 500 μl VS were collected from 30 females and mixed together to ensure that each male was exposed to the same stimulus. Approximately 15 μl VS were applied to water-moistened cotton gauze packed into a 2-ml Eppendorf tube, 1 tube for each male. Immediately before testing, the tube was placed out of reach from the male at the top of the back wall in the initially nonpreferred compartment in VS-paired conditioning sessions for the VS group. Empty Eppendorf tubes were used for the control group in all conditioning sessions and for the VS group in the no-stimulus conditioning sessions. To ensure exposure to nonvolatile components of VS, the remaining ~200 μl VS were mixed with 1.5 ml of mineral oil, and approximately 10 μl of this mixture was applied with a metal spatula directly onto the nose of hamsters in the VS group immediately before the hamsters were placed in the VS-paired compartment. Clean oil was applied to the nose of hamsters in the control group for all conditioning sessions and those in the VS group for no-stimulus conditioning sessions.

Twenty-four hours after the last conditioning session, hamsters were tested for their place preference following the same procedure used for the pretest. As in the pretest, no stimulus was present, and preference and difference scores were calculated for each animal.

Experiment 1: Are testicular hormones necessary for formation of a CPP to VS in adult hamsters?

This experiment tested whether circulating testicular hormones are required for the display of a CPP to VS in adult ham-

sters. Pilot studies in this laboratory indicated that male hamsters formed a CPP to VS when conditioning began 1 wk after gonadectomy (32), suggesting that putative activational effects of testicular hormones do not wash out acutely, similar to the gradual decline in sexual behavior that occurs over many weeks after gonadectomy in male rodents (33). Therefore, in this experiment, we studied hamsters that had been GDX 10 wk before the start of conditioning. All adults arrived in the laboratory at postnatal day P56-63, but arrivals were staggered so that groups could be tested at the same time. No-stimulus control animals were left gonad-intact and pretested at P64–71. Hamsters in the GDX+0 group were GDX at P57–64, remained unmanipulated for 10 wk, and then were implanted with blank capsules at P127–134, 1 wk before pretest at P134–141. The GDX+T group was GDX and given testosterone capsules at P57–64, 1 wk before pretest at P64–71, to serve as positive controls to demonstrate a significant CPP. This arrangement required conditioning and testing animals at different young adult ages, but we have never observed age-related differences in behavioral or neural responses to testosterone in prior experiments that controlled for this variable in young adults (34). Additionally, GDX/testosterone-treated male hamsters of ages similar to those in the GDX+0 group reliably form a CPP to VS (35). Therefore, we thought that maintaining the no-stimulus control and GDX+T groups for 10 weeks in the laboratory was unnecessary and could not justify the costs of doing so.

Experiment 2: Are testosterone and dopamine receptor activation necessary for a CPP to VS in juvenile hamsters?

This experiment tested the involvement of dopamine in testosterone-facilitated CPP to VS in juvenile male hamsters. All animals arrived at P12, were pretested at P20, and were run in 3 cohorts. Gonad-intact hamsters were used as no-stimulus controls, whereas other groups were GDX and given blank or testosterone capsules at P13, 1 week before testing. The GDX+0 group was included to confirm that juveniles with low levels of testosterone (as in gonad-intact animals) do not show a CPP to VS. A GDX+T group was included to determine if testosterone treatment can induce a CPP to VS. The remaining groups were all GDX+T and were given intraperitoneal injections of haloperidol (0.05, 0.15, and 0.45 mg/kg) or propylene glycol vehicle 30 minutes before VS and no-stimulus conditioning sessions, respectively. Haloperidol is a potent D2 antagonist but also can bind the D1, adrenergic, and sigma receptors less effectively (NIMH Psychoactive Drug Screening Program, http://pdsp.med.unc.edu/). No-stimulus, GDX+0 and GDX+T control groups received propylene glycol vehicle injections 30 min before both conditioning sessions.

Experiment 3: Does dopamine receptor antagonism alone alter place preference in juvenile hamsters?

This experiment was designed to determine if the doses of haloperidol used in experiment 2 had any intrinsic aversive qualities in testosterone-treated hamsters, such that they would induce a conditioned place aversion (CPA). If they did, prevention of CPP for VS in experiment 2 could be attributable to avoidance of the haloperidol-conditioned environment. All animals arrived at P11 or P12, were GDX+T at P13, pretested at P20, and run in 2 cohorts staggered by 1 day. A similar conditioning paradigm was used as that described, but haloperidol was given in the initially preferred chamber in an attempt to reduce initial preferences, and no VS were used. Locomotor movement (number of changes in infrared beam breaks) and fecal boli output during conditioning sessions were also quantified as indicators of physiological effects of haloperidol.

Unconditioned attraction test

Experiment 4: Does dopamine receptor antagonism affect attraction to VS in juvenile hamsters?

This experiment determined whether haloperidol reduces attractive properties of VS. Animals that were excluded from experiment 3 after the pretest (and before any haloperidol exposure) because of insufficient exploration were used here; thus, these males arrived at P11–12, were GDX and testosterone-treated on P13, and tested over 5 days on P28–32. VS were collected from stimulus females 1 day before the first day of testing, as described; VS from ~14 females were mixed together with 100 μl mineral oil into 1 of 5 Eppendorf tubes. Tubes were stored at 4°C until 1 tube was thawed 30 minutes before onset of testing each day. A metal spatula was used to smear approximately 15 μl clean mineral oil or VS mixture onto a glass slide, 1 per hamster, immediately before the test. A clean and VS-smeared slide were taped approximately 5 cm up the wall at opposite sides of glass aquaria (51 × 26 × 31.5 cm) in a procedure adapted from (36, 37). The location of the smell was counterbalanced across groups and within an animal.

On days 1 and 5, animals were injected with intraperitoneal propylene glycol vehicle 30 minutes before the test. On days 2 to 4, animals were injected with 0.05, 0.15, or 0.45 mg/kg haloperidol, in counterbalanced order. Animals remained in their colony room until immediately before testing. To begin testing, the hamsters were placed in the middle of the aquarium and their behaviors live-scored and video recorded for 5 minutes. Upon test completion, hamsters were returned to their colony room, the slides removed, and aquaria cleaned with 75% ethanol. The length of time a hamster spent investigating each slide, with nose less than 0.5 cm from the slide, was quantified from video recordings by a scorer blind to the location of the VS tube. An attraction score (time with VS slide − time with oil slide) was calculated for each animal.

Statistical analysis

To confirm that all control and experimental groups had similar initial preference and difference scores, a one-way ANOVA was used. To assess whether the stimuli induced a CPP or CPA in experiments 1 to 3, changes in preference and difference scores were analyzed, as reported previously (7). Changes in preference and difference scores were determined by subtracting pretest measures from test measures for each hamster. In the control animals, average change measures for preference score and difference score were determined to provide a standard for unconditioned change. Control change measures in preference and difference scores was then subtracted from each experimental animal's scores to correct for any unconditioned change. Therefore, control measures are not shown in figures. Corrected changes in preference and difference scores were then used in 1-sample *t* tests within each group, comparing the value to zero to evaluate significant differences from chance preference. These

statistical procedures are similar to those of previous studies that used paired *t* tests to determine changes in preference and difference scores within a group (6, 38–43). In addition, correcting for unconditioned changes observed in control animals reduces the chances of false positive results, as any initial preferences for an outer compartment can sometimes be reduced after repeated equivalent exposures to those chambers (6, 7). Significant changes in both preference and difference scores were required to conclude that a CPP had been established. To assess effects of haloperidol on physiological variables in experiment 3, paired samples *t* tests were used to compare movement and fecal boli output in the haloperidol- and vehicle-paired chambers, within each haloperidol dose group.

To assess whether the dopamine receptor antagonist haloperidol affected unconditioned attraction to VS in experiment 4, a repeated measures ANOVA was used to test the effect of haloperidol dose on attraction score, with *t* test follow-ups and Bonferroni corrections. In addition, 1-sample *t* tests were used to determine if each dose group's preference and difference scores were significantly different from chance, half or zero, respectively. Measures from vehicle injections on the first and last day of testing did not differ and were averaged together per animal. A repeated measures ANOVA was used to determine the effects of drug on the number of line crossings, to indicate effects of drug on locomotor activity. In all analyses, $P < .05$ was considered significant, and all statistical analyses were done with SPSS software (PASW Statistics 20; SPSS, An IBM Company, Chicago, Illinois).

Results

Experiment 1: Are testicular hormones necessary for formation of a CPP to VS in adult hamsters?

Long-term GDX adult hamsters failed to form a CPP for VS (Figure 1). No changes in preference or difference score of the GDX+0 group were seen as a result of conditioning with VS, as 1-sample *t* tests showed that neither the corrected change in preference ($t_{(9)} = -1.98$, NS) or difference ($t_{(9)} = 1.19$, NS) scores were significantly different from zero. In contrast, the GDX+T group did show a CPP to VS, as 1-way *t* tests showed that the corrected change in preference ($t_{(9)} = 4.06$, $P < .01$) and difference ($t_{(9)} = -4.23$, $P < .01$) scores were significantly different from zero. Groups did not differ in their initial preference score ($F_{(2,29)} = 2.17$, NS) or difference score ($F_{(2,29)} = 1.95$, NS). Therefore, recent exposure to testicular hormones is necessary for VS-induced CPP.

Experiment 2: Are testosterone and dopamine receptor activation necessary for CPP to VS in juvenile hamsters?

Testosterone was sufficient to promote a CPP for VS in juvenile hamsters (Figure 2). The GDX+T VS group that received vehicle injection showed a CPP to VS, as 1-way *t* tests found that the corrected change in preference ($t_{(5)} = 3.11$, $P < .05$) and difference ($t_{(5)} = -2.77$, $P < .05$) scores were significantly different from zero. The GDX+0 VS group did not show a significant corrected change in either preference or difference score as a result of conditioning

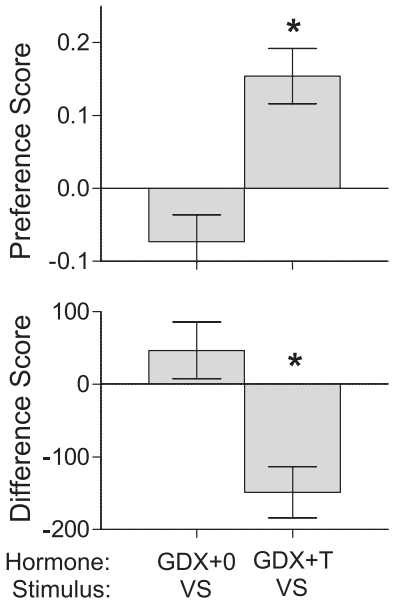

Figure 1. Conditioned place preference (CPP) to vaginal secretions (VS) in hormone-manipulated adult hamsters. Corrected changes in preference and difference scores are shown, mean ± SE. * Indicates difference from no change (zero), $P < .05$. Long-term gonadectomy with blank hormone capsules prevented the CPP for VS observed in the gonadectomized (GDX) + testosterone group.

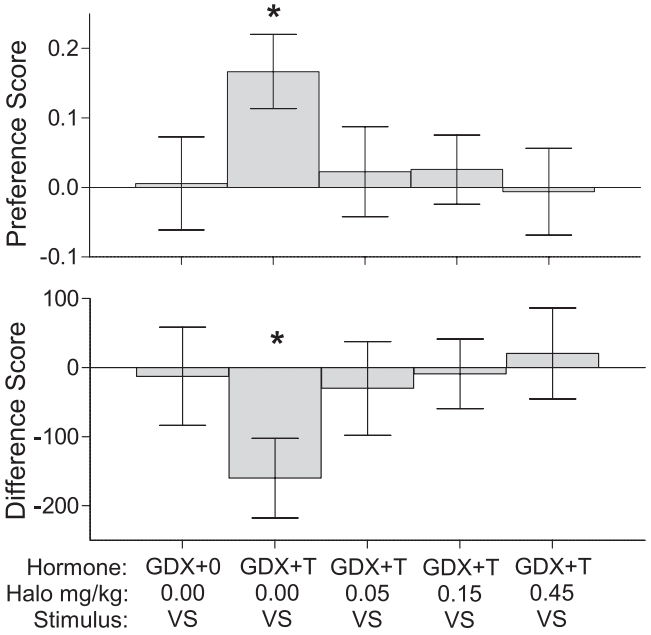

Figure 2. Conditioned place preference (CPP) to vaginal secretions (VS) in hormone- and dopamine-manipulated juvenile hamsters. Corrected changes in preference and difference scores are shown, mean ± SE. * Indicates difference from no change (zero), $P < .05$. Testosterone treatment in juvenile hamsters facilitated an adult-like CPP for VS, and dopamine antagonism prevented the CPP for VS at multiple doses.

($t_{(6)}$ = 0.09 [NS] and $t_{(6)}$ = −1.74 [NS], respectively), replicating effects seen in gonad-intact juveniles with similar concentrations of circulating hormone (7). Additionally, dopamine receptor antagonism blocked the CPP for VS in T-treated juvenile hamsters (Figure 2). The CPP was blocked by haloperidol at all 3 doses: the 0.05-, 0.15-, and 0.45-mg/kg GDX+T VS groups did not show corrected changes in preference scores ($t_{(7)}$ = 0.35 [NS], $t_{(6)}$ = 0.52 [NS], and $t_{(7)}$ = −0.10 [NS], respectively) or difference scores ($t_{(7)}$ = −0.44 [NS], $t_{(6)}$ = −0.18 [NS], and $t_{(7)}$ = 0.31 [NS], respectively) that were significantly different from zero as a result of conditioning. Groups did not differ in their initial preference score ($F_{(5,47)}$ = 0.27, NS) or difference score ($F_{(5,47)}$ = 0.26, NS).

Experiment 3: Does dopamine receptor antagonism alone alter place preference in juvenile hamsters?

The lower 2 doses of haloperidol were not aversive (Figure 3). Neither the 0.05 nor 0.15 mg/kg group showed a CPA to haloperidol, as 1-way *t* tests showed that neither the corrected change in preference ($t_{(7)}$ = −0.23 [NS] and $t_{(8)}$ = 0.55 [NS], respectively) nor difference ($t_{(7)}$ = −0.02 [NS] and $t_{(9)}$ = −0.54 [NS], respectively) scores were significantly different from zero. A CPA to the highest dose of haloperidol was detected. One-way *t* tests showed that the corrected change in preference score was significantly different from zero ($t_{(7)}$ = 2.55, $P < .05$), but the corrected change in difference score was not ($t_{(7)}$ = −1.88, NS). Groups did not differ in their initial preference score

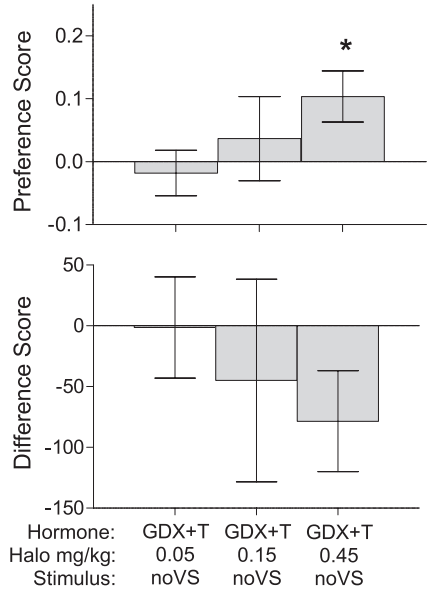

Figure 3. CPA to 0.45 mg/kg haloperidol in testosterone-manipulated juvenile hamsters. Corrected changes in preference and difference scores are shown; mean ± SE. * Indicates difference from no change (zero), $P < .05$. The 2 lower doses of dopamine antagonist did not result in a CPA.

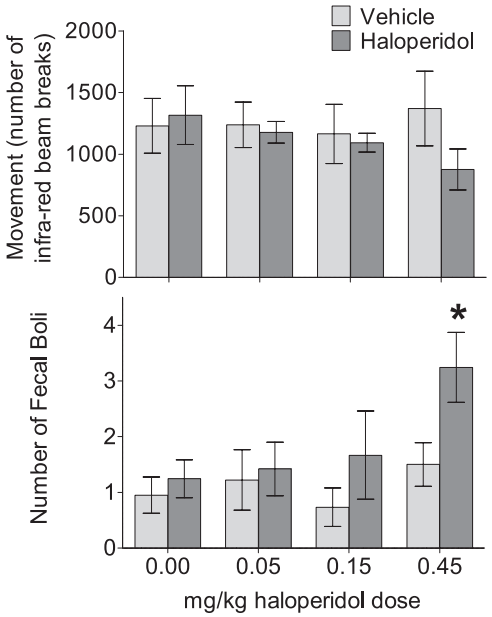

Figure 4. Movement (top) and fecal boli output (bottom) of hamsters in vehicle- and haloperidol-paired chambers, mean ± SE. * Indicates differences between chambers within an animal, $P < .05$. Haloperidol did not affect movement but did increase fecal boli output at the highest dose.

($F_{(3,32)}$ = 0.01, NS) or difference score ($F_{(3,32)}$ = 0.14, NS). Haloperidol had little effect on locomotor activity and number of fecal boli (Figure 4). Paired samples *t* tests demonstrated that movement was not affected by haloperidol at 0.00-, 0.05-, 0.15-, or 0.45-mg/kg doses ($t_{(8)}$ = −0.26 [NS], $t_{(8)}$ = 0.28, [NS], $t_{(8)}$ = 0.26 [NS], and $t_{(8)}$ = 1.21 [NS], respectively). Fecal boli output was increased at the 0.45-mg/kg dose ($t_{(8)}$ = −2.67, $P < .05$), but not at the 0.00-, 0.05-, or 0.15-mg/kg doses ($t_{(8)}$ = −1.10 [NS], $t_{(8)}$ = −0.59 [NS], and $t_{(8)}$ = −1.74 [NS], respectively).

Experiment 4: Does dopamine receptor antagonism affect attraction to VS in juvenile hamsters?

Dopamine receptor antagonism affected attraction to VS in a dose-dependent manner (Figure 5). In repeated measures analysis, a significant effect of dose was observed in attraction score with Greenhouse-Geisser correction, $F_{(1.42,11.38)}$ = 9.802, $P < .01$, such that in follow-up *t* tests, vehicle scores were significantly different from 0.05-, 0.15-, and 0.45-mg/kg dose scores ($t_{(8)}$ = −4.74, −3.46, and −3.80, all $P < .01$, respectively). However, 1-sample t tests, comparing difference scores to chance preference between the slides (zero), indicate that attraction to VS was still intact in the 0.15-mg/kg group, as in the vehicle group: the 0.00- and 0.15-mg/kg dose attraction scores were significantly different from chance ($t_{(8)}$ = 4.22, $P < .01$ and $t_{(8)}$ = 2.81, $P < .05$, respectively), whereas 0.05- and 0.45-mg/kg dose scores were not dif-

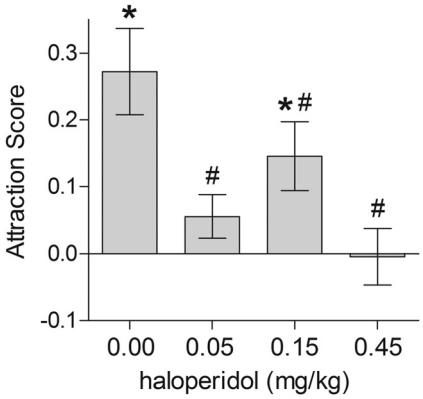

Figure 5. Attraction score to vaginal secretions (VS) in haloperidol-treated hamsters, mean ± SE. # Indicates difference from vehicle. * Indicates difference from no preference (zero), $P < .05$. Haloperidol reduced attraction to VS at all doses but less so at the 0.15-mg/kg dose.

ferent from chance ($t_{(8)} = 1.72$ and -0.11, both NS, respectively). No effects of dose were found on number of line crossings by repeated measures ANOVA ($F_{(3,24)} = 0.11$, NS), data not shown. Thus, haloperidol significantly reduced attraction to VS at some doses.

Physiological measures

Physiological measures are shown in Table 1 and confirm efficacy of testosterone capsules in increasing circulating testosterone in both ages. Groups of the same age did not differ in body weight.

Discussion

These studies demonstrate that the perception of a species-specific chemosensory stimulus as rewarding is testosterone dependent and involves activation of dopamine receptors. Specifically, we found that long-term GDX adult male hamsters do not form a CPP to VS, whereas testosterone treatment of juveniles is sufficient to enable them to form a CPP to VS. In addition, the primarily D2 receptor antagonist haloperidol prevented expression of a CPP to VS in testosterone-treated juvenile hamsters. We infer from these findings that adolescent maturation of social information processing is the result of the pubertal increase in circulating testosterone that, via yet unidentified influences on dopaminergic circuits, results in the perception of female chemosensory stimuli and environments associated with those stimuli as rewarding.

Testosterone and social reward

Given the necessity of testosterone in VS reward in adulthood and the ability of testosterone to promote VS reward in juvenile animals, we surmise that 1) the adult-like rewarding responses to VS come about normally because of the pubertal increase in circulating testosterone, and 2) no other hormone-dependent or -independent adolescent developmental processes are necessary for VS reward. Indeed, organizational effects of testosterone during puberty are not required for VS reward, as animals deprived of gonadal hormones during puberty and treated with testosterone in adulthood show a robust CPP to VS (35). The activational effects of testosterone in VS CPP mirror those seen in studies of attraction to VS in both juveniles and adults and sexual response behaviors that normally increase during adolescence (5, 9, 44). Although the mechanism by which testosterone facilitates reward responses to VS has not been identified specifically, we propose that it promotes dopaminergic tone via D2 receptor activation.

Dopamine and social reward

Our study demonstrates a role for D2 receptor activation in the rewarding interpretation of VS, as the primarily D2 receptor antagonist haloperidol blocked the CPP to VS. This blockade is due to a reduction in the attractive and rewarding properties of VS, as demonstrated by the unconditioned attraction test. Although these effects theoretically could be attributable to a haloperidol-induced reduction in olfactory abilities (45), D2 receptor activation previously has been shown to decrease olfactory sensitivity and discrimination (46–48). In addition, in pilot studies, hamsters exposed to even the highest dose of haloperidol were still readily able to detect food olfactory cues (49). Moreover, the blockade of a CPP was not attributable to aversive properties of haloperidol that caused the animal to avoid the haloperidol-associated CPP compartment because experiment 3 demonstrated that the 2 lower doses of haloperidol, 0.05 and 0.15 mg/kg, were not aversive. Additionally, haloperidol did not affect movement and affected fecal boli output only at the highest dose. Because fecal boli output classically has been used as an indicator of anxiety and aversion (50), these findings are in parallel with the formation of a CPA to the highest dose of haloperidol, although one caveat is that D2 receptor activation inhibits gut motility in the enteric nervous system (51). Taken together, it is unlikely that haloperidol interfered with sensory detection of VS or that it is in and of itself aversive at the lower doses used in this study; therefore, we conclude that D2 receptor activation is required for VS to be perceived as rewarding.

Dopamine previously has been implicated in multiple aspects of sexual behavior, including anticipatory or appetitive behaviors (52), copulatory or consummatory behaviors (53), and the reinforcing responses to sexual interaction (23). In addition, dopaminergic action at D2

receptors likely is important for associating sociosexual stimuli with environmental or other cues. Systemic low doses of a nonspecific dopamine antagonist block conditioned mate preference in female rats (54), and a D2 agonist during cohabitation with a scented same-sex partner induces a same-sex partner preference for similarly scented males in male rats (55). Work in monogamous prairie voles further supports the importance of D2 receptor in associating sexual reward with stimuli or individuals, as systemic injections of D2, but not D1, receptor agonist and antagonist facilitate and disrupt partner preference in male voles, respectively (56). The current study supports the role for D2 receptor activation in reinforcing responses to unconditioned social cues in sexually naïve animals and parallels the effects of haloperidol in reducing motivation for primary female visual, auditory, and chemosensory cues in sexually naïve male rats (57).

Because we have found that multiple dopamine-sensitive brain regions, including the amygdala, MPOA, and Acb, are involved in behavioral responses to VS (7, 18), systemic intervention was used to antagonize dopamine receptors at multiple putative sites of action. Although the site(s) of action of dopamine cannot be determined from this study, there are several likely candidates. Dopamine agonists and antagonists into MPOA facilitate and reduce the performance of sexual behavior, respectively, in male and female rats (58–61). In addition, the MPOA is implicated in anticipatory sexual behaviors and female preferences (62, 63). The mesolimbic system does not seem to be involved in the performance of copulatory behaviors, except for general motor abilities (63, 64). However, dopaminergic action in the Acb may be involved in anticipatory sexual behavior, such as increased locomotor activity and erections in response to female cues, independent of motor effects (62, 65). In addition, the Acb is important in pair bonding and mate-cue association, as evidenced by work in voles (66, 67). Thus, dopamine action in the MPOA, Acb, or both regions may be important for CPP to VS.

Testosterone modulation of dopaminergic systems

Previous research demonstrates puberty-related changes in dopamine content, transporters, receptors, and synaptic responses in the Acb (68–73). Whether these changes are dependent on the pubertal rise in testosterone has not been studied, with the notable exception that the adolescent pattern of initial overproduction and subsequent pruning of D1 and D2 receptors in the rat Acb occurs independently of the presence or absence of gonadal hormones (74). Although developmental changes in MPOA dopamine have been well-studied in female rodents (75), less is known about adolescent changes in dopaminergic tone in the male MPOA. However, the hormone sensitivity of the adult MPOA is well-established. Several studies have demonstrated that long-term (2–8 wk) gonadectomy results in an increase in several measures of dopaminergic tone in the MPOA, including tissue content and amphetamine-induced dopamine release, but a decrease in extracellular dopamine in rats at rest (27, 76–79). Importantly, MPOA dopaminergic responses to female stimuli in adult male rats are similarly modulated by testosterone (11, 28). Although effects of castration in the ventral striatum are less consistent than those in the MPOA, 28 d gonadectomy generally reduces dopamine and DOPAC concentrations in Acb tissue (27, 80, 81). Thus, it is plausible that the normative increase in circulating testosterone during adolescence promotes dopaminergic release in response to VS, in MPOA, Acb, or both, thereby promoting VS reward. However, many of these studies were conducted in adult animals, and more work is needed to confirm this hypothesis in developing brains because the effects of testosterone exposure in juvenile animals may be different from those in adults (34).

Taken together, these studies demonstrate the importance of testosterone and dopamine in rewarding responses to an unconditioned social stimulus. Both testosterone and dopamine systems mature during adolescence, when the rewarding quality of VS typically is acquired. It should be noted that the dopaminergic circuit could be functional in juvenile animals to mediate CPP to VS, but that testosterone-dependent activation of some other neural circuitry is also necessary for VS reward. However, the most parsimonious explanation, given the supporting evidence, is that testosterone treatment in juvenile animals mimics the normative elevation in pubertal testosterone, which in turn affects the dopaminergic system to permit VS reward.

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