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Rapid effects of testosterone on social decision-making in a monogamous California mice (*Peromyscus californicus*)

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A B S T R A C T

Social animals must cope with challenges and opportunities by adjusting how they react to a salient stimulus. Here we use California mice (Peromyscus californicus) and investigate the mechanisms underlying social decision-making by studying (i) rapid effects of testosterone (T) pulses on a male's decisions to approach a novel male (challenge) versus a receptive female (opportunity), and (ii) whether social experience shapes how such effects are manifested. In Experiment 1, we found that sexually naïve males administered saline injections preferentially approached unfamiliar females over unfamiliar males, in contrast, 10 min after receiving a single T-injection, males expressed a preference for approaching unfamiliar males. Such an effect of T only occurred in sexually naïve males, but not pair-bonded males, suggesting that the rapid effects of T on approach behavior may rely on the pair-bonding experiences. Experiment 2 investigated social decision-making across three repeated exposures to the challenge/opportunity situations. Only the initial decision, approach to the challenge, predicted future aggressive behaviors, and such an effect relied on the rapid actions of T. We also found that experience with the controlled challenge situation (the male intruder was restrained behind a wire mesh) dampened the approach to the male side (potential threat) when later exposed to the same conditions. This suggests that a resident's motivation to defend against a threatening individual may decrease as the threat posed by the "neighbors" is reduced. Overall rapid effects of post-encounter T pulses may play important roles in influencing behavioral decisions during social interactions.

1. Introduction

Diverse animals must contend with varied forms of social interactions that occur in different contexts and phases of life. Appropriate decision-making abilities to cope with these events is therefore critical to an animal's individual fitness (Arnott and Elwood, 2008; Chittka et al., 2009; Couzin et al., 2005). Accordingly, O'Connell and Hofmann (2011a) classified such social interactions into two straightforward categories: (1) opportunities, such as finding or guarding mates, which require animals to approach an individual for reproductive purpose; and (2) challenges, such as territorial defense, which require animals to approach and expel an intruder. In nature, animals likely find themselves in situations where the acquisition of opportunity entails simultaneously dealing with a challenge. Individuals must decide how to behave when such conflicting stimuli are presented together, and this process is likely the net result of multiple (conflicting) motives (Christy, 1987; Luyten and Liley, 1991; Wynne-Edwards and Lisk, 1988). Past work suggests that an animal's hormonal state and social experience play a powerful role in regulating these events (Hau and Goymann, 2015; Koolhaas et al., 2010), yet how hormones interact with the social experience to influence social decision-making remains largely unclear.

Testosterone (T) pulses, natural transient increases in hormone levels following social interactions with both male and female conspecifics, lie at the core of social interactions by facilitating social approach and dominance-seeking behavior across species (Gleason et al., 2009). For example, a previous study reveals that the post-victory T pulses and the winning experience are capable of influencing future winning ability independently (Fuxjager et al., 2011b), suggesting the unique roles of these T pulses in modulating behavior. According to the challenge hypothesis (Wingfield et al., 1990), T levels are elevated in response to a challenging encounter in which social status might be threatened, thereby initiating approach, motivation and simultaneously reducing fear (Archer, 2006; Bos et al., 2011). Evidence from different animal taxa also demonstrates that T pulses can rapidly modulate behavioral responses in the challenge context, such as increased territorial vocal signaling within 30 min (Remage-Healey and Bass, 2005); reduced urinary marking in subordinate male white footed mice within 20 min (Fuxjager et al., 2015); modulation of men's perceptions of their own physical dominance within 2 h (Welling et al., 2016); enhanced responsiveness to social threat (Hermans et al., 2008) and reduced personal distance towards angry/aggressive individuals (Wagels et al., 2017) in human subjects within 3.5 h. The potential role of brain aromatase in mediating T's behavioral effects in the challenge situations also has been demonstrated in fish (e.g. Huffman et al., 2013), birds (e.g. Soma et al., 2000) and mammals (e.g. Trainor et al., 2004). Furthermore, the rapid effects of T can depend on the pair-bonding status of the individual. For example, T-pulses rapidly decrease ultrasonic

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vocalizations towards a novel female in male California mice (*Peromyscus californicus*) that have pair-bonded with a female, whereas it rapidly increases courtship vocalizations in unpaired males (Pultorak et al., 2015). Pair-bonding also influences reinforcing effects of T (Zhao and Marler, 2014, 2016) that can act upon the neural circuits controlling social decision-making (Frye et al., 2002; Ikemoto and Panksepp, 1999; King et al., 1999; O'Connell and Hofmann, 2011b). We therefore hypothesized that T can rapidly influence social decision-making in the challenge/opportunity situations and that such an effect will be subject to the pair-bonding status.

Adding a layer of complexity to this idea is the fact that prior experience with the conspecific threats may influence hormonal responses and social decision making. For example, familiarity with the "neighbor" can dampen circulating androgens and elicit lower levels of aggression in cichlid fishes (Aires et al., 2015; Weitekamp and Hofmann, 2017). Familiarity with an opponent also decreases calling behavior and chases in Thomas langurs (*Presbytis thomasi*) (Wich and Sterck, 2007), while suppressing aggression in male collared lizards (*Crotaphytus collaris*) (Husak, 2004). These findings suggest that previous experience of dealing with the challenge/opportunity may affect future decision-making when exposed to the same situation. Therefore, we also explored 'animals' social decision-making and T's effects on the decision-making with repeated exposure to the challenge/opportunity situations. We speculated that either the preference would be repeatable and independent of context or that the preferences would change once the males assessed the controlled challenge situation with social stimuli behind a wire mesh; in particular the removal of the male threat via restraint behind the wire mesh barrier. We had no a priori predictions regarding which preference test(s) would predict the future levels of social interaction with an intruding male.

In the current study, we tested our hypotheses using a two-choice assay commonly employed as an indirect measure of social decision-making in different taxa (Henley et al., 2010; Hetta and Meyerson, 1978; Merkx, 1983; Meyerson and Lindström, 1973; Portillo and Paredes, 2003). Accordingly, the measure of preference of a given stimulus is the time spent in proximity to that same stimulus. Research shows that, in the context of opportunity, sexually naive and experienced male rats spend more time near sexually receptive versus non-receptive females or males (Ågmo, 2003; Edwards and Einhorn, 1986). However, in the context of a challenge, a focal mouse's level of aggressive motivation has been measured by time spent near a conspecific male, which correlates positively with aggressive behavior in subsequent physical confrontations in mice (Kovalenko and Kudryavtseva, 2016; Kudryavtseva, 2003; Kudryavtseva et al., 2004). Indeed, animals often show sex-specific aggression when defending a territory or a mate, with males targeting other males and females targeting other females (Adkins-Regan and Robinson, 1993). We therefore conducted two separate studies to test how T rapidly influences social decision-making. In Experiment 1, we investigated both the effects of transient T pulses and pair-bonding experience on social decision-making (approach preference) when in the presence of both a male intruder and a receptive female, which reflects a challenge/opportunity context. In Experiment 2, we explored how the effect of establishing residency influences this process. We therefore only used sexually naïve males in Experiment 2 and allowed males to establish residency for the sake of assessing the relationship between the decision-making and future aggression measured with a resident-intruder paradigm. We also examined changes in social decision-making with repeated exposures to the challenge/opportunity situations.

2. Method

2.1. Overview of experiments

Two experiments were conducted in this study. In Experiment 1, sexually naive and pair-bonded males were injected with either T or saline, and then subjected to a two-choice test assay (see below) to assess their preference to either a novel male (conflict) or a receptive female (mating). In Experiment 2, we allowed the sexually naïve males to establish residency (characteristic of territoriality) in the middle, neutral chamber of the two-choice test apparatus before the preference tests. In this final experiment, on the day after the last preference test, we examined each focal male's aggressive behavior to an unfamiliar male using a resident-intruder paradigm and assessed the relationship between the decision-making through approach preferences and the aggression levels.

2.2. Experiment 1

2.2.1. Animals

Animals were maintained in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. We used 52 male *P. californicus* aged 6–12 months. They were group-housed (2–3 per cage; 48 × 27 × 16 cm) under a 10 L: 14D light cycle with lights off at 01:30 pm. Purina 5001 mouse chow and water were given ad libitum. All behavioral tests were conducted at least 1 h after the dark cycle's onset and under dim red light. Male mice were randomly assigned to either a) a sexually naïve group ($n = 26$), which consisted of sexually naive males that were housed with two other male cage mates (housed together after weaning), or b) a pair-bonded group (n = 26), in which males were paired with a female 1-week before the experiment.

2.2.2. Testosterone dose

We used 36 μg/kg T-injections (T-cyclodextrin inclusion complex) because in a previous study this injection produced an increase in T-levels approximately 3–5 times higher than the baseline, reaching a maximum of 4–5 ng/ml and lasting for approximately 10 min (Trainor et al., 2004). This dose mimics natural changes in T found in intact California mice after winning an aggressive encounter (Oyegbile and Marler, 2005) and in male-female encounters (Zhao and Marler, unpublished data); in keeping with this, the same dosage enhances aggression and future winning ability (Fuxjager et al., 2011a; Fuxjager et al., 2011b; Trainor et al., 2004), induces conditioned place preferences (Zhao and Marler, 2014, 2016), and can rapidly influences ultrasonic vocalizations (Pultorak et al., 2015). In the current study, half of the sexually naïve group and half of the pair-bonded group were randomly selected to receive T-injections (T-group). As T-cyclodextrin was dissolved in saline, the other half of the animals constituted the controls and received injections of saline (saline group).

2.2.3. Testing apparatus

Preference testing took place in a large polycarbonate apparatus (91 × 46 × 43 cm) that was divided equally into three chambers (Zhao and Marler, 2014). Access to each side chamber was controlled through sliding doors (Fig. 1a). The rear third of each side chamber was separated from the front two thirds by a metal wire mesh. During the preference test, stimulus animals were placed in the area behind the wire mesh, which prevents physical interaction between the focal and stimulus animals, while visual, auditory as well as olfactory cues were accessible.

2.2.4. Procedure

Experiment 1 consisted of three phases: habituation (30 min), injection (10 min) and preference test (15 min) (Fig. 1b). Immediately after selection, each focal male was placed into the middle chamber of a testing apparatus. At this time, the sliding doors were opened, permitting access to the side chambers. The focal males were then given 30 min to habituate to their new environment, including the side chambers. After this period, the focal male was gently guided into the middle chamber and the sliding doors were sealed. The focal males then received either T or saline. In each group, half of the randomly selected animals received a weight-adjusted subcutaneous injection of T (36 μg/kg), while the other half received an injection of saline as a control. We then allowed 10 min before testing for the injection to take effect. We chose 10 min because a previous study showed that the most significant rapid effects of 17β-estradiol on sexual behaviors occurred at 15 min, but not 5 or 30 min after the injection (Cornil et al., 2006). We therefore assumed the rapid effects of T should occur between 5 and 15 min. Ten minutes after the injection, stimulus males and females were placed behind the wire mesh partition in each apparatus such that the focal male and the stimulus could see, hear, smell, and maintain proximity,

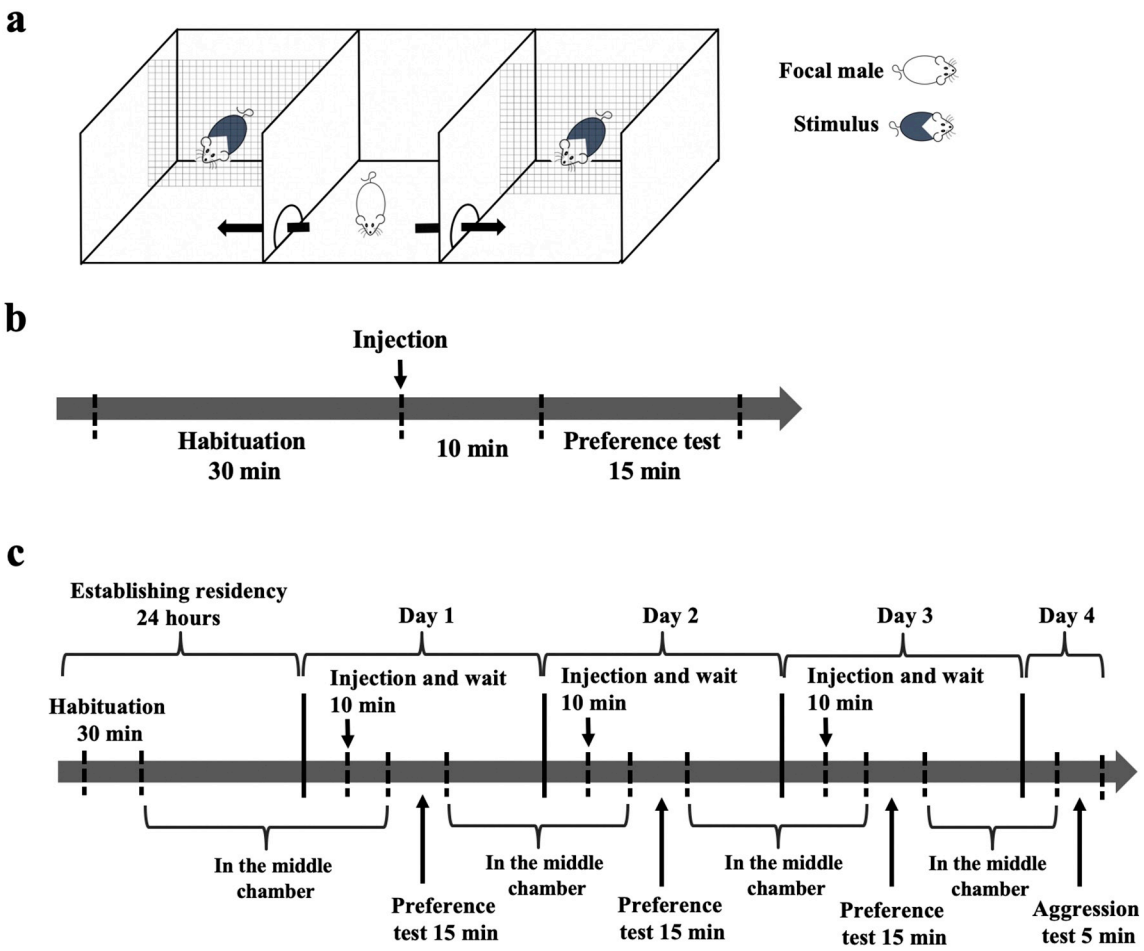

Fig. 1. Schematic of the experimental apparatus and workflow. (a) During the preference test, a focal male was allowed to explore all three chambers (see methods for description). Each side of the chamber housed either one stimulus male or one stimulus female. (b and c) Timeline of both Experiment 1 and Experiment 2.

but could not engage in physical interactions such as agonistic fighting or mating. Both the male and the female stimulus were age-matched non-siblings (separated by at least two generations) and none of them had been used in previous studies. Only females in estrus were used as stimuli, and we determined this by collecting vaginal secretions smeared onto a Superfrost Plus microscope slide and observe the cell types under a light microscope at 10×. Estrous state was identified by the predominant presence of cornified squamous epithelial cells in densely packed clusters (Byers et al., 2012). To avoid pre-existing side preferences, we counterbalanced the side of the apparatus in which the stimulus male was placed; in each treatment (T or saline) group, half of the male (or female) stimuli were placed in right side and the other half were placed in the left side.

All preference tests were conducted within 2 h after the lights off and recorded using Panasonic HD cameras on the low-light setting. Each test was run for 15 min after the sliding doors were opened. At the conclusion of each test, the focal male was gently guided back into the middle chamber and the doors were closed.

2.3. Experiment 2

2.3.1. Animals

We used 34 sexually naïve male *P. californicus* aged 6–12 months. Animals were randomly assigned to receive T or a saline vehicle as a control. Prior to testing, each animal was group-housed with two other males (cage dimensions: 48 × 27 × 16 cm) under a 10 L:14 D light cycle. Twenty-four hours before the two-choice test, the randomly selected focal males were moved to the middle chamber of the testing apparatus until the end of the aggression test on the fourth day. In the middle chamber, they were housed with one of their two former cage-mates to avoid isolation-induced stress. Shelter (a plastic tube), food (Purina 5001 mouse chow) and water were also provided. We have shown that > 24 h is sufficient to establish residency status in California mice (Bester-Meredith and Marler, 2001; Fuxjager et al., 2010; Oyegbile and Marler, 2005).

We selected stimulus females and males (including intruders) based on the same criteria as in Experiment 1 and the estrous cycle stage was assessed at least 4 h prior to testing on each day. Only females in estrus were used. Each intruder male was shaved prior to testing so that they could be distinguished from the focal male. Stimulus males and females were used up to three times, but never more than once in the same treatment group.

2.3.2. Procedure

We first habituated the focal males to the testing apparatus for 30 min. After this period, the focal male was gently guided into the middle chamber and the sliding doors were sealed. The cage-mate was then added to the middle chamber. The animals were left undisturbed for 24 h. The focal male and his cage-mate were housed in the middle chamber with a red plastic tube toy, and food and water ad libitum.

The procedure of injection (10 min) and two-choice test (15 min) were the same as in Experiment 1 except that we repeated the same procedure three times (once per day) on three consecutive days (Fig. 1c). On each testing day, the cage-mate was removed from the testing apparatus and placed in a small cage. All focal males received the same treatment and the same dosage on each of the testing days. We then waited 10 min before assessing rapid effects of T on behavior. The side placement of stimulus males and females was counterbalanced across groups.

At the conclusion of each test, the focal male was gently guided back into the middle chamber and the doors were closed. We then returned the cage-mate to the middle chamber, and removed the stimulus animals. We tested the animals only once per day, and tested each focal male in this way a total of three times.

2.3.3. Aggression tests

On the fourth day of testing, the focal males were subjected to a resident-intruder test as a proxy for territorial aggression. This test used only the middle chamber of the testing apparatus and the side chamber in which stimulus males had been presented to each focal male. The cage-mates were removed from the cage and the testing room before each trial as in the male-female two-choice tests described above. We allowed the focal male to freely explore the side chamber for 2 min. We then shut the door, and transferred a weight-matched intruder (± 3 g) (one per focal male) into the side chamber, and gave it 2 min to habituate to the side chamber. We then opened the sliding doors and recorded the next 5 min of interaction between the two males with Panasonic HD cameras. After 5 min had elapsed, the two animals were separated. After the end of aggressive tests, all males were returned to their original cages.

2.4. Data analysis

All videos were coded by an observer blind to both the treatment group of the animal (T or S) and the male/female arrangement of the side chambers in each test. In each male/female choice test, the amount of time the focal male spent in each chamber was recorded. The choice of approaching either the male or female stimulus was measured by a difference score (time spent in the male side minus time in the female side), a measure of approach preference that has been used in two-choice tests (Cummings et al., 2008; Henley et al., 2010). A positive difference score means more time was spent with the stimulus male, whereas a negative difference score indicates more time was spent with the stimulus female. The normality of the data was determined by the Shapiro-Wilk test. For Experiment 1, two-way ANOVA was used to analyze the interaction between treatment (saline vs. T) and pair-bonding status (sexually-naive vs. pair-bonded). If ANOVA revealed a significant interaction, an independent *t*-test was used to compare the two treatments (T vs. saline). For Experiment 2, the effect of interaction between trial and treatment was analyzed using two-way repeated measures ANOVA followed by pairwise comparisons. For aggression tests, the winner was defined as the individual who initiated at least three consecutive attacks that eliciting avoidance or freezing behavior of the intruder (Oyegbile and Marler, 2005). The effect of treatment on the probability of winning a contest was analyzed using a modified Fisher's exact test adjusted for data arranged in a 2 × 4 table (Freeman and Halton, 1951). The attack latency data were not normally distributed according to a Shapiro-Wilk test and therefore Spearman tests were used to analyze the correlation between attack latency and difference score. Three males in Experiment 1 and four males in Experiment 2 were excluded from analysis due to a complete absence of exploratory behavior in the two-choice test.

3. Results

3.1. Experiment 1

In Experiment 1, the two-way ANOVA yielded a significant interaction ($F_{(1,45)} = 4.152$, $p = 0.047$, eta-squared = 0.084) for the difference score, but no significant main effects of pair-bonding status ($F_{(1,45)} = 0.244$, $p = 0.624$, eta-squared = 0.005) and drug treatment ($F_{(1,45)} = 2.110$, $p = 0.153$, eta-squared = 0.045) (Fig. 2). Independent *t*-tests revealed that the difference score was significantly higher in T-treated sexually-naive group compared to saline-treated sexually-naive males ($t_{21} = 2.179$, $p = 0.041$, Cohen's d = 0.906); no such significant effects of treatment occurred in the paired groups ($t_{24} = 0.472$, $p = 0.641$, Cohen's d = 0.185) (Fig. 2). For the time spent in the chamber containing the male stimulus, the two-way ANOVA did not reveal any significant interaction (Table 1; $F_{(1,45)} = 1.395$, $p = 0.244$, eta-squared = 0.030) or main effects of either pair-bonding status (Table 1; $F_{(1,45)} < 0.0001$, $p = 0.989$, eta-squared = 0.000) or drug treatment (Table 1; $F_{(1,45)} = 1.380$, $p = 0.246$, eta-squared = 0.030). For the time spent in the female chamber, the two-way ANOVA yielded a significant interaction (Table 1; $F_{(1,45)} = 6.134$, $p = 0.017$, eta-squared = 0.120), but no significant main effects of pair-bonding status (Table 1; $F_{(1,45)} = 0.802$, $p = 0.375$, eta-squared = 0.018) or drug treatment (Table 1; $F_{(1,45)} = 1.987$, $p = 0.166$, eta-squared = 0.042). Independent t-tests revealed that, compared to saline-treated pair-bonded males, the saline-treated sexually-naive males spent significantly more time in the female side (Table 1; $t_{22} = 2.235$, $p = 0.036$, Cohen's d = 0.89); no such significant effect of experience was found in the T-treated groups (Table 1; $t_{21} = 1.197$, $p = 0.243$, Cohen's d = 0.47). Also, compared to saline-treated sexually-naive males, the T-treated sexually-naive males spent significantly less time in the female side (Table 1; $t_{21} = 2.30$, p = 0.03, Cohen's d = 0.95); no such significant effects of treatment occurred in the paired groups (Table 1; $t_{24} = 0.934$, $p = 0.359$, Cohen's d = 0.36). The above results suggest that sexually-naive males show an overall tendency to approach females in the absence of a T pulse; however, a T pulse appears to motivate sexually-naive males to allocate more of their time to the novel males, which is a typical behavioral pattern observed in all pair-bonded males. For the time spent in the middle chamber, the two-way ANOVA did not reveal any significant interaction (Table 1; $F_{(1,45)} = 1.556$, $p = 0.219$, eta-squared = 0.033) and main effects of pair-bonding status (Table 1; $F_{(1,45)} = 0.833$, $p = 0.366$, eta-squared = 0.018) and drug treatment (Table 1; $F_{(1,45)} = 0.022$, $p = 0.882$, eta-squared = 0.000).

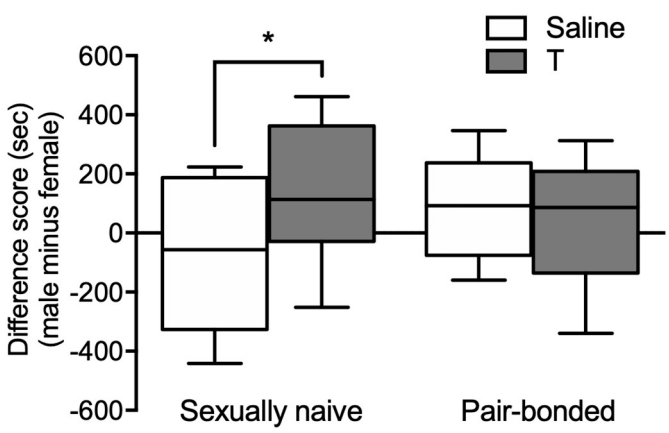

Fig. 2. Average difference scores (time in male side – time in female side) for sexually naive and pair-bonded males in the two-choice test. Horizontal axis represents animals that spend an equal amount of time in the male and female side, whereas positive scores represents male-biased side preference and negative scores represents female-biased side preference. * $p = 0.041$ independent *t*-test. n = 11–13 in each group. Data are presented as Mean ± SEM.

3.2. Experiment 2

Experiment 2 examined the consistency of the preference of choice and the correlation between the preference of choice and the aggressive behavior.

3.2.1. Two-choice test

Two-way repeated measures ANOVA on the difference score revealed a significant overall effect of trials (Trial 1 vs. 2 vs. 3) ($F_{(2,56)} = 4.489$, $p = 0.016$, eta-squared = 0.138), with no significant effect of interaction ($F_{(2,56)} = 0.583$, $p = 0.561$, eta-squared = 0.020) and treatment ($F_{(2,56)} = 0.257$, $p = 0.616$, eta-squared = 0.009). This suggests that the time allocation to the male and female chambers is not consistent across trials. Pairwise comparisons showed that the

Table 1
Time spend in the three chambers in sexually naïve and pair-bonded males that received either saline or T.

	Sexually naïve		Pair-bonded	
	Saline	T	Saline	T
Time in female side (s)[a]	398.82 ± 43.83*†	271.92 ± 34.45	288.69 ± 26.17	323.54 ± 26.57
Time in male side (s)[NS]	329.91 ± 33.35	412.75 ± 36.66	371.92 ± 33.31	371.69 ± 36.31
Time in middle chamber (s)	171.27 ± 23.46	215.33 ± 34.62	239.38 ± 34.64	204.76 ± 31.34

Data are mean ± SE.
[NS] No significant effects on behavior.
[a] Significant interaction (pair-bonding status × drug treatment) by the two-way ANOVA ($p < 0.05$).
* Significant effect compared to pair-bonded saline group by independent *t*-test ($p < 0.05$).
† Significant effect compared to sexually-naïve T group by independent t-test ($p < 0.05$).

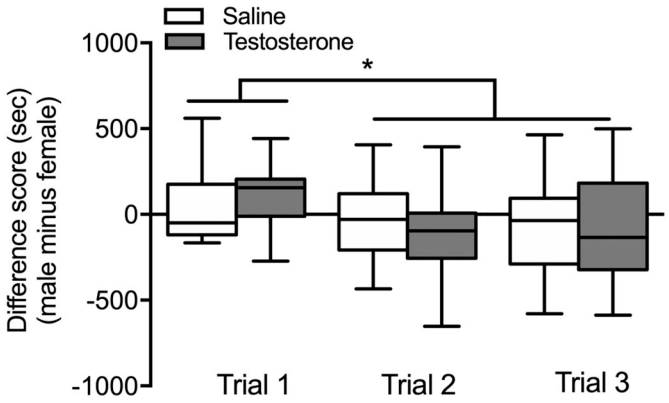

Fig. 3. Average difference scores for sexually naive males. Horizontal axis represents animals that spend an equal amount of time in the male and female side, whereas positive scores represents male-biased side preference and negative scores represents female-biased side preference. * $p = 0.016$ significant effects of two-way repeated measures ANOVA. Data are presented as Mean ± SEM.

Table 2
Time spend in the female and male sides, and the middle chamber across the three trials in saline and T groups.

		Saline	T
Time in female side (s)	Trial 1[a]	315.02 ± 29.70	304.77 ± 29.04
	Trial 2[b]	388.55 ± 37.08	442.96 ± 38.94
	Trial 3[b]	402.36 ± 43.39	430.12 ± 44.67
Time in male side (s)[NS]	Trial 1	377.30 ± 43.96	403.74 ± 28.01
	Trial 2	348.00 ± 32.17	302.47 ± 38.39
	Trial 3	344.51 ± 42.69	336.76 ± 40.74
Time in middle chamber (s)	Trial 1[a]	207.68 ± 40.55	191.48 ± 25.59
	Trial 2[ab]	163.44 ± 32.14	154.56 ± 29.45
	Trial 3[b]	153.12 ± 32.72	133.12 ± 22.06

Data are mean ± SE.
Different letters (a and b) represent significant difference ($p < 0.05$) by the pairwise comparison.

difference score of Trial 1 was significantly higher than that of both Trial 2 ($p = 0.012$) and 3 ($p = 0.029$), suggesting animals decreased the preference to the male side across the trials; no significant difference between Trial 2 and 3 was found ($p = 0.791$). Also, in the sexually naïve males that had established residency, T did not influence the approach preference towards males in each trial (Fig. 3).

For the time spent in the chamber containing the female stimulus, the two-way repeated measures ANOVA revealed a significant overall effect of trials (Trial 1 vs. 2 vs. 3) (Table 2; $F_{(2,56)} = 7.243$, $p = 0.002$, eta-squared = 0.206), with no significant effect of interaction (Table 2; $F_{(2,56)} = 0.509$, $p = 0.604$, eta-squared = 0.018) and treatment (Table 2; $F_{(2,56)} = 0.396$, $p = 0.534$, eta-squared = 0.014). Pairwise comparisons showed that the time spent approaching the female stimulus in Trial 1 is significantly lower than that of Trial 2 (Table 2; $p = 0.003$, Cohen's d = 0.809) and 3 (Table 2; $p = 0.004$, Cohen's d = 0.744), suggesting animals increased the preference to the female side across the trials; no significant difference between Trial 2 and 3 was found (Table 2; p = 0.791, Cohen's d = 0.003). For the time spent in the chamber containing the male stimulus, the two-way repeated measures ANOVA did not reveal any significant interaction (Table 2; $F_{(2,56)} = 0.549$, $p = 0.581$, eta-squared = 0.019) and main effects of trials (Table 2; $F_{(2,56)} = 1.971$, $p = 0.149$, eta-squared = 0.066) and drug treatment (Table 2; $F_{(2,56)} = 0.060$, $p = 0.808$, eta-squared = 0.002). For the time spent in the middle chamber, the two-way repeated measures ANOVA revealed a significant overall effect of trials (Trial 1 vs. 2 vs. 3) (Table 2; $F_{(2,56)} = 3.929$, $p = 0.025$, eta-squared = 0.123), with no significant effect of interaction (Table 2; $F_{(2,56)} = 0.037$, $p = 0.964$, eta-squared = 0.001) and treatment (Table 2; $F_{(2,56)} = 0.168$, $p = 0.685$, eta-squared = 0.006).

Pairwise comparisons showed that the time spent in the middle chamber in Trial 1 is significantly lower than that of Trial 3 (Table 2; $p = 0.006$, Cohen's d = 0.476). There was no significant difference between Trial 1 and 2 (Table 2; $p = 0.081$, Cohen's d = 0.328), and between Trial 2 and 3 (Table 2; $p = 0.450$, Cohen's d = 0.141).

3.2.2. Aggression tests

According to Fisher's Exact Test, the probability that sexually naïve males won their aggressive encounter did not differ between saline and T groups ($\chi^2 = 2.143$, df = 1, $p < 0.05$, Cramer's V = 0.267). However, based on the treatment (saline or T) and preference of choice (spending more time in male or female side), animals were further sorted into four groups: saline + female (S + F), saline + male

Table 3
Difference score (s) for males that prefer either male or female in the saline and T groups.

	Saline		T	
	Prefer male	Prefer female	Prefer male	Prefer female
Trial 1	299.28 ± 93.03 ($n = 6$)	−95.722 ± 14.70 ($n = 9$)	192.42 ± 34.71 ($n = 11$)	−158.03 ± 66.68 ($n = 4$)
Trial 2	213.00 ± 69.51 ($n = 5$)	−167.32 ± 49.07 ($n = 10$)	163.20 ± 94.00 (n = 4)	−250.92 ± 65.01 ($n = 11$)
Trial 3	227.83 ± 76.36 ($n = 6$)	−248.31 ± 68.73 (n = 9)	229.62 ± 66.16 (n = 6)	−308.68 ± 59.94 (n = 9)

Data are mean ± SE.

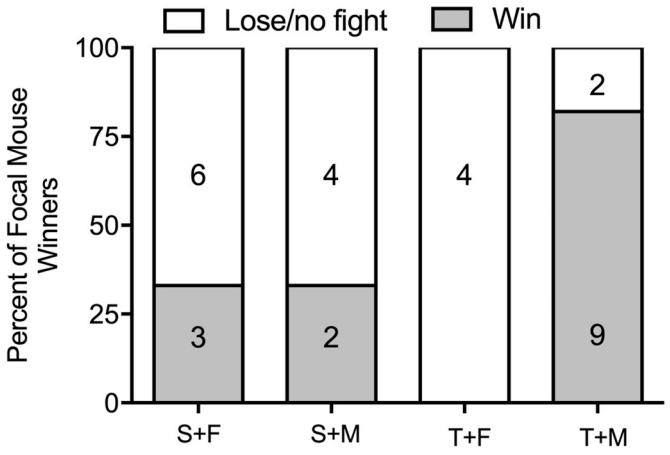

Fig. 4. Percent of focal mouse winners from the saline (S) or testosterone (T) groups that either preferred females (F) or males (MS) in Trail 1. Accordingly, individuals in the S + F group were treated with S and preferred to associate with F, and so on. Numbers in the bars represent the number of individuals that won or lose/no fight in the agonistic encounters.

(S + M), T + female (T + F), T + male (T + M) (Table 3). We found that only in Trial 1, the percentage of mice that won the test encounter was significantly different among these four groups ($\chi^2 = 9.579$, df = 3, $p = 0.021$, Cramer's V = 0.596), with the T + M group obtaining significantly higher proportion of wins than the other three groups (Fig. 4).

For males that received T (combined T + M and T + F), we found that attack latency during the aggressive trial was negatively correlated with the difference scores in Trial 1 ($r = -0.79$, $P = 0.001$, $n = 15$), but not with difference scores in Trials 2 or 3 ($p > 0.05$) (Fig. 5). Also, we found no correlation between attack latency and difference scores in the saline group ($p > 0.05$). The results suggest that the persistency of approaching the male stimulus in Trial 1 may predict the motivation of aggression of sexually naïve mice that have become residents, but is not revealed without exposure to T.

4. Discussion

To our knowledge, this study is the first to show that T pulses can rapidly mediate social decision-making processes, but in a way that hinges on an individual's pair-bonding status. We found, for example, that sexually naïve male California mice normally preferred to associate with receptive females. However, a single pulse of T (similar to ones that occur in response to social stimuli) reversed this effect, causing males to instead associate with potential competitors. We also found that social decision making under the challenge/opportunity choice tests relied on an interaction between the T pulse and pair-bonding status. Compared to the effects in sexually naïve males, T injections did not further enhance the approach preference to the male side in pair-bonded males. To this end, we showed that when a resident male was repeatedly exposed to challenge/opportunity situations, the approach preference in Trial 1 (and not Trials 2 and 3) may indicate the internal motivation of coping with a challenge, which is consistent with the notion that the time spent near the stimulus male correlates positively with the levels of aggression in the subsequent physical confrontations (Kovalenko and Kudryavtseva, 2016; Kudryavtseva, 2003; Kudryavtseva et al., 2004). Importantly, this motivation in the current paradigm is only revealed in the presence of a T pulse (i.e., in mice that preferred males and also received T); this was manifested in the later aggressive behavior towards the male intruder. Furthermore, multiple exposures to the challenge/opportunity situation appeared to cause the adjustment of decision-making when exposed to the same situation. We speculate that the resident male could assess that his male "competitor" was less of a threat because the wire mesh physically separated the two individuals. Thus, the resident male could allocate more time to the opportunity situation.

The rapid effects of T on the decision making may be a hormonal strategy to help animals cope with immediate threats (Nyby, 2008). The approach preference to receptive females in sexually-naive males may reflect the tendency of seeking reproductive opportunities, whereas T rapidly changes the goal and redirects decision-making to motivate males to focus on the potential threat of the same-sex conspecifics within 25 min after the injection. We speculate that the rapid effects of a T pulse may support the endogenous mechanisms that underlie sexually-naive males' drive to begin establishing a territory. We previously demonstrated the rewarding effects of T pulses by showing that sexually-naive males can form conditioned place preferences to the environment wherein they received T injections (Zhao and Marler, 2014, 2016). Such preferences to a specific environment may contribute to the formation of a territory, and the transient T pulse may initiate this process particularly when in the presence of a same-sex intruder. Several nodes of the social decision-making network such as the bed nucleus of the stria terminalis, preoptic area, lateral septum, hippocampus and medial amygdala are embedded with high levels of steroid hormone receptors and are subject to the modulation of androgens (O'Connell and Hofmann, 2012; Oliveira and Oliveira, 2014). The cellular mechanism underlying T's rapid effects on social decision-making is still unclear, but through conversion to 17β-estradiol or 3α-androstanediol, T can rapidly modulate reproductive behaviors in Japanese quail (*Coturnix coturnix*) (Cornil et al., 2006) and rats (*Rattus norvegicus*) (Frye, 2001). In cichlid fish (*Astatotilapia burtoni*), aggressive behaviors can be facilitated by the aromatase activity in the preoptic area (Huffman et al., 2013). We suggest a similar mechanism of converting T to estrogens or 3α-androstanediol may also underlie its effects on social decision-making in the challenge/opportunity situations. Alternatively, the winner effect appears to involve experience-induced changes in aggression that seem to function through androgen and not estrogen receptors (Trainor et al., 2004).

Variation in the social decision-making between the sexually naïve and pair-bonded males may reflect different life history stages. In the wild, sexually naïve males that disperse from the natal area are motivated to explore novel environments and establish their own territories (Ribble, 1992), and conditioned place preferences induced by T pulses have the potential of facilitating territorial establishment (Zhao and

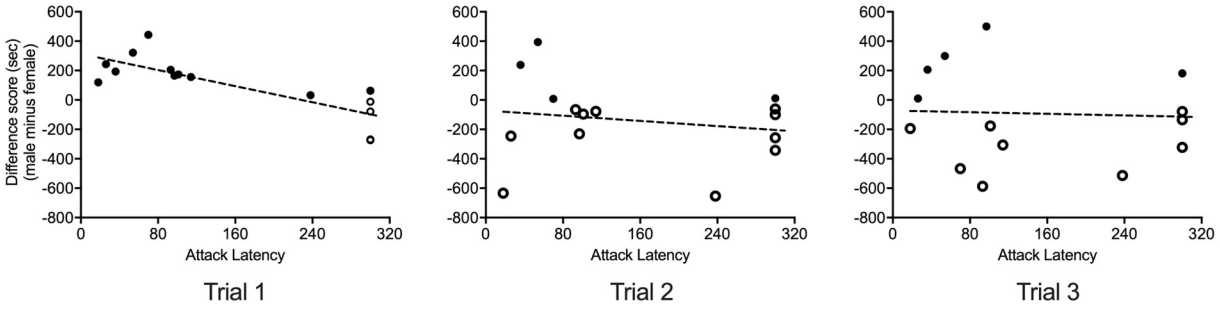

Fig. 5. Correlation of attack latency during the resident-intruder test and difference score (sec) in animals that received T. Filler (black) circles represent individuals that preferred males in Trial 1–3, whereas open circles represent individuals that prefer females in Trial 1–3.

Marler, 2016). In the current study, T pulses may further contribute to behavioral changes during the initiation of territoriality by rapidly altering the social decision-making of the sexually naïve males to approach unfamiliar males. Compared to sexually-naive males, pair-bonded males decreased time spent on the unfamiliar female's side, which may be because the pair-bonded males already have long term mates. In a study by Ribble (1991) conducted in the field, no extra-pair mating was detected in California mice. In the laboratory, male sexual fidelity in male California mice appears to be more self-imposed by the male mice who do not copulate with the estrous females when given the opportunity regardless of whether their partner is present or not (Gubernick and Nordby, 1993). Mechanisms for sexual fidelity in the male are further supported by the inhibitory effect of a T pulse on rapid vocal responses of a paired male to an unfamiliar male (Pultorak et al., 2015).

Although the effect of residency is not directly examined in the current study, some evidence hints that the effects of T pulses on decision making differed between the Experiment 1 and Trial 1 of Experiment 2 might be accounted for by the residency status of the sexually naïve males. The significant rapid effect of T is only displayed in sexually naïve males from Experiment 1, which is conducted in an environment in which residency has not been established. As discussed above, we speculate that the rapid effect of T in sexually naïve males may mainly function to initiate territory establishment but not to maintain the territory. In such a scenario, the T pulse would not further enhance the approach preference to males in Experiment 2. We previously demonstrated that in sexually naïve males, the T-induced conditioned place preference (CPP) was only produced in unfamiliar environments, and not in conditions where males had established residency (thereby mimicking territoriality); while in pair-bonded males, the T-induced CPP was only produced at home but not in an unfamiliar environment (Zhao and Marler, 2014, 2016). Thus, compared to unfamiliar environments, we speculate that home is a more salient environment to pair-bonded males and if we allowed pair-bonded males to become residents, the T pulse may have a more significant effect on the approach preference to a male intruder, which may function to enhance territorial defense.

Neither the approach preference to the male nor T's rapid effects alone predict the level of future aggression towards the novel male intruder. When the animals were repeatedly exposed to the challenge/opportunity situations, the decision-making in Trial 1, but not Trial 2 and 3 predicted the aggressive motivation but this effect relied on the actions of T. We speculate that the approach preference to the male stimulus in Trial 1 may reflect the variability in aggressive motivation that is related to the way individual males react to environmental challenges (Koolhaas et al., 1999). Such variation in aggression may be achieved, at least partially, through hormonal mechanisms. For example, previous studies showed that the prenatally circulating T levels (Compaan et al., 1994) and T secreting capacity of the testes (De Ruiter et al., 1993) are greater in aggressive (short attack latency) than in less-aggressive (long attack latency) males. Expanding upon these previous findings, our results reveal that the rapid effects of T may facilitate the manifestation of the individual variation in the aggressive propensity. For the males that received T injections but preferred the female side, their aggressive motivation might be lower and therefore the time spent on the male side does not predict later aggression. The direct evidence for the mechanisms underlying the variation of the approach preference is currently lacking. Previous studies in birds and mice demonstrated that the expression of androgen and estrogen alpha-receptors (Rosvall et al., 2012; Sperry et al., 2010; Trainor et al., 2006) and the aromatase activity (Compaan et al., 1994) in distinct brain areas may explain the variation in aggression. The similar plasticity in the levels of enzyme and receptor may also account for the difference in the preference to the male stimulus; the more time spent in the male side might indicate the higher levels of enzymes and receptors for T to act on and therefore behave more aggressively after repeated T injections. Also, the repeated T injections may also allow animals to form a preference to the chamber which they preferred. In that same chamber (male side), males later behave more aggressively.

The effect of T on decision-making is not only influenced by males' mating status, but also the previous experience of being exposed to the challenge/opportunity situations. We can only speculate that such plasticity in social decision-making echoes the “dear enemy” hypothesis, which posits that a resident's motivation to defend against a threatening individual decreases when the opponent is a “neighbor” (Getty, 1987; Temeles, 1994). In Experiment 2, animals were housed in the middle chamber of the apparatus for 24 h before Trial 1. During the 30-min exploration in Trial 1, the focal males may have assessed that the threat of the male intruder was limited because of being restricted to part of a side chamber via a wire mesh. Therefore, the time spent in the male side did not change. Meanwhile, focal males allocated more time in the female side, which is likely accounted for by a decrease in the time allocation in the middle chamber. Although the focal males in the current study did not engage in any sexual behaviors, previous studies in mice and hamsters have shown that female chemosensory stimuli are a natural reward and can induce a preference to the location associated with the females (Bell et al., 2010; Pankevich et al., 2006). Such plasticity in the decision-making may be an evolved mechanism that could help territorial animals minimize the energy expended on aggression towards the secured neighboring territories (Ydenberg et al., 1988). According to the “dear enemy” hypothesis, a resident should invest more in defending against a more threatening individual that is capable of inflicting greater losses on the resident (Getty, 1987) while the energy spent dealing with the challenge will be reduced when the threat posed by the “neighbors” is low (Temeles, 1994). Such a scenario could be tested by comparing resident's behavioral responses to an activity-restricted versus non-restricted intruder.

Altogether, the current study reveals that the T pulses, that naturally occur following male-male agonistic encounters or male-female sexual encounters (Gleason and Marler, 2010), can rapidly affect animal's social decision-making in response to challenge/opportunity situations. Moreover, we find that this response varies depending on whether males are sexually naïve or pair bonded. We also have evidence that indirectly suggests that residency status may influence T's rapid effects on social decision-making, at least in sexually naïve males. Finally, plasticity in the social decision-making process may also occur when males are repeatedly exposed to the same challenge/opportunity situation, possibly by assessing the limited threat of an intruder introduced behind a wire mesh barrier. All these findings depict a complex interplay between hormonal, environmental and prior social experience, which may act as a mechanism for animals to cope with the immediate situation following the agonistic or sexual encounters in nature.

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