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Testosterone facilitates nonreproductive, context-appropriate pro- and anti-social behavior in female and male Mongolian gerbils

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ABSTRACT

A growing body of literature suggests that testosterone (T) rapidly modulates behavior in a context-specific manner. However, the timescales in which T can rapidly mediate distinct types of behavior, such as pro- vs. anti- social responses, has not been studied. Thus, here we examined acute T influences on social behavior in male and female Mongolian gerbils in nonreproductive contexts. Females and males received an injection of either saline or T and were first tested in a social interaction test with a same-sex, familiar peer. 5 min after the peer interaction, subjects then underwent a resident-intruder test with a novel, same-sex conspecific. After another 5 min, gerbils were tested in a novel object task to test context-specificity (i.e., social vs. nonsocial) of T effects on behavior. Within 1 h, males and females injected with T exhibited more huddling with a peer but more active avoidance of and less time spent in proximity of an intruder than did animals injected with saline. T effects on behavior were specific to social contexts, such that T did not influence investigation of the novel object. Together these findings show that T rapidly promotes pro-social responses to a familiar peer and anti-social responses to an intruder in the same individuals within 5 min of experiencing these disparate social contexts. This demonstrates that T rapidly facilitates behavior in a context-appropriate manner outside the context of reproduction and reveals that rapid effects of T on behavior are not restricted to males.

1. Introduction

Many animals live in dynamic social environments that require adapting one's behavior to best fit a particular social context. Indeed, the ability to rapidly modulate behavior in a context-appropriate manner is crucial to the survival and fitness of most organisms (Kelly and Vitousek, 2017). Candidate mechanisms for understanding the regulation of rapid behavior are steroids, which can be peripherally derived or produced in the brain. Steroids act through slow, genomic (transcription dependent) and rapid, non-genomic (transcription independent) receptor mechanisms to modulate cellular processes that likewise influence multiple physiological and behavioral responses (Simon, 2002; Frye, 2009).

Transient increases in testosterone (T) in response to social, particularly aggressive, stimuli were originally proposed to modulate dynamic tradeoffs between parental and aggressive/competitive efforts (Wingfield et al., 1990); indeed, several studies have demonstrated that T can rapidly increase aggression (Beeman, 1947; Carre and Olmstead, 2015; Carre et al., 2017). Since then, in recognition of the complex patterns of T pulses observed across species and social/reproductive contexts (Goymann et al., 2019; Wingfield et al., 2019), as well as causal studies demonstrating diverse behavioral effects of rapid increases in T, the function of such pulses have been expanded to include the promotion of mating behaviors and physiological processes that likely promote fertilization success in competitive mating contexts (Nyby, 2008; Thompson and Mangiamele, 2018), as well as priming animals to win future fights and to establish place preferences related to mating/reproduction (Marler and Trainor, 2020). Further, recent studies have also begun to highlight even more complex roles of T pulses on behavior. Whereas T typically promotes sexual responses, it can rapidly suppress sexual communication with a novel female in pairbonded, but not unpaired, male California mice, possibly to promote mate fidelity (Pultorak et al., 2015); this finding illustrates that T can suppress sexual responses if they are not appropriate in a particular context. Additionally, in sexually naïve California mice, the preference to approach a novel female over a novel male reverses 10 min after a T injection, suggesting that a T surge may facilitate an animal's decision to rise to a challenge; notably, this effect was not observed in pairbonded males, demonstrating that T does not uniformly facilitate competitive behavior (Zhao

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et al., 2019). Further, studies in humans suggest that T may not only promote aggressive responses, but also foster pro-social behaviors, particularly when there is an advantage of gaining social status. For example, T administration increases altruistic behavior when subjects are being watched (known as the audience effect) (Wu et al., 2020), and although not a demonstration of rapid effects of T on behavior, high levels of T are associated with increased ingroup cooperation during intergroup competition (Reimers and Diekhof, 2015). Thus, T has been shown to promote pro-social as well as aggressive/anti-social responses, though our recent study in male Mongolian gerbils (*Meriones unguiculatus*) was the first to demonstrate that T can rapidly promote such divergent responses in the same individuals as a function of social context. Specifically, we demonstrated that T rapidly increases nonsexual, pro-social behaviors during social interactions with a pair-bond partner but decreases pro-social behavior during interactions with a novel, same-sex intruder (Kelly et al., 2022).

Although rapid effects of T have been widely studied in the context of reproduction, their influences on behavior in nonreproductive contexts have been less well explored. Such influences could be a by-product of surges induced by recently encountered sexual/competitive stimuli, though still functionally meaningful (e.g., increased huddling with a sibling inside the nest/home after an encounter with a rival outside the nest/home that elevated T), or a function of surges induced by stimuli that are not sexual nor direct challenges to dominance/a territory/access to mates. For example, skydiving induces T surges in men and women (White et al., 2019), as does being part of a successful cooperative hunt, even in those who do not make the kill, among male hunter gatherers (Trumble et al., 2014). The latter effect is not modified by an audience, thereby suggesting the surge is not functionally related to sexual signaling but rather to provisioning associated with the kill. Such stimuli, like those associated with the physical and psychological competitions associated with sport and gaming now widely recognized for their ability to induce T surges, may be indirectly, if not directly, related to reproductive success/dominance. However, they are nonetheless more closely linked to the autonomic arousal and stress associated with psychological challenge, especially if perceived positively, and they are potentially generated by adrenal rather than gonadal output. T surges are therefore likely to modulate a variety of behaviors above-and-beyond those directly related to reproduction, especially given the reinforcing nature of T surges (Johnson and Wood, 2001). Thus, it is possible that they can enhance appetitive responses toward whatever environmental stimuli are present during the surges, even if those stimuli did not trigger them.

Further, while we know a great deal about rapid effects of T on behaviors related to reproduction in males, we know virtually nothing about how fluctuations of T might regulate female social behaviors except that T has rapid effects on reward processes in females like those in males, at least in rodents (Triemstra and Wood, 2004). Our limited knowledge of how T fluctuations may regulate female social behavior aside, we do know that T can rapidly fluctuate in response to social stimuli in females, particularly in our own species. Not only are there elevations of T in contexts associated with dominance/aggression in women (van Anders et al., 2015) but also in response to participation in, and especially winning, competitive sports (Oliveira et al., 2009; Edwards and Casto, 2015; Casto et al., 2017), as well as in the non-social, autonomically arousing context of skydiving, as discussed above, when responses in women are as large as those of men (White et al., 2019). Although we are unaware of studies demonstrating socially-driven surges of T in non-human female mammals, a related androgen in fish, 11-ketotestosterone (11-KT), does increase in circulation in a sex-changing fish (*Ballan wrasse*) in response to changes in social structure (typically the death of a male on a territory) that drive the transition from female to male (Muncaster et al., 2013). Interestingly, gonads do not appear necessary for the rapid behavioral changes that accompany sex change in the related blue-headed wrasse (Godwin et al., 1996). However, 11-KT increases in the brain, but not the periphery, in female gobies induced to change sex by altering the social environment, and those elevations are at least correlated with behavioral changes (Lorenzi et al., 2012). Similarly, recent work in female birds has demonstrated that T fluctuates rapidly in the brain in response to socially salient stimuli (de Bournonville et al., 2020). Together, these studies suggest that socially-driven changes in androgens in females, be they peripheral or central, may be more widespread across vertebrates than previously thought, and thus it is possible that androgens may have the ability to rapidly influence behavior as well.

To determine if T influences context-appropriate behavior in nonreproductive contexts, as well as in females, we used Mongolian gerbils (henceforth referred to as gerbils) to examine the rapid effects of exogenous T on pro- and anti-social behaviors during interactions with a familiar, same-sex peer and a novel, same-sex conspecific (i.e., intruder). We chose gerbils because they are a socially monogamous species that live in extended family groups and are highly affiliative with mates, offspring, and siblings but are aggressive toward novel, same-sex individuals (Roper and Polioudakis, 1977; Gromov, 2008; Liu et al., 2009; Pan et al., 2020; Gonzalez Abreu et al., 2022). This behavioral phenotype allows us to examine behavioral flexibility outside the context of reproduction in presumably positive (interacting with a familiar peer) and presumably negative (interacting with an intruder) contexts. We injected adult male and female gerbils with either saline or T to adult male and female gerbils and tested subjects in back-to-back social interaction tests, first with a same-sex familiar peer and then with a same-sex intruder. To determine if T rapidly influences nonsocial behavior, animals were then exposed to a novel object after the social interaction tests. If T rapidly facilitates context-appropriate behavior, we hypothesized that gerbils that received a T injection would exhibit heightened pro-social behavior with a peer and heightened anti-social behavior with an intruder. Alternatively, if T's rapid behavioral effects are selectively related to the context in which the T surge is initiated, then heightened pro-social responses with a familiar peer would carry over to the resident-intruder test, such that T-injected animals would behave pro-socially with an intruder. If T's behavioral effects are not selectively related to social contexts, we predicted it would also influence the time spent investigating a novel object.

2. Methods

2.1. Animals

All Mongolian gerbil subjects were offspring of breeders in our colony at Emory University. Breeders were obtained as young adults (postnatal day (PND) 50–65) from Charles River Laboratories. At testing, subjects were aged PND 75–110 and sexually naive. Gerbils were group housed (2–4) with same-sex littermates in standard rat polycarbonate cages (40.64 cm × 20.32 cm × 20.32 cm) prior to housing in same-sex sibling pairs. Subjects were co-housed with just one same-sex sibling for 3 weeks prior to the start of testing. All cages were lined with Sani-Chips bedding and included nesting material, chewing blocks, and shepherd shacks. Animals were able to obtain food and water ad libitum and were kept on a 14-h light: 10-h dark cycle. Ambient temperatures were maintained at 24 ± 2 °C. Gerbils are diurnally active and were tested during the day (Refinetti and Kenagy, 2018). All procedures were approved by the Institutional Animal Care and Use Committee of Emory University (Protocol 202,000,131).

2.2. Experimental design

Prior to experimental testing, all subjects ($n = 8$ males and 8 females) were tested in a peer preference test to confirm that sexually naive adult male and female gerbils form a bond with their same-sex cagemate/sibling. The following week subjects were randomly assigned to either a Saline group or a Testosterone group. We previously demonstrated that behavior in gerbils significantly changes 20 min after injection of a

physiologically-relevant dose of exogenous T (Kelly et al., 2022). Therefore, 20 min after injections or either saline or T, gerbils were first tested in a peer interaction test in their homecage (a standard rat cage) and freely interacted for 10 min. The peer was then removed from the homecage and the subject rested for 5 min. A novel, same-sex intruder (age- and weight-matched) was then placed into the homecage for a resident-intruder test for 10 min. The intruder was then removed, and the subject was allowed to rest for an additional 5 min. Finally, a novel object (a rubber duck) was placed into the homecage for 5 min to test context-specificity (i.e., social vs. nonsocial) of T effects on behavior. All behavioral tests occurred within 1 h of IP injection of saline or T.

2.3. Intraperitoneal injections

T (Steraloids) was dissolved in beta-cyclodextrin (Sigma Aldrich) and diluted to 50μg/kg (0.004 % beta-cyclodextrin) in sterile isotonic saline and injected IP. This dose of T elevates T within the range of values previously reported in adult gerbils (Pina-Andrade et al., 2020; Kelly et al., 2022). T in beta-cyclodextrin quickly delivers T to the bloodstream for rapid metabolization, resulting in circulating T that resembles natural T pulses (Taylor et al., 1989; Fuxjager et al., 2011). The amount of beta-cylcodextrin diluted in sterile saline for T injections was added to sterile saline (without T) for saline injections so that Saline and T Group animals were both exposed to equivalent amounts of beta-cyclodextrin.

2.4. Peer preference test

The peer preference test was conducted in a three-chamber testing apparatus (76.2 cm × 25.4 cm × 30.48 cm) that contained their same-sex cagemate/sibling (i.e., familiar peer) tethered in one end chamber and a novel, opposite-sex conspecific tethered in the other end chamber. Stimulus animals were allowed to acclimate to tethering for 20 min prior to the start of testing. The chamber location for the familiar peer and novel conspecific were counterbalanced across subjects. After the stimulus acclimation period, subjects were transferred via a plastic beaker to the center chamber and were allowed to freely explore the entire apparatus for 3 h. The time spent in the middle room, novel conspecific room, and familiar peer room was scored as well as the amount of time spent huddling and exhibiting aggressive behavior with either the familiar peer or novel conspecific using Behavioral Observation Research Interactive Software (BORIS; Friard & Gamba, 2016). No subjects exhibited aggression, and therefore data are not presented for this behavior.

2.5. Peer interaction test

At the beginning of testing, food and water were temporarily removed from the homecage. Subjects were then administered an IP injection of either saline or T and were then immediately placed back in the homecage with their same-sex sibling/cagemate (i.e., familiar peer). Subjects and their familiar peer were video recorded for 30 min, and the last 10 min (i.e., corresponds to 20 min after IP injection when T actions are expected to take effect) of videos were scored for the time spent huddling, investigating (nose-to-body contact and following), and exhibiting overt aggression (chasing, biting, lunging, pinning) using BORIS. No aggressive behavior was observed in any peer interaction test, and thus no data on aggression is reported.

2.6. Resident-intruder test

The resident-intruder test occurred 5 min after the peer interaction test. Same-sex cagemate/siblings were temporarily removed from the homecage and placed in holding cages immediately after the peer interaction test. A novel, age- and size- matched, same-sex conspecific (i.e., an intruder) was then placed into the subject's homecage and behavior was video recorded for 10 min. Intruders were group housed in same-sex sibling groups and removed directly from their homecage; intruders were allowed 3 h to rest before being used a second time as a stimulus animal. Subjects and intruders were video recorded for 10 min and videos were scored for the time spent near the conspecific (i.e., within two body lengths), investigative behavior, active avoidance (i.e., running away from the approaching intruder), and overt aggression (chasing, biting, lunging, pinning) using BORIS.

2.7. Novel object test

The novel object test occurred 5 min after the resident-intruder test. The intruder was removed from the homecage and after 5 min the subject was temporarily placed under a plastic beaker on one side of the cage. A rubber duck was then placed on the opposite side of the cage; the subject was then released from underneath the beaker and the latency to approach the rubber duck was scored as well as the time spent investigating (i.e., nose, mouth, or front paws in contact with the object) the rubber duck during a 5 min test. Thus, all testing was completed within 1 h of injections, a time-course generally consistent with rapid, non-genomic steroid mechanisms, especially for effects of systemic injections on behavior (Cornil et al., 2006a), as well as within a window in which levels of T remain elevated following intraperitoneal injections, at least in rats (Sodersten et al., 1980). However, it should be noted that levels begin decreasing after 30 min, so if rapid behavioral effects depend upon increasing levels of T at the time of testing, rather than elevations above baseline, then we should only see effects on behavior in the first peer interaction test and not on behavior in the resident-intruder or novel object test.

2.8. Statistical analysis

Behavioral data from the peer preference test were analyzed using general linear models (GLMs) with sex and stimulus type (familiar peer or novel, same-sex conspecific) as fixed factors. For the peer interaction, resident-intruder, and novel object tests, behavioral data were analyzed using GLMs with sex and treatment (T or saline) as fixed factors. Effect sizes for GLMs are reported as partial eta squared. All posthoc pairwise comparisons were adjusted using the Sidak correction with effect sizes reported as Cohen's d. All data were analyzed using SPSS 28 (IBM Analytics, USA) and graphs were made using Prism 8 (GraphPad, USA).

3. Results

3.1. Virgin female and male adult gerbils form a bond with familiar peers

To determine whether male and female Mongolian gerbils form nonsexual bonds with adult peers, we conducted a peer preference test that modeled the 'partner preference test' that is commonly used for assessing sexual pair bonds between males and females of socially monogamous species (Beery, 2021). We examined the preference for female and male gerbils to spend time with and huddle with their same-sex, familiar peer (i.e., their cagemate/sibling) or a novel, same-sex conspecific in a 3 h test (Fig. 1A). A GLM with sex and stimulus type (familiar peer or novel, same-sex conspecific) yielded a main effect of stimulus type for the percentage of test time spent in stimulus chambers ($F_{(1,28)} = 28.03$; $P < 0.01$; partial eta squared = 0.49; Fig. 1B), and showed that both males and females spent a significantly greater percentage of the test in the chamber that contained their familiar peer. Similarly, GLM analyses revealed a main effect of stimulus type for huddling ($F_{(1,28)} = 16.37$; $P < 0.01$; partial eta squared = 0.35; Fig. 1C), such that both sexes spent a greater percentage of the test huddling with their familiar peer compared to the novel, same-sex conspecific. We observed no effects of sex or interactions between sex and stimulus type (all $P > 0.21$). This confirms that adult gerbils form nonreproductive bonds with familiar adult peers.

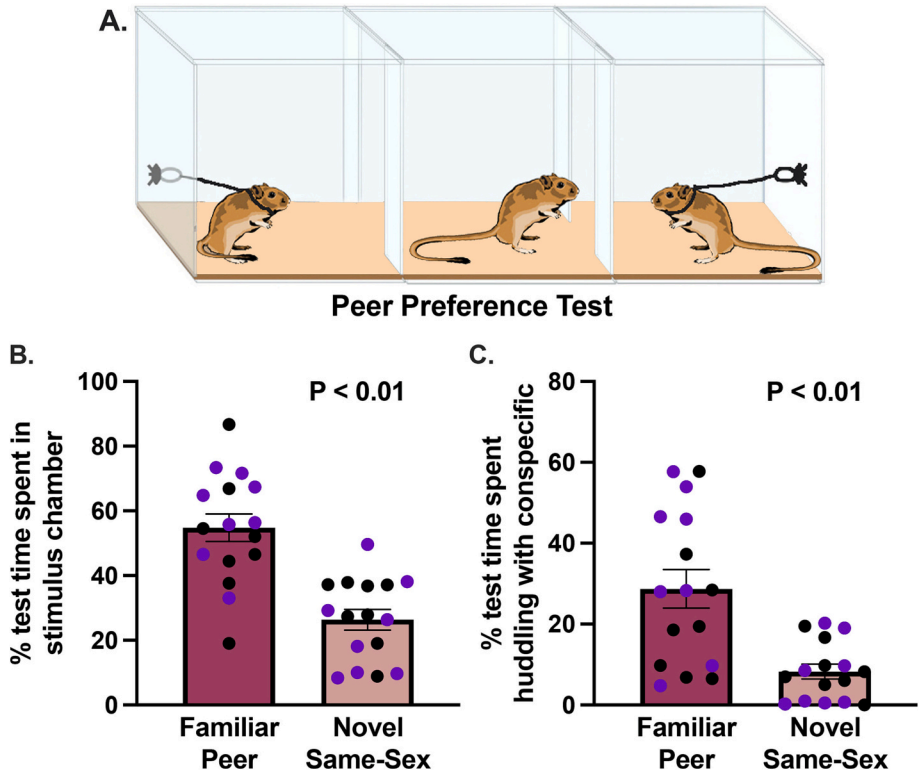

Fig. 1. (A) Peer preference test setup. Female (black dots) and male (purple dots) gerbils spent a greater percentage of test time (B) in the chamber of their familiar peer and (C) huddling with their familiar peer.

3.2. Testosterone enhances huddling with a familiar peer

To determine if T enhances pro-social behavior in nonreproductive contexts, we conducted a peer interaction test in which male and female subjects interacted with their familiar peer following an injection of either saline or T. A GLM with sex and treatment (T or saline) as fixed factors yielded a main effect of treatment for huddling ($F_{(1,12)} = 5.17$; $P = 0.04$; partial eta squared = 0.30; Fig. 2A), with females and males that received T spending significantly more time huddling with their peer than saline-injected animals. This finding is consistent with our previous study in which T increased male gerbil pro-social contact with a female pairbond partner (Kelly et al., 2022). We did not observe an effect of or interaction with sex for huddling (all $P > 0.36$). Additionally, we examined investigative behavior, however GLM analyses yielded no effects or interactions (all $P > 0.41$; Fig. 2B). These findings demonstrate that T influences on pro-social behavior are not specific to reproductive contexts or to male gerbils.

3.3. Testosterone facilitates anti-social behavior with novel, same-sex intruders

To determine if T influences anti-social behavior in sexually naive, adult gerbils, we conducted a resident-intruder test in which male and female subjects interacted with a novel, same-sex conspecific in the

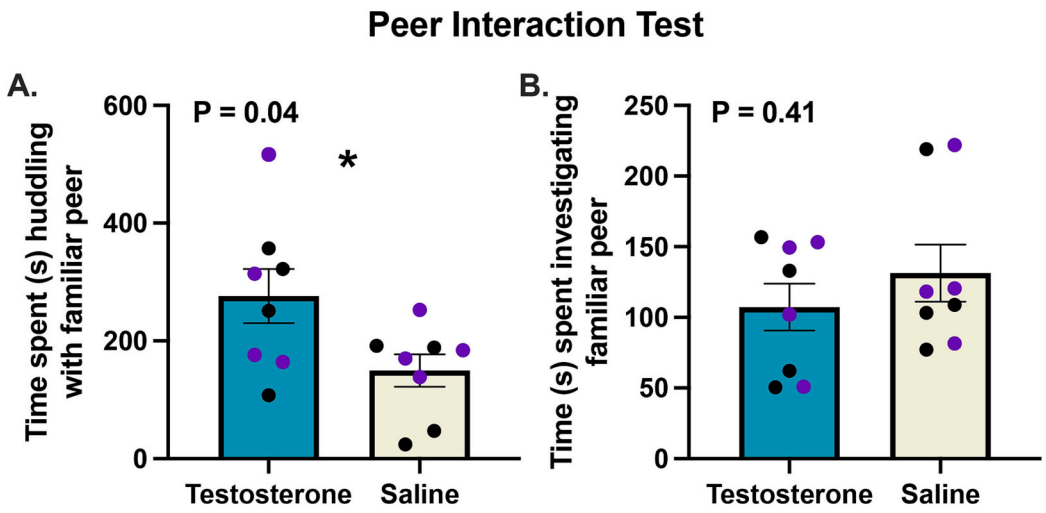

Fig. 2. Male (purple dots) and female (black dots) gerbils injected with T (A) spent more time huddling with their peer compared to saline-injected animals. (B) Investigation did not differ between animals injected with T or saline.

subjects' homecage. A GLM with sex and treatment as fixed factors yielded a main effect of treatment for active avoidance of the intruder ($F_{(1,12)} = 5.44$; $P = 0.04$; partial eta squared = 0.31; Fig. 3A), with females and males that received T spending significantly more time actively avoiding the intruder compared to saline-inject animals. Additionally, we observed a main effect of sex ($F_{(1,12)} = 6.57$; $P = 0.03$; partial eta squared = 0.35), such that females exhibited more active avoidance of intruders than males. However, we did not observe an interaction between sex and treatment ($F_{(1,12)} = 4.12$; $P = 0.07$; partial eta squared = 0.26).

GLM analyses for the time spent near the intruder also yielded a main effect of treatment ($F_{(1,12)} = 87.86$; $P < 0.01$; partial eta squared = 0.88; Fig. 3B); both males and females injected with T spent significantly less time near the same-sex, novel intruder compared to animals injected with saline. Yet, we observed no effect of or interaction with sex for time spent near the intruder (all $P > 0.21$).

When examining overt aggression, a GLM revealed a main effect of sex ($F_{(1,12)} = 7.28$; $P = 0.02$; partial eta squared = 0.38; Fig. 4A), such that females exhibited significantly more overt aggression than males. In fact, no males exhibited overt aggression whereas 6 out of 8 females exhibited overt aggression. Interestingly, T did not influence overt aggression, and we found no effects of or interactions with treatment (all $P > 0.17$).

Lastly, a GLM yielded a main effect of treatment for investigation of the intruder ($F_{(1,12)} = 9.22$; $P = 0.01$; partial eta squared = 0.44), but no effect of sex ($F_{(1,12)} = 3.53$; $P = 0.09$; partial eta squared = 0.23). We also found a significant interaction between treatment and sex ($F_{(1,12)} = 8.00$; P = 0.02; partial eta squared = 0.40; Fig. 4B). Sidak-corrected posthoc analyses showed that within males, those that received T investigated the intruder more than males that received a saline injection ($P < 0.01$; Cohen's d = 4.35). Further, males that received a T injection exhibited more investigation than females that received a T injection (P < 0.01; Cohen's d = 3.55). Together, these findings demonstrate that while T promotes pro-social behavior in the context of interacting with a familiar peer, T facilitates anti-social behavior in the context of interacting with an intruder.

3.4. Testosterone had no effect on nonsocial behavior in the presence of a novel object

Finally, for the novel object test, a GLM with sex and treatment as fixed factors revealed no main effects or interactions for the latency to approach (all $P > 0.12$; Fig. 5A) or the time spent investigating the rubber duck (all $P > 0.60$; Fig. 5B), suggesting that T does not simply enhance behavior in any context, such as facilitating investigation of a novel object, but rather T specifically enhances social behaviors in a context-specific manner.

4. Discussion

The present study revealed that T rapidly promotes pro-social responses to a familiar peer and anti-social responses to an intruder in the same individuals within 5 min of experiencing these disparate social contexts. This demonstrates that T rapidly facilitates behavior in a context-appropriate manner, even outside of reproductive contexts. Additionally, our findings show that rapid effects of T on behavior are not restricted to males, as T rapidly facilitated context-appropriate behavior in female gerbils as well as males. We also observed that females were more overtly aggressive than males, regardless of T, and that T may influence investigation of an intruder in males, but not females. While T similarly increased active avoidance of and decreased time spent in proximity of an intruder in both sexes, larger sample sizes might still reveal sex differences in how sensitive the sexes may be to T.

4.1. Testosterone facilitation of context-appropriate behavior

Studies in human subjects make up the bulk of the findings on T-mediated pro-social behavior. For example, higher T levels are associated with increased ingroup cooperation during an intergroup competition (Reimers and Diekhof, 2015) and with higher pro-social conformity upon observing a peer behave pro-socially (Duell et al., 2021). On the other hand, during a test meant to explore T's relationship to initial friendship formation, higher baseline T levels were associated with decreases in perceived closeness when forced to disclose personal information to a stranger, and decreases in T after the test were associated with higher reports of closeness (Ketay et al., 2017). These findings suggest that T may not promote affiliative responses even in contexts in which they might be considered appropriate, though it should be noted the interaction was with a stranger and not an already established friend. Further, some findings from human studies are contradictory. For example, one study found that 3 h after topical T gel application altruistic behaviors increased in the presence of an audience (known as the audience effect) (Wu et al., 2020). Yet, a recent study found that 2 h after a topical T gel application strategic pro-sociality was eliminated and submission to audience expectations decreased (Kutlikova et al., 2023). The primary difference between these studies is that

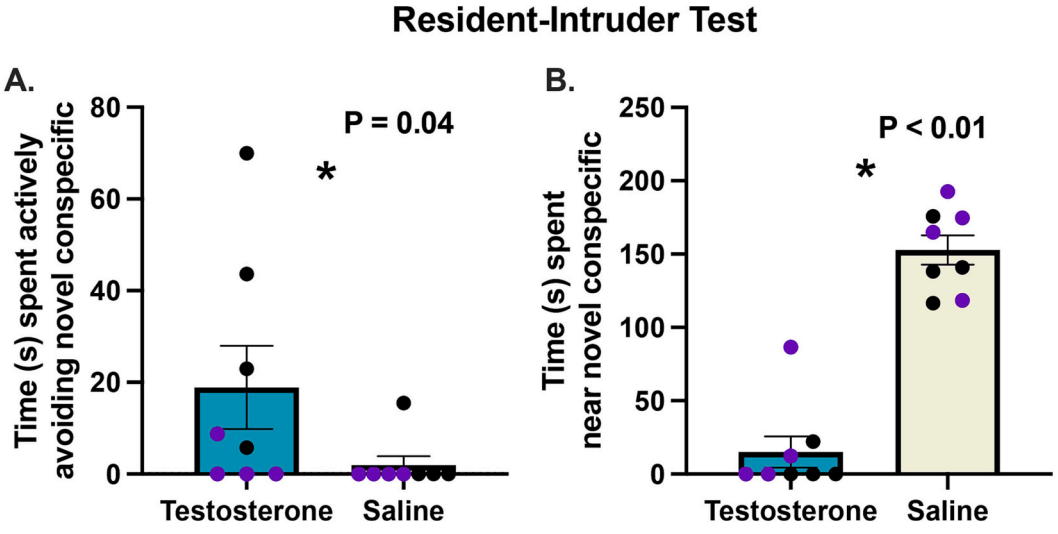

Fig. 3. Male (purple dots) and female (back dots) gerbils injected with T spent (A) more time actively avoiding and (B) less time near the intruder compared to saline-injected animals.

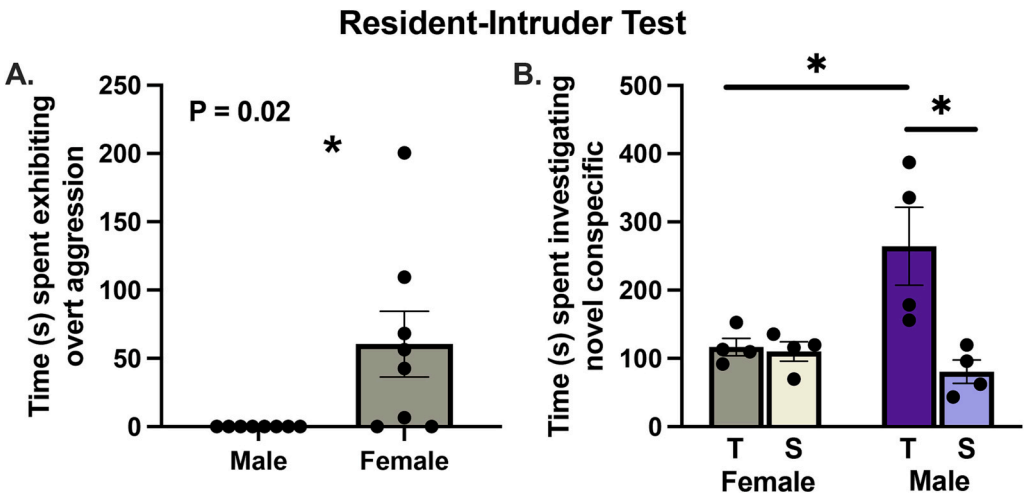

Fig. 4. (A) Female gerbils exhibited more overt aggression than males. (B) Males that received T investigated the intruder more than saline-injected males. T-injected males exhibited more investigation than T-injected females.

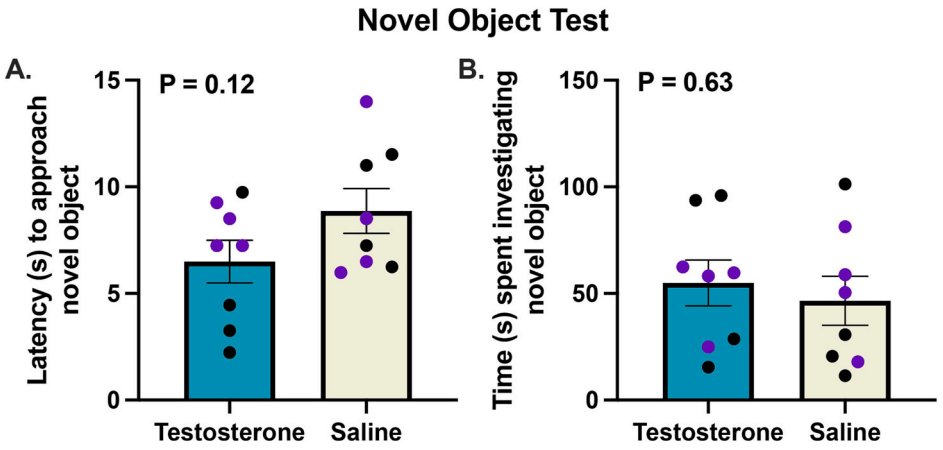

Fig. 5. In the novel object test, treatment did not significantly influence (A) the latency to approach the novel object or (B) time spent investigating the novel object in male (purple dots) or female (black dots) gerbils.

Wu et al. used a modified dictator game in which participants were explicitly asked if they wanted to donate a certain monetary amount to charity, whereas the Kutlikova et al. study used a design where the donation to charity was measured indirectly by the participant's performance in a reinforcement learning task. One could interpret the results from both studies as T enhancing self-beneficial behavior, and nuanced differences in context made a substantial difference in participants interpreting the consequences of their choices and/or perception of how their actions may be perceived by others. Indeed, it has been proposed that, while T promotes pro-social behavior under some conditions, its direction of effect is ultimately the result of mechanisms that facilitates status promotion or protection, i.e., that T promotes self-serving behaviors (Eisenegger et al., 2011; Geniole and Carre, 2018). This begs the question of what gerbils gain from T-induced increases in huddling with peers (this study) or pairbonded mates (Kelly et al., 2022). A recent study in adult male ICR mice found that T rapidly facilitated group huddling in mice, but only in the presence of a predator odor (Zhao et al., 2023), suggesting that the pro-social response was only increased by T in a context where there was something for individuals to gain – in this case, protection. In our studies, however, T increased huddling with their familiar peer in the absence of any overt stressors (i.e., predator odor). Studies have shown that T influences body temperature in quail, lizards, and humans (Hanssler and Prinzinger, 1979; Rusch and Angilletta, 2017; Cintron-Colon et al., 2019), so it is possible that the T-induced effects on prosocial behavior in our studies may not directly reflect a motivation to affiliate, and instead could reflect a disruption of thermoregulatory abilities that drive animals to seek physical contact with a familiar peer or mate. Indeed, rodent pup group huddles function in a manner to provide warmth and insulation to all its members (Alberts, 1978, 2007), and it has been argued that the continuation of huddling behavior into adulthood, despite the ability to sustain a basal metabolism in isolation, persists due to early life associations that huddling reduces thermodynamic entropy from brown adipose fat tissue metabolism and increases access to social information (Wilson, 2017). However, T induction of warmth-seeking behavior would not explain the active avoidance of, and decreased time spent near, an intruder in our study. Thus, if T does rapidly influence thermoregulation, which would need to be tested in gerbils, it is not sufficient to drive an animal to seek warmth in every context.

Alternatively, moderately social species like gerbils, and other socially monogamous species such as California mice and prairie voles, physically and emotionally benefit from co-housing with mates or peers (Grippo et al., 2007; Donovan et al., 2020), so it is perhaps more likely that T specifically enhances affiliation with appropriate conspecifics, with the benefits being those inherent to species that evolved some form of group living. Indeed, huddling with a peer or a pairbond partner is likely rewarding for gerbils, and studies have shown that T can rapidly increase reinforcement mechanisms that result in prolonged place

preferences (Zhao et al., 2020; Petric et al., 2022); thus, in the present study T may have enhanced the rewarding properties of pro-social contact with a familiar peer. Whether T would rapidly enhance nonsexual, pro-social responses in a solitary species remains unknown; because pro-social behavior outside the context of reproduction is rare for solitary and/or territorial species, there simply may never be an appropriate context in which a solitary animal would exhibit nonreproductive pro-sociality that T would be able to enhance. Future studies could explore how widespread T-mediated pro-sociality may be across species, albeit with consideration of ethologically-relevant constraints. Further, implementing a behavioral test to assess consolation behavior as has been done in prairie voles (Burkett et al., 2016) could shed light on the extent to which T influences pro-social behavior that primarily benefits the self or another conspecific.

Our findings that T rapidly enhanced anti-social responses is consistent with the broader literature demonstrating that T can facilitate aggression and competitive efforts in appropriate contexts (Wingfield et al., 1990; Carre et al., 2009; Batrinos, 2012). For example, T rapidly increases aggressive behavior in impulsive and dominant men (Carre et al., 2017) and male mice (Beeman, 1947). Interestingly, in humans, a single dose of T decreases gaze aversion and physical avoidance of angry faces, suggesting that T can enhance an individual's willingness to engage in a challenge (Terburg et al., 2012; Enter et al., 2014). Similar findings have been found for male California mice that are not pair-bonded (Zhao et al., 2019). In contrast, we found that T increased active avoidance of and decreased physical proximity to an intruder in non-pair bonded male and female gerbils. California mice are quite territorial and more aggressive than gerbils (Trainor and Marler, 2001; Deng et al., 2017; Gonzalez Abreu et al., 2022), so it is possible that these disparate findings reflect species differences in the propensity to exhibit overt aggression. While it may be species-typical for a male California mouse to engage in a fight, it may be more typical for gerbils to essentially exhibit more conflict avoidance. If true, this would suggest that T's ability to rapidly mediate context-appropriate behavior has been plastic over evolutionary time, resulting in the promotion of different behavioral outputs across species as a function of what is most adaptive to the fitness and survival of individuals within a particular species. This possibility highlights the importance of using comparative approaches to determine what is generalizable about T mechanisms and how to then predict their effects on behavior across species, including humans, in evolutionary and ethological contexts.

Because an increasing number of studies have demonstrated that T dynamically mediates behavior in a variety of contexts, we questioned whether T may be acting on general attentional mechanisms to focus an animal's behavioral responses toward the most salient stimuli in the environment. Therefore, we tested gerbils in a novel object task to determine if animals injected with T would exhibit more investigation of the object – the most salient stimuli for that given context, and which animals from numerous species have been shown to recognize and actively investigate in paradigms similar to ours (Gaskin et al., 2010; Lueptow, 2017; Gaspary et al., 2018; Kyne et al., 2019). While we observed rapid effects of T on social behaviors, we observed no such effect of T on the latency to approach or investigate a novel object, suggesting that T may not rapidly modulate behavior in a nonsocial context. Although T injections induce elevations of the steroid for at least 1 h in rats, circulating levels begin rapidly decreasing after their peak at 30 min (Sodersten et al., 1980), and thus were likely declining similarly during the resident-intruder and novel object tests in our experiment. Yet, T nonetheless enhanced social avoidance in the resident-intruder test, which concluded only 5 min before the novel object test, thereby suggesting that rapid, presumably non-genomic mechanisms may be activated by elevations of circulating steroids even as they decline. Thus, the lack of an effect in the novel object test was probably not a function of declining T levels. However, while our data therefore suggest that T's ability to promote flexible behavioral responses across rapidly changing social contexts is not mediated by general increases in attention to and the amplification of typical responses toward all salient environmental stimuli, further tests in which the order of testing contexts is reversed should be conducted to verify that conclusion.

4.2. Effects of testosterone across sexes

Few previous studies have explored whether exogenous T or androgen receptor antagonism can rapidly influence social behaviors and/or neurophysiological responsiveness in adult females, with primarily negative results in those that have (Filova et al., 2015; Mohandass et al., 2020; Zubizarreta et al., 2020). Our findings are therefore novel in demonstrating that T can rapidly influence social behaviors, both pro- and anti-social responses, depending on context, in females. Of course, if the effects of T observed here depend on its local aromatization to E2, as we suspect is likely (see further discussion below), then T's effects in females may reflect mechanisms typically activated by increases in circulating E2. If so, future studies examining rapid, nonsexual behavioral effects of E2 in females will be important to explore. However, even if the receptor mechanisms are estrogenic, our data still raise the intriguing possibility that T surges themselves could play roles in behavioral regulation in females, potentially providing the substrate for local, high elevations of E2 as they do in males. Although pulsatile T secretions do not appear to have been widely assessed/reported in non-human female animals, human females do exhibit T pulses. Women show pulsatile secretion of T, with no pattern differences across the three phases of the menstrual cycle (Nobrega et al., 2009), and variability in T levels relates to social context and behavior (van Anders, 2013). Further, acting in a socially-dominant context rapidly increases T levels in women (van Anders et al., 2015), as do sport competitions (Edwards and Casto, 2015; Casto et al., 2017) and even nonsocial, autonomically arousing stimuli like sky-diving (White et al., 2019). Additionally, recent studies in birds have also demonstrated that T fluctuates rapidly in the brain in response to socially salient stimuli in females, suggesting that locally-derived neuro-androgens may also rapidly influence behavior (de Bournonville et al., 2020). Our data, together with the aforementioned studies, indicate that further studies of rapid T effects on female social behaviors should be undertaken across species, as well as studies to determine if there are more widespread peripheral and/or brain fluctuations of T in female vertebrates. If any such surges are observed, whether in response to social and/or generally arousing stimuli, we should also finally determine if they are dependent on the adrenals or the gonads, in males as well as in females.

We found that sexually naïve female gerbils were more overtly aggressive toward an intruder than sexually naïve males. In fact, none of the males in our study exhibited overt aggression. However, T did not rapidly influence aggression in either sex, nor did it affect any of our measures of aggression in pair bonded males in our previous study (Kelly et al., 2022). In contrast to the current study, though, pairbonded males in our previous study exhibited overt aggression toward an intruder, and resident-intruder tests had to be terminated due to intense aggression only in some of the male subjects that received T (Kelly et al., 2022), suggesting measures of aggressive intensity may be required to detect rapid effects of T on aggression in pairbonded males. That T might be able to rapidly influence aggression in pairbonded males (and perhaps females, which were not tested in the previous study), but not non-pairbonded animals, would be consistent with studies in California mice showing T effects depend on pairbond status. Those studies found that T rapidly suppresses sexual communication with a novel female in pairbonded, but not unpaired, males (Pultorak et al., 2015) and T influences social approach preferences to novel males or females in unpaired, but not pairbonded, males (Zhao et al., 2019), which together suggests that T pulses may facilitate bond maintenance in that species. It would therefore be interesting to explore whether T's potential to rapidly influence social response, including aggression, may depend on pairbond status in either or both sexes, and if its effects toward peers

change as a function of pairbond status, potentially inhibiting pro- and promoting anti-social responses toward peers after bonding with a mate. Of course, it is also possible, since we have not yet detected any rapid effects on overt aggression in either sex and, at least in males, in two testing contexts, that T simply does not rapidly affect overt aggression in this species.

4.3. Mechanisms of testosterone actions

T can have rapid, non-genomic effects on behavior through direct actions on androgen receptors (Remage-Healey and Bass, 2004, 2007; Mohandass et al., 2020). Multiple membrane androgen receptors exist, including ZIP9, GPRC6A, TRPM8, and a splice variant of the classical androgen receptor that gets trafficked to membranes (Berg et al., 2014; Thomas, 2019; Mohandass et al., 2020), any of which could mediate the rapid, presumably non-genomic behavioral actions of T that we have demonstrated in gerbils. However, behavioral functions of membrane androgen receptors are poorly understood. Instead, T might induce the rapid behavioral effects on pro- and/or anti-social behaviors via its local conversion to estradiol (E2) in the brain and the subsequent activation of estrogen receptors that have been shown to mediate rapid E2 behavioral effects. These include a g-protein coupled estrogen receptor (GPER) and membrane versions of classical estrogen receptor alpha and beta (Seredynski et al., 2015; Micevych et al., 2017; Kumar and Foster, 2020).

Although all classes of steroids can have rapid effects on behavior, the effects of estrogens have been best characterized, particularly for sexual behaviors. For example, a single injection of estradiol (E2) rapidly activates male sexual behavior in quail (Cornil et al., 2006b) and rats (Cross and Roselli, 1999). In C57BL/6 J mice, an injection of an aromatase inhibitor suppresses male sexual behavior (mounts and intromissions) expressed 10–20 min later, demonstrating that T's conversion to E2 is necessary for rapid modulation of male sexual behavior (Taziaux et al., 2007). Similarly, injections of T or E2 rapidly promote the social approach of male goldfish to female stimuli, stimulate milt production, and increase retinal responses to visual stimuli, and administration of an aromatase inhibitor blocks these effects, indicating all are mediated by estrogenic receptor mechanisms (Lord et al., 2009; Mangiamele and Thompson, 2012; Yue et al., 2018). Additionally, several studies examining the rapid influence of steroids on behavior have also focused on E2 effects on aggression (Heimovics et al., 2015b). E2 administration rapidly increases aggression during an intrusion in non-breeding male song sparrows (Heimovics et al., 2015a) as well as in male California mice that are housed on a short-day photoperiod (Trainor et al., 2008). Similarly, an infusion of E2 directly into the anterior hypothalamus increases agonistic behavior within 20 min in male hamsters (Hayden-Hixson and Ferris, 1991). Together these studies demonstrate that steroid-mediated rapid modulation of behavior via estrogen signaling is widespread across vertebrates. Thus, it is possible, and perhaps probable, that the T effects on behavior in gerbils in the present study are due to actions at estrogen binding sites following its local aromatization.

4.4. Contexts for rapid T effects

Importantly, we do not yet know when and where T elevations that may trigger these behavioral effects in natural contexts occur. Social context does moderate T levels in gerbils (Clark and Galef Jr., 2001), but rapid changes in response to sexual, aggressive, or any other social interactions have not yet been investigated in this species, peripherally or in local brain areas, in males or females. T is responsive to both sexual and aggressive cues in many species, and its effects have typically been studied in relation to the particular context in which the elevations occur. But social interactions are fluid, and animals can be faced with rapidly changing social inputs, or even simultaneous, competing inputs (an affiliative partner and an intruder that enters the home territory). Thus, it seems reasonable to hypothesize that T surges and/or the downstream elevations of E2 that follow in specific brain regions may generally amplify social responses to the most salient social cues present in the environment, thereby promoting context-appropriate behavioral responses. Our data are consistent with that possibility, as a single elevation of peripheral T was able to rapidly promote pro- and anti-social responses across different contexts within 5 min of one another. In contrast, long-term consequences of hormone surges in particular contexts may prime animals to behave similarly in the future, as evidenced by the winner effect in California mice (Oyegbile and Marler, 2005; Fuxjager et al., 2011) and by our previous study, which showed that a T-induced increase in affiliation with a pairbond partner subsequently led to greater prosocial behavior with an intruder several days later, indicating a carry-over effect of T until another T surge rapidly facilitated the transition to antisocial responses with an intruder (Kelly et al., 2022).

5. Conclusion

Here we showed that T rapidly promotes pro- and anti-social responses in a nonreproductive context in male and female gerbils. Rapid T effects on behavior occurred in a context-specific manner within individuals in social contexts with opposing valences, such that T increased huddling with a familiar peer but 5 min later decreased proximity near, as well as increased active avoidance of, a novel, same-sex intruder. Further, we found that rapid T effects on behavior were specific to social contexts given that T did not influence behavior in a novel object task. To our knowledge, these are the first findings to demonstrate rapid T effects on pro-social responses in females. Notably, though, while our study included both sexes, future studies are required to specifically examine effects of sex on T-mediated rapid behavior. These findings support our initial hypothesis that T rapidly facilitates context-appropriate behavior, suggesting that T's context-dependent effects are not restricted to the context in which a T surge is initiated, thus enabling an animal to behave flexibly in dynamic environments.

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Author contributions

AMK conceptualized the study, conducted the behavioral tests, scored behavioral videos, conducted data analysis, and wrote the paper. RRT conceptualized the study and reviewed/edited the paper.

Data availability

The data presented here are available from the corresponding author upon reasonable request.

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