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Testosterone release and social context: When it occurs and why

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ABSTRACT

The functions of rapid increases in testosterone seem paradoxical because they can occur in response to different social contexts, such as male–male aggressive encounters and male–female sexual encounters. This suggests that context may impact the functional consequences of changes in testosterone, whether transient or long term. Many studies, including those with California mice (*Peromyscus californicus*), have addressed these issues using manipulations and species comparisons, but many areas remain to be investigated. We report a study here that suggests transient increases in testosterone after social competition influence future competitive behavior, but social experience alone may also be critical in determining future behavior. In other rodents, a comparable testosterone surge occurs in response to sexual stimulation, but the function is not entirely understood. In addition to competitive and sexual behavior, testosterone impacts other systems instrumental to social behaviors, including paternal behavior and degree of monogamy. Thus, mechanisms regulated by testosterone, such as the vasopressin and aromatase systems, may also be influenced by rapid surges of testosterone in aggressive or sexual contexts. We discuss how the functions of testosterone may overlap in some contexts.

1. Introduction

Plasticity in testosterone (T) function provides individuals with a mechanism for altering their behavioral responses to the environment. As is frequently stated, hormones such as androgens change the probability that a behavior will be expressed based on the environment. Plasticity in hormonal control of behavior hearkens back to seminal papers that examined a variety of non-traditional species (e.g. [27,86]) and argued for between- and within-species variation in hormone–behavior relationships. Wingfield and other researchers have elegantly exemplified this view through a number of studies demonstrating elevations in T in response to challenges from other male conspecifics [126]. This framework is referred to as the 'Challenge Hypothesis' [124,126,129] and is applicable to a diverse range of animal species (reviewed in [56]). In birds, increases in T following an aggressive contest or social interaction vary according to mating system and the degree of male–male aggressiveness (reviewed and analyzed in [55]), such that monogamous species (but not necessarily more paternal) display greater increases in T. Additionally, aggression and paternal behavior can be independent of T because of a variety of environmental factors (reviewed in [71,134]), yet a positive association between androgens and paternal behavior has been found as well [73,74,105,113,114].

Mechanistic studies of plasticity have also been extended to the level of the brain, particularly in regard to neuroendocrine control of behavior. Adaptations at a neural level such as conversion of hormones, density of receptors, cofactors and other neural mechanisms allow the degree of association between hormones and behavior to vary [55] and permit more precise control of specific brain areas and consequently behavior. For example, T is converted to estrogens via aromatase; variation in aromatase levels in specific brain areas allows for plasticity in the control of aggressive and paternal behavior such that dependency on plasma hormone levels is decreased (reviewed in [46,116]).

Despite extensive progress in these areas, there has nonetheless been a dearth of studies demonstrating that plasticity in T response can influence individual variations in behavior as a result of varying social and physical environments [131]. The results below suggest that T can underlie changes in an individual's behavior, but that social experience may also add to the complexity of the relationship. A significant question for understanding how this process occurs is to understand how the social and physical environments, both past and present, can influence current effects of androgens for behavior. We discuss how a steroid hormone such as T can respond similarly to dissimilar social contexts (Fig. 1) and how this response can have different functional consequences for behavior. For example, we examine how transient increases in T following male–male competitions relate to the winner effect (how past win-

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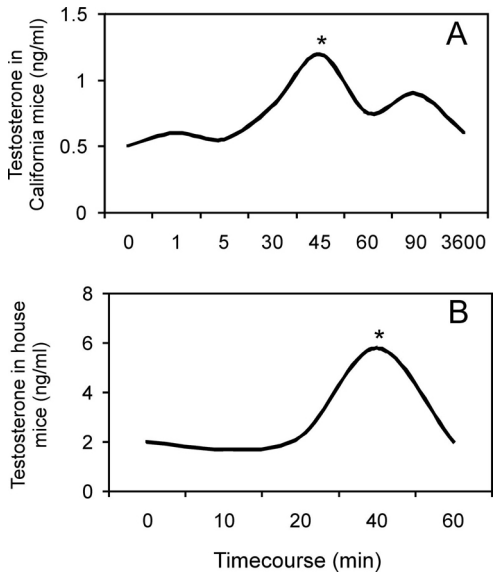

Fig. 1. Transient testosterone spikes following different types of social encounters. (A) Representation of testosterone levels after a single antagonistic male–male encounter in California mice (modified from [75]). (B) Representation of male testosterone levels following the presentation of a sexually receptive female from behind a partition and during the summertime in house mice (modified from [6]). In both graphs, the '*' indicates that testosterone levels at this time point after the social encounter are significantly ($p < 0.05$) higher than baseline.

ning experience influences future ability to win), with an emphasis on the monogamous and territorial California mouse (*Peromyscus californicus*). We then discuss similarities between the T response after male–male encounters in California mice, and male–female encounters in house mice (*Mus musculus*) (Fig. 1), and speculate on potential functions of the increased T during courtship for future behavior. This is then extended to other social behaviors, and we consider how androgens can impact neuroendocrine systems that influence paternal behavior and breeding strategy.

2. Testosterone pulses and male–male antagonistic encounters

The ability of androgens to promote and facilitate aggressive behavior has been studied extensively in many species [88,111]. As described above, this relationship was expanded by the 'Challenge Hypothesis' [126], which considered how in some animals plasma T influences aggression during times of breeding when T levels are expected to otherwise be low. Researchers have continued to investigate the inter-play between T and aggression and have shown that the effect of male–male antagonistic encounters can influence androgen release (reviewed in [130]). This point is demonstrated by experiments with the stoplight parrotfish (*Sparisoma viride*), whereby males in the process of establishing a territory have higher plasma androgens than males that have already acquired a territory [16]. Moreover, by simulating territorial intrusions the researchers revealed that the increase in plasma T and 11-KT was due to male–male aggressive disputes, which occur more frequently during territory settlement [16].

Additional work has investigated the specific time-course in which T increases relative to a competition or other aggressive interactions. These experiments typically involve collecting blood samples from individuals at various intervals following a simulated contest. For example, in a study with male song sparrows (*Melospiza melodia*), experimenters placed a live, caged male conspecific in the center of a resident male's territory and simultaneously broadcasted a tape of a conspecific song. Blood was then collected from the resident male at different times following the staged intrusion. The results indicated that T levels only increased above baseline 10–60 min following the encounter [125]. Numerous other studies in an array of species have also shown similar evidence of a transient, post-encounter spike in T (reviewed in [56]).

There is also a growing body of research in human athletes and competitors showing a degree of temporal variation in T surges associated with an aggressive encounter or competition. For instance, judo competitors show high pre- and post-competition T levels that are associated with increased fights and attacks [109]. A similar study in tennis players found that not only did pre-match plasma T predict the eventual winner, but also that the winner experienced an additional surge in T immediately after the victory [13]. Moreover, T spikes are not exclusively dependent on physical struggle during competition, as winners of chess tournaments also show elevations in pre- and post-competition plasma T compared to losers [78].

The difference between pre- and post-competition T spikes is not fully understood, and is difficult to evaluate. Increased T prior to a competition might be related to the 'Challenge Effect' in a manner not yet explored in non-human animals. Alternatively, these two pulses might have independent effects on aggression and winning ability. For instance, pre-encounter elevations in T could be an anticipatory physiological response that helps individuals prepare for an upcoming encounter [75]. This is consistent with a study in undergraduate men showing that increases in salivary T predict a willingness to engage in competition [18]. In some species, the individual initiating combat is the fight's eventual winner [59,63]. Thus, an anticipatory T surge might increase the odds of winning by preparing an individual to more readily engage in a fight or competition. If increases in plasma T reflect an expectation of an upcoming aggressive interaction, then this implies a degree of anticipatory learning [75]. It is easy to conceive of how humans predict their involvement in an upcoming competition, but other research has shown that in some circumstances, rodents and fish are also capable of this ability [58,118].

Other work demonstrates inter-individual variability in the expression of T pulses related to a contest. For instance, in both African cichlid fish (*Oreochromis mossambicus*) and Japanese quail (*Coturnix japonica*), individuals engaged in aggressive disputes with their mirror image do not show a spike in T at the end of the encounter [57,94]. Thus, these data imply that a fight's social context can regulate an individual's subsequent hormonal response. Also consistent with this notion is the so-called 'audience effect,' whereby bystanders watching a fight but not participating in it also show an increase in T. The most notable examples of this phenomenon are cichlid fish [92] and humans [11]. Interestingly, the latter example comes from a study in World Cup spectators, in which individuals rooting for the competition's ultimate winner have higher T levels after the game compared to individuals rooting for the team that lost [11].

How and why T pulses in response to a fight are mediated by social context remains unclear, but the answer likely depends on the function of T spikes themselves (discussed below). If, for example, surges in T modify behavior, then social regulation of this process would likely adjust behavior in a context-dependent manner [93]. Specifically, behavior would be adjusted to an individual's surrounding social environment, which is further influenced by factors such as competition or density. This naturally begs the question of whether other contextual factors associated with an aggressive interaction might similarly influence transient increases in T. Research rooted in the field of behavioral ecology has shown over time that extrinsic factors, such as residence status or resource availability, have potent effects on animal aggression and can greatly bias a fight's outcome [8,9,38,41,67,69,95,121]. The relationship among T, contest location, and winning ability is further supported by studies showing that in soccer and hockey play-

ers T increases prior to a competition only when the match occurs at the home venue [17,87]. Thus, how a fight's physical location influences changes in T should be examined in future work.

2.1. Functional significance of contest-related testosterone pulses

Even though it is widely documented that aggressive male–male encounters can induce elevations in androgens, the function of these hormonal titers remains largely unclear. One hypothesis that explains the possible effects that contest-related T surges have on variations in behavior proposes that T reinforces learning associated with an aggressive encounter [75]. Numerous studies in rodents have shown that T has rewarding properties and elicits a conditioned place preference (CPP) [4,132]. Moreover, androgen-induced CPPs are blocked by dopamine receptor agonists [100], suggesting that T might activate dopamine receptors to create a reward and induce a CPP [75]. Additional experiments in reptiles and rodents have demonstrated that individuals can form CPPs for locations in which they have previously won fights [40,76,83]. Thus, this body of research collectively suggests the possibility that elevations in T following a fight might induce a CPP for the location in which a given encounter occurs [75]. In effect, this process would likely influence territoriality by adjusting site preferences during instances of territory settlement. To our knowledge, this hypothesis has not yet been tested directly, but doing so would provide interesting insight into the adaptive function of not only the 'Challenge Effect,' but also of CPPs.

A second hypothesis proposes that transient increases in T following an aggressive encounter modulate plasticity in winning behavior (i.e. the 'Winner–Challenge Hypothesis' [97]). Winning aggressive contests can enhance an individual's ability to win future encounters. This phenomenon is called the winner effect [37], and it occurs in a number of different species such as fish [19], mammals [75], birds [36], and invertebrates [59,122]. Our laboratory has studied the link between post-encounter T and the winner effect in two closely related species of *Peromyscus* mice. The first is the California mouse, which is strictly monogamous [52,53] and defends territories year-round [103]. Not only is this species highly aggressive, but it also shows a robust winner effect (Fig. 2) [97] and experiences a transient surge in T following a win (Fig. 1A) [75,97]. The second species is the white-footed mouse (*Peromyscus leucopus*), which is promiscuous and substantially less territorial than the California mouse [34]. Interestingly, this species shows a substantially diminished winner effect and does not experience a T pulse 45 min after winning a fight [99]. When examined together, these two studies demonstrate a close association between the winner effect and temporary T increases following an aggressive interaction. Further support for this hypothesis comes from another study conducted in our laboratory using California mice. In this experiment, male individuals that experience a T pulse after winning a fight attack opponents more quickly in *future* contests, as compared to individuals that did not experience this hormonal change [115]. Along the same lines as the second hypothesis described above is a third explanation of the function of post-encounter T surges. Namely, elevated T following a competitive dispute might affect behaviors associated with winning and territoriality, without necessarily influencing aggressive behavior directly, *per se*. Evidence for this hypothesis comes from studies in song sparrows (*Melospiza morphna*), whereby T increases the degree to which males patrol their territory and sing following an aggressive dispute, but does not modulate aggression during the actual encounter [128]. Given these data, it is possible that post-encounter T pulses enhance the persistence of territorial aggression [127,128]. Further support for this notion comes from studies in male chicks (*Gallus gallus*) showing that T influences the persistence of search behavior and response to stimuli [7].

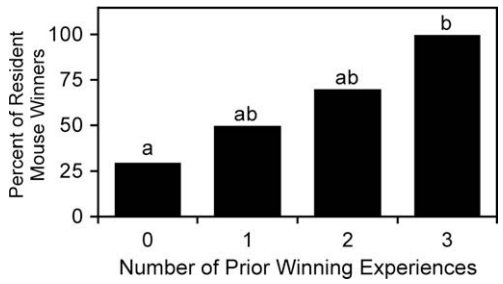

Fig. 2. Demonstration of winner effect in male California mice (modified from [97]). The ability of mice to win aggressive contests against intruders with a slight competitive advantage increases as a function of the total number of prior victories. Differences in the letters above the bars indicate significant differences among treatment groups.

2.2. Experimental evidence for T-mediated plasticity in winning ability

Recently, our laboratory used the California mouse model to test the Winner–Challenge Hypothesis directly [98]. We randomly assigned sexually naïve, *intact* male mice to one of three experimental conditions ($n = 10$ per condition; Table 1), in which individuals received either three winning experiences that were each followed by single T injection, no winning experiences but three separate T injections, or no winning experiences but three separate saline injections (controls). Animals not experiencing wins *per se* were instead provided with a handling experience, whereby each individual was subjected to the same handling by the experimenter associated with a given aggressive contest, but without encountering a conspecific [97,99]. All individuals received injections of either T (T: 36 mg/kg T-cyclodextrin inclusion complex [115]) or sterile saline 30 min after the respective win or handling experience. Giving injections at this time ensures that individuals experience a transient surge in T 45 min following an encounter, as demonstrated by prior research [115]. This mirrors the post-encounter physiological response that naturally occurs in this species (Fig. 1A; [75]). To confirm that the T injections elevated endogenous T levels above baseline, blood samples used to measure plasma T were collected from a separate group of individuals 45 min after being subjected to the same experimental conditions as described above. Testosterone assays were conducted using an enzyme immunoassay technique described previously [12,97]. The resulting intra- and inter-coefficients of variation were 2.4% and 12.5%, respectively. Both a detailed description of this study's protocol [98] and the manner in which animals were housed and maintained [97,99] has been described earlier.

As expected, T injections significantly increased the concentration of plasma T 45 min after an encounter (Table 2: one-way ANCOVA; $F(1, 29) = 2.61$, $p = 0.044$). Testosterone was significantly higher in individuals that won encounters and received T injections compared to individuals that received saline injections (controls) (Fisher's LSD; $p = 0.027$). Additionally, individuals that received handlings and T injections showed a non-significant trend of higher T compared to controls (Fisher's LSD; $p = 0.081$), whereas plasma

Table 1
Treatment groups to which sexually naive and intact male California mice were assigned (T = testosterone).

Treatment group	Experience during training phase (days 1, 2 and 3)	Testing phase (day 4)
Condition 1	Win + T injection	Test encounter
Condition 2	Handle + T injection	Test encounter
Control	Handle + saline injection	Test encounter

Table 2
Plasma testosterone (mean ± SE) of individuals in each of the three conditions after receiving the corresponding experimental treatment. Significant differences among testosterone levels are indicated by different letters following the given value. (T = testosterone).

Condition	Plasma T levels (mean ng/ml ± SE)
Win + T	7.5 ± 18.67[a]
Handle + T	6.1 ± 9.33[a]
Handle + saline	3.4 ± 4.01[b]

T between individuals that received T injections, regardless of whether winning experience was acquired, were not significantly different (Fisher's LSD; $p = 0.565$), indicating that endogenous T levels were not adding significantly to the overall T level.

The percentage of mice that won the test encounters differed significantly among the treatment groups (Fig. 3A; Fisher's exact test; $p = 0.0034$). Mice that received T injections after each of three victories won more test encounters than controls (Fisher's exact test; $p = 0.0031$), but no further significant differences between treatment groups for the percentage of test encounters won were detected. The average attack latency among the three treatment groups was also significantly different (Fig. 3B; one-way ANOVA; $F(2, 27) = 8.58$, $p < 0.001$). Individuals that acquired winning experience *and* received T injections attacked their opponents faster than individuals in the other two conditions (T injections: Fisher's LSD; $p = 0.035$, control mice: Fisher's LSD; $p < 0.001$). Interestingly, individuals that received only T injections attacked their opponents faster than controls (Fisher's LSD; $p = 0.0.014$), despite no increase in ability to win. Finally, the total number of attacks that the focal mouse initiated differed significantly among the experimental conditions (Fig. 3C; one-way ANOVA; $F(2, 27) = 9.85$, $p < 0.001$). Controls individuals attack their opponents less than individuals that received T injections, regardless of their status as a past winner (Win and T injection: Fisher's LSD; $p < 0.001$, T injection: Fisher's LSD; $p = 0.004$).

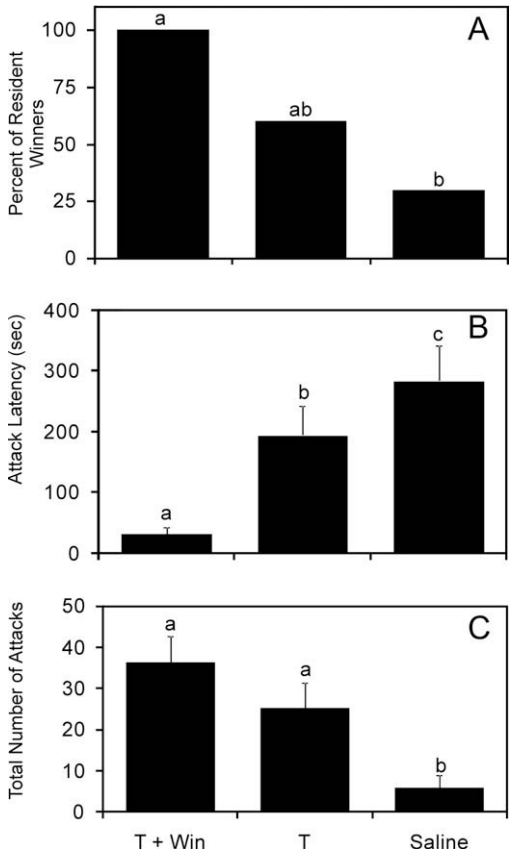

Fig. 3. Individual variation in winning ability and aggressive behavior as a result of prior winning experience and testosterone pulses. (A) The percent of mice in each treatment group that won the final test encounter. (B) Average attack latency of focal mice (i.e. duration between the test encounter's onset and the focal animal's first attack) in each treatment group. Data are presented as mean ± SE. (C) Average total number of attacks exhibited by the focal animal and directed to the intruder. 'T + Win' indicates individuals that received three winning experiences each followed by a testosterone injection; 'T' indicates individuals that received three handling experience each followed by a testosterone injection; 'Saline' indicates individuals that received three handling experiences each followed by a saline injection. Data are presented as mean ± SE.

These data provide strong support for the Winner–Challenge Hypothesis, as they show that in California mice a post-encounter T pulse is sufficient to mediate plasticity in winning behavior. Further support is found in a related study using African cichlid fish, as post-encounter T pulses were necessary to produce a winner effect [93]. However, these findings should be interpreted with caution because the focal fish were selected to participate in the experiment by having already won an aggressive encounter [93]. Such "self-selection" of focal animals in any study involving of winner effects confounds the role of winning experience with intrinsic fighting ability, such that focal individuals are inherently better fighters on average than they would have been otherwise if selected randomly [10,19,60]. Thus, because we randomly selected focal participants in this study, intrinsic fighting ability is controlled for and it can be concluded that T modulates the winner effect regardless of an animal's natural ability to fight.

Another interesting aspect of our results is that the effect of T alone does not induce a full and robust winner effect. Thus, it is likely that T and winning experience interact to enhance future winning, but the nature of this interaction is uncertain. For example, animals that won encounters and received T injections might have had higher endogenous T levels because their gonads could have released T beyond the T that was administered to them. However, given that plasma T of individuals that received T injections, regardless of winning experience (Table 2), was statistically indistinguishable, this explanation is unlikely. Thus, an alternate explanation is that the effects of both T and winning experience are independent. As such, they might each contribute to the winner effect separately. To date, no experiments have investigated how winning experience itself could enhance future winning ability independent of androgen action, although preliminary studies in our laboratory support this assertion (Fuxjager, Oyegbile, and Marler, unpublished data).

These data also suggest a complex relationship between the effects of T and winning experience on individual aggressive behaviors. For example, individuals that received only T injections attacked their opponent faster than control animals, but not as quickly as individuals that received winning experience and T injections. Furthermore, T injections prior to the test encounter caused individuals to increase the number of attacks towards their opponents, regardless of whether an animal had prior winning experience. Examined together, these findings suggest that the effects of T and winning experience might differentially influence aggression. Thus, whereas T might exclusively mediate behaviors like the total number of attacks, both winning experience and T combined might influence other behavioral parameters, such as the latency to attack an opponent.

3. Testosterone pulses and male–female sexual encounters

Having observed rapid increases in male T following aggressive male–male encounters, it is interesting and important to consider that rapid T release also occurs in other contexts (Fig. 1B). In con-

trast to the study of male–male encounters, T pulses in sexual contexts have been most extensively examined in mice and rats, and for this reason our discussion will focus on rodent models.

Males of a variety of species, including humans, have repeatedly been shown to respond to female stimuli by triggering an increase in luteinizing hormone (LH) immediately followed by a rise in plasma T [20,24,72,77,106]. Rapid T release occurs in at least two reproductive situations, the first upon initial exposure to a female, and also following ejaculation should the interaction lead to mating; generally, hormone levels rise within 10 min of encountering a female, peak within 20–30 min, and return to basal levels within 80 min [25,104]. The amplitude and duration of the rise in T is equivalent to "spontaneous" T pulses that occur in adult male house mice in the absence of any detectable stimulus [24,25]. The T reflex is observed in a wide range of species, and does not rely on previous sexual experience [20]. Moreover, behavioral stimulation by the female is not required; a female's urinary scent is enough to trigger an identical T reflex [77]. Testosterone response is pheromonally driven, and can be blocked in male rats by removal of the vomeronasal organ [26,133]. Several authors have noted that given how commonly the phenomenon is observed across species, an adaptive function is likely [91].

Sexually-stimulated T release occurs in naive males, but can also be shaped by experience and learning processes. Clancy et al. [20] found that while female urine elicited LH response in sexually naïve male mice, the response was more robust when the animals were sampled again after they became sexually experienced. Work with rams is in agreement, showing that the most consistent endocrine responses to estrus ewes are observed in sexually experienced males [14]. Although the exact mechanism is unknown, it seems likely that males with sexual experience are able to associate mating behavior with a female stimulus, and produce a stronger or more specific LH and T signal. Classical conditioning also impacts rapid T release. Graham and Desjardins [51] illustrated that by pairing exposure to a sexually receptive female with wintergreen oil, the scent of the oil was subsequently effective in triggering LH release. One apparent gap in the literature thus far is the integration of T response to female stimuli and location preference. Male rodents permitted access to an estrous female in a place preference apparatus have consistently shown a preference for the chamber in which mating occurred [15,61,82,84]. Likewise, peripheral injections of T, DHT or 3α-diol also induce place preferences in rats [4,107]. As discussed above [75], the role of T elevations in sexual contexts remains poorly understood. In a monogamous, territorial mammal such as the California mouse, mating behavior, care of young and territory defense are highly interrelated. In this species, T release in response to a potential mate or to sustained stimulation from the female partner may also influence the development of territorial behavior. In a species with a polygynous breeding system, rapid neuroendocrine response may also facilitate "memory" for the locations in which females are found. If females are localized to areas rich in resources, for example, such a mechanism could be highly relevant to male reproductive success.

While a close association between T and male sexual behavior across species suggests that rapid increases in T may be conserved for reproductive functions, the nature and specificity of stimuli that produce the response can vary considerably by the social system in which they are observed. In the cooperatively breeding and socially monogamous Common marmoset (*Callithrix jacchus*), social context modulates male responses to the ovulatory odors of novel females [135]. While single males showed T elevations in response to the odors, mated males with offspring, exhibited a non-significant reduction in T. These results suggest that ovulatory odor is not only ineffective in triggering T release, but potentially suppresses reproductive functioning. The authors suggest that since "family" males are highly invested in their mates and infants, the ovulatory cues that trigger neuroendocrine response have become uniquely associated with their own mates, or that female and infant stimuli actively inhibit neuroendocrine response to other females [135]. These findings make strong intuitive sense, given that an increase in sexual behaviors supported by reflexive T would be inappropriate for a species that forms pairbonds. Male stimuli can also influence rapid T release in reproductive contexts. Clancy et al. [20] presented naive and experienced male CF-1 mice with urine from a sexually mature male, a mature female, or a mixture of both types of urine. Regardless of sexual experience, the inclusion of urine from intact and castrated adult male mice entirely blocked LH response to female urine. From these data, an interesting picture begins to take shape. Male mice avoid the urine marks of other males [20], presumably to avoid aggressive male–male encounters as a result of intrusion onto a resident male's territory. Urine marking by a territory holder may, as suggested by the suppression of LH, serve to block rival males from inducing sexual behavior in females within the territory, or by inhibiting sexual advances by the intruding male [20]. Within the context of territorial behavior, we can begin to see how reproductive and aggressive behaviors are closely knit together in ways that may explain why two seemingly distinct types of stimuli elicit, at least superficially, very similar neuroendocrine responses.

3.1. Putative functions of rapid T release in sexual contexts

Despite the widespread nature of a rapid T release in male–female encounters, the exact function of this phenomenon remains unclear. Although the idea that a T increase functions to support the male in impending sexual behavior seems obvious, several lines of evidence suggest that the story is more complicated. Not all strains of mice exhibit reflexive T release, but nonetheless express normal sexual and courtship behavior [5,65]. To account for some of the complications that arise, male sexual behavior might be better conceptualized as a composite behavioral response to various environmental modulators, including the affective state of the male, the degree to which he is sexually stimulated, his physical condition, the location in which he encounters the female, and breeding system.

3.1.1. The case for male sexual behavior

We begin by first considering the evidence for how rapid T release supports the mechanics of sexual behavior. Stepping away from the large literature on the supportive role of baseline T, rapid T response requires two unique considerations. First, rapid reinstatement of sexual behavior by androgens in a castrated male does not directly address the function of reflexive T in intact males, particularly given that sexual behavior is maintained with relatively low concentrations of T and is not graded in nature [3,54]. Secondly, LH and T increase rapidly upon exposure to a female, suggesting that any influence on impending sexual behavior must also occur rapidly. Some evidence indicates that T rapidly influences sexual behavior in the short-term. Sachs and Leipheimer [108] demonstrated that T injections could rapidly (6–30 min) induce electromyographic activity in the bulbocavernosus muscle of castrated rats, indicating that androgens may be able to quickly influence penile reflexes. Male mice that have been primed by exposure to female urine have shorter mount latencies when given access to a receptive female, as do males given injections of T that mimic endogenous T pulses [64]. Similar results have been found in rats, where pre-exposure of a male to an estrous female causes a reduction in ejaculation latency in mating tests [3]. Moreover, maximum LH secretion seems to occur around the same time at which the male begin mounting a stimulus female [23].

Testosterone may also indirectly support sexual behavior via the ability of its androgenic metabolites to reduce anxiety. Aikey et al. [1] demonstrated in a series of comprehensive studies that the anxiolytic effects of T in male mice are attributable to the actions of 3α and 5α-reduced metabolites on the $GABA_A$ receptor. Importantly, these metabolites reduce anxiety within a time course consistent with a role for rapid T release in facilitating mating behavior. In this study, anxious behavior was indexed using an elevated plus maze, and by measures that account for both location of the animal in the maze as well as entries into open and closed arms. $GABA_A$ antagonists bicucculine and picrotoxin were shown to block anxiolysis, but also depressed locomotor activity.

Several important messages arise from these findings; first, the authors suggest that rapid T functions to help males achieve matings by suppressing distractions, such that males are able to approach and mate with a female more successfully [1]. Reduction of anxiety may be particularly relevant for reproductive success for polygynous species such as the house mouse, in which sexual partners may be less familiar. However, surprisingly little is known about the relationship between the $GABA_A$ receptor and male sexual behavior. Another interpretation of these results is that reduced T-metabolites increase locomotor activity, impacting exploratory behavior and space use coincident with locating a potential mate. In either case, neuroendocrine response of LH release habituates with continued exposure to the same female [23]. Should a novel female be encountered, LH and T are again rapidly increased. Whether the function is to ease anxiety associated with a new individual and mating behavior, or to increase exploration of the location in which the female was encountered, rapid T may enhance reproductive success.

3.1.2. The case for communication

One argument against a supportive role for T pulses in mechanics of sexual behavior, somewhat counter-intuitively, is the relative robustness of the response. For example, the presence of the female herself is not necessary, as urinary pheromones alone are effective in triggering the T response [77]. Male mice also respond to urinary odors from females at any stage of the estrus cycle, as well as females that have been ovariectomized [66]. If reflexive T functions primarily to support the mechanics of sexual behavior, then these data suggest that the neuroendocrine response to female stimuli is not particularly parsimonious; many of the instances in which T pulses occur will not result in a mating. Alternatively, if the timeline in which we are considering mating behavior is extended, T may well increase mating success by inducing receptivity in species where ovulation is induced, or by increasing the male's attractiveness to females.

3.1.3. Bringing a female into receptivity

A basic tenet of behavioral endocrinology is that by and large, female animals have constrained receptive periods outside of which mating and fertilization of ova will not occur. While some animals cycle freely without intervention, others require induction of ovulation by stimuli from adult males. Female prairie voles, for example, will not come into estrus unless exposed to the urinary or salivary chemosignals from an adult male [85]. Likewise, reproductively immature animals may even be brought into reproductive condition earlier. In his classic work, Vandenbergh demonstrated that puberty in female house mice can be accelerated by approximately 20 days via exposure to urine from adult, intact males [119]. Puberty acceleration is caused by the actions of a volatile substance in male urine that is T dependent, and thus is a reliable signal that a reproductively active male is present [110]. While these relationships are clear regarding basal levels of T, a rapid, single increase in T would need to quickly impact urine content for acceleration of receptivity to be a function of rapid T release in sexual contexts. Unfortunately, very little evidence is available to support the plausibility of this hypothesis, but it would be an interesting direction for future research.

Beyond supporting pheromonal induction of receptivity, androgens impact male physiology in ways that permit females to discern male quality. Female rodents can distinguish between the urine of males with varying levels of circulating T, and seem to prefer the signals from the highest T males [42,50,112]. Another signal associated with T is ultrasonic courtship vocalizations, which male mice uniquely emit upon encountering female stimuli and are proposed to be an index of sexual arousal [65,123]. Ultrasonic courtship vocalizations in rats, hamsters and house mice are dependent on androgens, and as little as one T injection can restore courtship calling in a castrated animal for six days or longer [35,45,89]. While signals sustained by basal steroid levels are accepted as influential in mate choice, virtually no research has addressed the impact that rapid T might have on these signals. We do know that in most cases, ultrasonic courtship calls are closely associated with T response to female stimuli [65,101]. For example, male CF-1 mice begin eliciting ultrasonic courtship calls seconds after exposure to female urine, and calls within the first minute are predictive of plasma T 30-min later [65]. While male mice emit courtship calls the most heavily upon introduction to a female, they continue to call up to copulation and following ejaculation [90]. It has been suggested that one function of courtship calling is to stimulate the female, and at least one study found that female mice prefer vocalizing males to those who have been devocalized [102]. In this scenario, the androgen-dependence of courtship vocalizations suggests that rapid T response may underlie a male's ability to sustain courtship vocalizations, and to secure matings with females [101].

3.2. Individual variation and mate choice

To this point, rapid T release in sexual contexts has been discussed in terms of its consistency within and across species, with a focus on mammals. Exciting new research has extended the concept of rapid T response to include the magnitude to which the reflex occurs. If individual differences in T response to females occur and are honest indicators of an important male trait such as territorial aggression or parenting, "responsiveness" may serve as a metric with which to assess mate quality. In birds, T commonly mediates a trade-off between paternal investment and mating effort, since more energy expenditure towards either activity may come at the expense of the other. In a 2007 study, McGlothlin and colleagues administered gonadotropin-releasing hormone (GnRH) injections to free-living male dark-eyed juncos (*Junco hyemalis*), a species in which both parents care for young but mating commonly occurs outside of the pair [80]. GnRH injections caused a short-term rise in T comparable to what is seen following a territorial intrusion (Fig. 1), but variation was observed between individuals. Males who responded to GnRH treatment with larger increases in T above baseline provisioned their young less often, and demonstrated more aggression during a simulated territorial intrusion. In the dark-eyed junco, greater HPG responsiveness, then, reflects a male who is more highly invested in mating effort than parental effort. In addition, GnRH responsiveness is positively correlated with amount of tail white [81], an ornament that is used by males in courtship and aggressive displays. Given the relationship between T responsiveness and tail ornament, it is feasible that female juncos use tail white to identify males who are the most likely to aggressively defend their territories.

Returning to rodent literature, preliminary data from our own laboratory (Gleason and Marler, unpublished data) suggests that rapid T response may serve as a signal of paternal quality in the strictly monogamous and biparental California mouse. Upon expo-

sure to a female, males respond differently such that approximately half experience a decrease in T, and half show an increase at 60 min post-pairing. California mice are unusual for mammals in that castration reduces, and T replacement maintains, huddling and grooming of young by fathers [113,114]. Both field and laboratory studies indicate that offspring survival is dependent on paternal care [53], suggesting that evaluation of paternal quality during courtship could be highly adaptive. In sharp contrast to the closely related, promiscuous white-footed mouse, it is interesting to note that California mice have very long intromission latencies (48 min average for California mice, 21 min average for white-footed mice), even when females are in estrus [32,33]. While the significance of a twofold increase in intromission latency is unknown, differences in mating system may underlie such differences in copulatory behavior. This extended precopulatory period in California mice combined with individual variation in rapid T release suggests that T "responsiveness" could be a honest signal of paternal quality during mate choice interactions. Clearly, T responsiveness as a signal of quality will depend on the relationship between T and male traits in a particular species.

4. Effects of testosterone pulses on neuroendocrine systems

While no consensus has yet been reached on the exact function of reflexive T release, there are clearly several exciting lines of research that continue to shape our understanding. One natural question that arises is how rapid T signals impact the neuroendocrine system, and how contextual information is integrated at the neural level. In male–male aggressive contexts, because the observed changes in aggression and winning ability persist after both a given fight's conclusion and the point at which T is eliminated from the bloodstream, it is likely that T and winning experience modify behavior by inducing long-lasting alterations in the brain [97,115]. The exact alterations, however, remain speculative. One possibility is that either or both T and winning experience influence neurochemical systems known to control aggression, such as serotonin [28,29,43], arginine vasopressin [2,12,48,49], and dopamine systems [28,118,120]. Such neurochemicals are pervasive in the nervous system, and their involvement in mediating aggressive behavior can be influenced by the presence of androgens [22,30,100,134]. Also possible is that post-encounter T spikes and winning experience, although associated, differentially influence distinct neuroendocrine mechanisms that control aggression, such as through activation of specific steroid hormone receptors. This effect is demonstrated by a study in California mice that found that T itself acts on the brain to reduce attack latency in future encounters, but it does not affect baseline levels of aggression. Instead, baseline aggression was influenced by aromatase inhibition, which in turn suggests that an estrogen-based pathway also controls the expression of baseline aggressive behavior [115].

Regarding the neuroendocrine influence of rapid T in response to female stimuli, several androgen-responsive systems impact sexual, parenting and mating behavior. The impact of T on paternal behavior varies significantly among species and can be tightly linked with the density and distribution of the aromatase enzyme such that specific brain areas influencing paternal behavior respond to T via conversion to estrogens [117]. Moreover, T promotes the extra-hypothalamic vasopressin system [31], which has also been linked with a variety of social behaviors, most notably pair bonding and paternal behavior [47,62]. A recent study with prairie voles (*Microtus ochrogaster*) demonstrated that variation in space use is related to the expression of the vasopressin 1a receptor (V1aR) in the posterior cingulate/retrosplenial cortex and the laterodorsal thalamus, brain areas implicated in spatial memory [96]. These brain areas are also activated in male rats presented with their female cage mate plus a novel male intruder [44]. The activity of the neural circuit that underlies aggression, identified using functional magnetic resonance imaging, is also suppressed by the V1a receptor antagonist SRX251. Moreover, androgen receptors are found in both of these brain regions [21,70]. It would be very intriguing if these different systems are tied together to shape spacing of individuals, particularly since spacing is one factor thought to promote the development of monogamy [68].

5. Conclusion

Several important ideas and questions emerge from this review and our reported study. The first notable observation is the striking similarity between the T surges seen in both competitive and sexual contexts (Fig. 1). Whether these T surges are equivalent in function or are distinct remains an open and important question. For California mice, the post-encounter T surge in winners influences future ability to compete, but could also occur in males as a result of exposure to females, preparing them to fend off rivals. Competition may also factor into mating behavior such that if there are other males present, it may be to a male's advantage to copulate as quickly as possible. Another similarity in function between rapid T release in male–male and male–female encounters could be the ability of T to induce CPPs, effectively centering a male on a physical location and the social context within that location. Males prefer sites where they have been exposed to females or have experienced aggressive encounters, so we can hypothesize that T-response to these situations shapes place preference in both [15,61,82,84]. Other evidence also indicates a potential role for T in reducing anxiety [1], which again could be critically important for approaching unfamiliar females or rival males. In both contexts, we would expect previous experience to be influential for behavior and endocrine response.

As suggested earlier, variation in T surges at the level of the individual may prove to be significant. Our data suggest that the separate effects of past winning experience and exposure to transient increases in T can induce changes in an individual's aggressive behavior. This relationship, however, is complex because these two factors interact; in effect, T itself may influence future winning ability, but this influence is much stronger when it is coupled with the experience of winning a fight. Within sexual contexts, relatively low circulating T maintains sexual behavior, again calling into question why rapid T increases are so commonly observed if not required for mating. While there is little strong evidence supporting any specific function, interesting speculations can be made. Many studies of sexual behavior are performed on rodents in small arenas where males primarily control the timing of mating [39]. When females have the opportunity to pace the interaction, they assert more control and mating takes longer [79]. The rapid rise in T may well be involved in courtship interactions prior to mating, and depend on interactions between the pair. The effects of a rise in T could become particularly apparent in a monogamous species in which the assessment period prior to mating is more prolonged. There are, however, intriguing hints that the ability of a male to mount a rapid rise in T is not simply a response to a social stimulus, but also a reflection of the quality of a male. Although additional work is needed to tease apart the potential functions of transient increases in T at a behavioral, physiological and neural level, the similarity of rapid T response in differing social contexts presents an exciting new direction for research.

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