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Experimental increase of testosterone increases boldness and decreases anxiety in male African striped mouse helpers

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HIGHLIGHTS

- We studied the role of testosterone in dispersal-like and social behaviors.
- We increased testosterone levels in captive philopatric male striped mice.
- Exogenous testosterone decreased basal corticosterone levels.
- Testosterone did not alter social behavior but enhanced dispersal-like behavior.
- We suggest that the up-regulation of testosterone is important to facilitate dispersal.

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ABSTRACT

Males of many species can adjust their behaviors to environmental conditions by changing reproductive tactics. Testosterone surges in adult breeding males typically inhibit the expression of paternal care while facilitating the expression of aggression during environmental changes. Similarly, in non-breeding philopatric males of cooperatively breeding species, up-regulation of testosterone may inhibit alloparental care while facilitating dispersal, i.e. males might become bolder and more explorative. We tested this hypothesis in philopatric male African striped mice, *Rhabdomys pumilio*. Striped mouse males can either remain in their natal groups providing alloparental care or they can disperse seeking mating opportunities. Compared to philopatric males, dispersed males typically show higher testosterone levels and lower corticosterone levels, and more aggression toward pups and same sex conspecifics. We experimentally increased the testosterone levels of the philopatric males kept in their family groups when pups were present. Testosterone-treated males did not differ significantly from control males in alloparental care and in aggression toward same-sex conspecifics. Compared to the control males, testosterone treated males were bolder, more active, and less anxious; they also showed lower corticosterone levels. The philopatric males were sensitive to our testosterone treatment for dispersal- and anxiety-like behavior but insensitive for social behaviors. Our results suggest a role of testosterone in dispersal.

1. Introduction

The ability of an individual to change its reproductive tactic as a response to changes in the social and non-social environment can provide numerous advantages [1]. In several species, males are unable to compete with larger males with greater competitive abilities adopting alternative reproductive tactics (ARTs) [2–4]. ARTs are discontinuous behavioral and other traits selected to maximize fitness in two or more alternative ways [5]. If the environment and the individual body conditions do not favor dispersal, the males of social species can remain in their natal groups as philopatric helpers providing care toward the breeder's offspring (i.e. alloparental care), instead of dispersing and seeking mating opportunities [6]. Males are predicted to disperse and follow reproductive tactics with higher fitness when certain conditions change, e.g. their environment or their competitive abilities [7]. For instance, a decrease in population density enhances dispersal in the philopatric African striped mice, *Rhabdomys pumilio* [8]. Dispersal implies essential behavioral shifts, i.e. decreased alloparental care and increased behaviors facilitating dispersal. However, less is known about the proximate mechanisms mediating these adaptive behavioral changes which are thought to rely on hormone-based mechanisms [9,10].

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Androgen levels, for instance testosterone, rise during puberty and correlate with dispersal in many species [11]. In Belding's ground squirrels, *Spermophilus beldingi*, early testosterone exposure caused inter-individual dispersal differences [12,13]. However, no study has demonstrated that testosterone surges cause dispersal at later life history stages (e.g. sexual maturity). Yet, dispersal is a risky undertaking [14–16] and the anxiolytic effect of testosterone [17] may facilitate dispersal. Similarly, testosterone may cause dispersal through the facilitation of exploratory behavior [12]. In the African striped mice, testosterone-treated juvenile males expanded their home ranges and showed decreased corticosterone levels [18]. As the final decision to disperse may rely on ecological factors (population density and reproductive competition) [7,8,19], an increase of testosterone levels may facilitate dispersal through both the inhibition of anxiety-like behavior and a decrease in glucocorticoid levels.

Changes in testosterone levels may also be a mechanism mediating the expression of alloparental care in cooperatively breeding species [20]. Elevated testosterone levels typically decrease paternal care in birds [21], although this testosterone action seems more species specific in mammalian fathers [22,23]. In prairie voles, *Microtus ochrogaster*, testosterone administrated postnatally decreased the alloparental responsiveness of males [24]. Thus, while up-regulation of the hypothalamus pituitary gonadal axis (HPG) resulting in increased testosterone levels may facilitate dispersal-like behavior, high testosterone levels may result in a decrease of alloparental care. Consistent with this hypothesis, meerkats male helpers, *Suricata suricata*, showed high testosterone levels and decreased pup feeding rates after prospecting for dispersing opportunities [25]. However, experimental demonstration of the role of testosterone in mediating both behaviors – dispersal and alloparental care – is lacking.

In the present study, we tested the role of testosterone in social behavior (alloparental care, affiliative and aggressive behaviors) and in non-social behavior (boldness, exploration, and anxiety-like behavior), which may facilitate dispersal, in the African striped mouse. Males striped mice can follow one of three ARTs, accompanied by changes in hormonal profile and parental care: 1) philopatric group-living males showing the lowest testosterone levels, highest corticosterone levels, and alloparental care; 2) solitary-living roamers showing the highest testosterone levels, low corticosterone levels, and no parental care; and 3) social dominant group-living territorial breeders showing intermediate testosterone levels, low corticosterone levels, and high parental care [26,27]. Juvenile males can disperse or alternatively they can remain in their natal groups to disperse at a later stage as adult [28]. The philopatric group-living males have to disperse from their natal groups to become either solitary-living roamers or dominant group-living territorial breeders [7,27]. Testosterone levels increased when philopatric group-living males changed into solitary-living roamers [29]. Furthermore, philopatric males became more aggressive toward same-sexed conspecifics and pups after they dispersed [30]. These studies suggest that an increase of testosterone levels may inhibit the expression of alloparental care and enhance the expression of aggressive behavior. As dispersing males showed increased testosterone and decreased corticosterone levels [31], we hypothesize that an increase of testosterone levels may cause the philopatric group-living males to be bolder (i.e. more prone to undertake risky behavior [32]), more explorative (i.e. more prone to approach a novel object [33]), and less anxious (i.e. more prone to spend time in the anxiogenic open arms than in the safer closed arms of an elevated plus maze [34]). We referred these different behaviors as "dispersal-like behavior". We tested these predictions under standardized conditions in captivity by experimentally the increasing testosterone levels of the philopatric group-living males.

2. Materials and methods

2.1. Animals and breeding conditions

The founder pairs of the striped mouse colony housed at the University of Zurich originated from individuals trapped in the Succulent Karoo in South Africa in 2002. Mice were housed under a 11:13 h light/dark regime with partly controlled temperature (approx. 23 °C). Family groups were housed in two glass tanks (50 × 30 × 30 cm) which were connected to one another with a flexible plastic tube. Additionally, one plastic cage (20 × 13 × 15 cm) was provided with nesting material. All tanks and cages had 5 cm of wood shavings for bedding. Each mouse received a 4 g seed mixture in the morning, a piece of fruit or lettuce at midday (1 g/mouse), and 2 mealworms in the afternoon. Water was provided ad libitum.

We used 11 family groups consisting of one dominant group-living territorial breeder, one breeding female and two litters. Philopatry was mimicked by leaving offspring in the family cage. Several previous studies demonstrated that the captive philopatric group-living males show similar hormonal profiles in comparison to males studied in field conditions +, with the philopatric males showing lower testosterone and higher corticosterone levels than solitary kept males, becoming sexually mature at an earlier age, developing larger testes, and producing more sperm [35–37]. When the offspring of their first litter were 21 days old, the litter was reduced in number to one philopatric group-living female and two philopatric group-living males. Each mouse was dyed for identification with a unique mark on the pelage (Rapido, Pinetown South Africa).

2.2. Experimental testosterone manipulation

We started the testosterone treatment on the day of birth of the second litter when the philopatric group-living males of the previous litter were 36.0 ± 2.4 days old. The philopatric group-living males were anesthetized with ether and implanted subcutaneously behind the neck using a precision trochar 10 gauge (Innovative Research of America, Sarasota, FL, USA). In each family, one of the two males randomly received one pellet of 3.5 mg testosterone (time-release pellets from Innovative Research of America, Sarasota, FL, USA) referred to as "test male" while his same-litter sibling received an empty pellet (placebo) referred to as "control male".

2.3. Blood collection

Blood samples were collected in the morning within one hour after the lights went on to control for a possible circadian rhythm of hormone secretions. Mice were anesthetized with ether and a blood sample of 200 μl was collected from the sub-lingual vein [38] within less than three minutes. After one hour, blood samples were centrifuged two successive times for 10 min. The resulting serum was frozen in aliquots of 50 μl for testosterone, and 10 μl for corticosterone assays until used. The blood samples were collected from each test and control male directly before the implantation (D0), a day after the implantation (D1), nine days after the implantation (D9), and 14 days (D14) after implantation.

2.4. Reproductive status and body mass monitoring

Reproductive status (scrotal, i.e. testes fully descended, or non-scrotal) and body mass (in grams) of the test and control males were recorded after each blood sample was taken.

2.5. Breeder aggression

In the African striped mice, breeding males and females could show aggressive behaviors toward the test males that could influence the expression of alloparental care. We daily recorded the frequency of aggressive behaviors (i.e. chasing, fighting, and biting) of breeding males and females toward both the test and control males during the whole experiment for 30 min.

2.6. Alloparental care

We performed daily alloparental care observations from D0 until D9. Observations were alternatively performed during the morning (between 9:00 am and 12:00 am) and the next day during the afternoon (between 03:00 pm and 06:00 pm) to cover the alloparental care observations during the whole active period of the African striped mice. Nests were videotaped for 30 min without any observers inside the animal room. The first five minutes of each video were ignored to minimize any effects of a potential disturbance as a result of the initial camera set-up. Using the software EthoLog 2.25© [39], we recorded the time spent (in seconds) by each test and control male in the nest, the time spent huddling, and the time spent licking the pups. We also recorded the frequency of carrying pups in the mouth and retrieving the pups. We considered the total amount of alloparental care provided by each test and control male as the sum of the time spent in the nest, huddling, and licking the pups. We finally considered the mean per day (%/day) of huddling, licking the time spent in the nest, and the total amount of alloparental care (i.e. huddling + Licking + time spent in the nest) for statistical analyses.

2.7. Behavioral tests

On D10, we performed three successive behavioral tests in the same order for every test and control mouse (see above). The order of these tests had no significant influence on boldness, activity, exploration, and aggression in the male African striped mice (unpublished data).

2.7.1. Boldness assessment: open field test

The subject was placed in the periphery of a neutral test arena (80 × 40 × 60 cm made of wood), and observed for five minutes. The time that the mice stay close to the wall (thigmotaxis) and the activity were used as an indicator of boldness/shyness: low thigmotaxis and high activity indicate an increase of boldness [40]. Thus, the amount of time the mouse spent with at least half a mouse length away from the arena's walls was recorded to assess increased boldness. We also measured the mouse activity. For this, we recorded every 15 s whether the mouse was moving (i.e. walking) or immobile in the open area.

2.7.2. Exploratory assessment: novel objects test

With the subject inside the same test arena, two novel objects (a fixed and a movable object: rubber tiger and table tennis ball) were placed at the opposite end of the arena. Direct observations were performed during 5 min to record latency to approach (seconds) and sniffing the object (frequency).

2.7.3. Aggressiveness assessment: dyadic encounters

Same sex encounter tests were performed in the same test arena. At the beginning, a partition in the middle divided the arena in two compartments. At one side a stimulus animal was placed. Stimulus animals were males (22–40 days old) housed in same-sex sibling groups consisting of two to three brothers from the same litter. They were removed from their families when they reached 16–21 days old and they were not genetically related to the focal males. In all cases, the focal animals (i.e. test and control males) were bigger than the stimulus animals (38.1 ± 2.6 vs. 22.1 ± 2.1; N = 16; V = 171; $p < 0.001$), as it is known that dominance is weight related in striped mice [41]. The focal animal (i.e. test or control males) was placed on the other side. After a habituation period of 5 min, the partition was removed and the focal animal was observed for 15 min. No damaging fights occurred during any dyadic encounters. The frequency of aggressive behaviors (chasing, fighting, and biting) was recorded. We also recorded the time spent in body contact and the frequency of sniffing and grooming the stimulus animal. This test has been used previously to measure aggression in the striped mice from the field [42] and in the captivity (Schradin, unpublished data).

2.7.4. Anxiety assessment: elevated plus maze

Test and control males were tested in an elevated plus maze on D12. The elevated plus maze consisted of two anxiogenic open arms and two safe enclosed arms with an open roof, arranged such that the open arms were opposite to each other. The maze was elevated to a height of 100 cm. We videotaped the number of entrance into each arm and the duration of visits inside each arm during 5 min. Two indices were used to measure the aversiveness of the open arm: the ratio of open arm entries to total arm entries (OER) [43] and the ratio of time spent in the open arms to total time spent in all arms (OTR) [34]. The activity of the mice was evaluated using the total arm entries during the trial.

2.8. Hormone assays

We performed the testosterone and corticosterone assays with commercial kits (IBL Hamburg, Germany), previously validated for striped mice serum [44]. Since corticosterone levels are very high in the philopatric group-living males [27], samples for the corticosterone assay were diluted (2:48) with the zero standard. For three samples of testosterone, the amount of serum aliquots was too small for hormone assay and was thus diluted (1: 1) with the zero standard. The intra-assay coefficient of variation was 8.98% for the testosterone and 14.84% for the corticosterone.

2.9. Data analysis

We stopped the experiments in two families because we observed wounds in test and control males, and these males had to be removed from their family units and euthanized. This reduced the sample size down to nine. Furthermore, in one family, the test male died after the third blood sample (D9). Thus, for this experiment, we did not obtain a last blood sample (D14) and we could not collect behavioral data about boldness, exploration, aggression, and anxiety.

Statistical analyses were carried out with R 2.15.0 [45]. Results are presented as mean ± SEM and significance was accepted at $\alpha \leq 0.05$. We used non-parametric statistical analyses due to small sample sizes. Each pairwise comparison (between test and control males) was performed with paired exact Wilcoxon signed rank tests and Fisher's exact test. To test for relationships of boldness and anxiety with activity, we performed Spearman rank correlations.

3. Results

3.1. Serum hormone levels

Before the treatment, the test and control males did not significantly differ in testosterone levels (0.97 ± 0.21 ng/ml vs. 0.85 ± 0.15 ng/ml; N = 9; V = 29 p = 0.50; Fig. 1a). On D1, D9, and D14, the test males showed significantly higher testosterone levels than the control males (D1: 47.08 ± 3.97 ng/ml vs. 1.88 ± 0.60 ng/ml; N = 9, V = 45, p = 0.004; D9: 20.32 ± 3.45 ng/ml vs. 1.82 ± 0.59 ng/ml; N = 9, V = 45, p = 0.004; D14: 18.64 ± 5.13 ng/ml vs. 1.64 ± 0.24 ng/ml; N = 8, V = 36, p = 0.008).

Before the treatment, the test and control males tended to differ in the corticosterone levels (876.78 ± 118.49 ng/ml vs. 510.49 ± 100.69 ng/ml; N = 8; V = 3; p = 0.08; Fig. 1b). On D1 and D9, the test males showed significantly lower corticosterone levels than the control males (D1: 517.97 ± 100.40 ng/ml vs. 1005.13 ± 251.80 ng/ml; N = 9; V = 6; p = 0.05; D9: 331.39 ± 31.82 ng/ml vs. 963,23 ± 218.41 ng/ml; N = 9; V = 5; p = 0.04). On D14, the test males tended to show lower corticosterone levels than the control males (D14: 259.65 ± 38.14 ng/ml vs. 971.38 ± 274.44 ng/ml; N = 7, V = 3, p = 0.08).

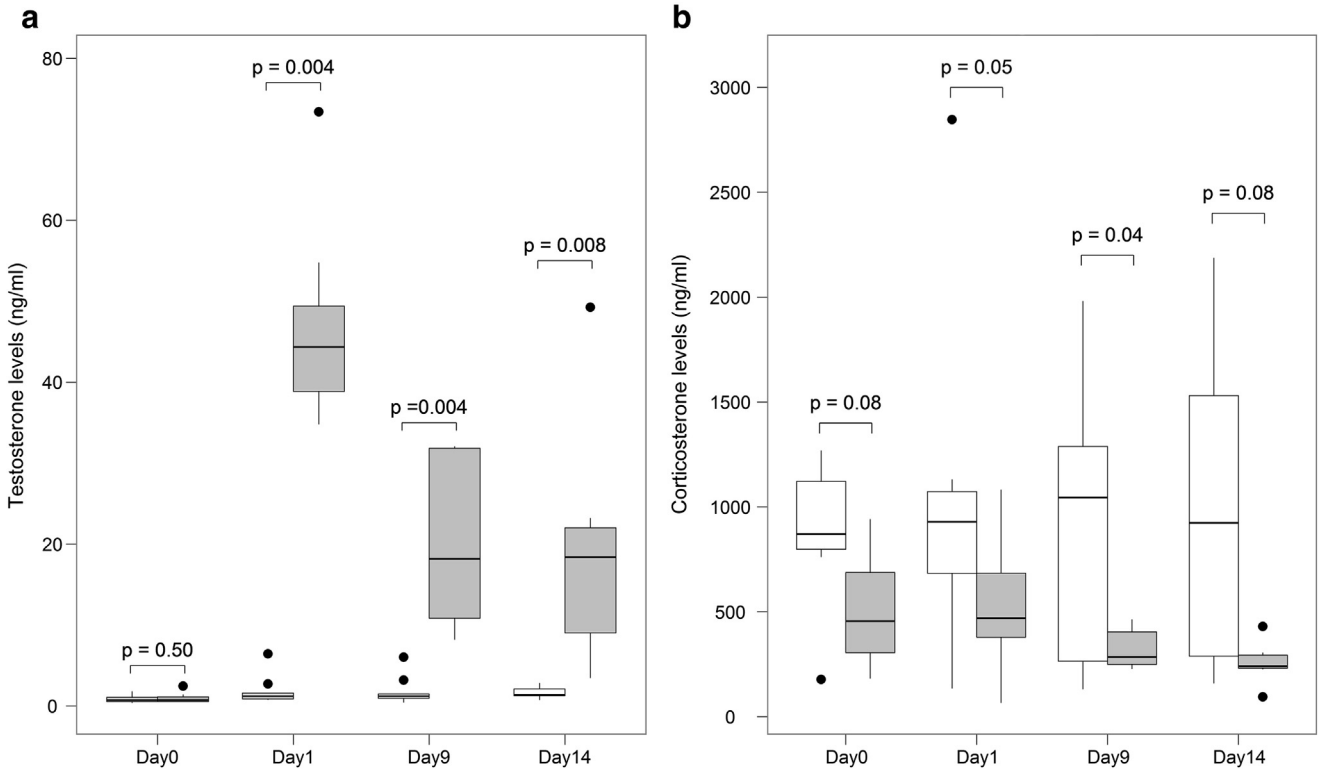

Fig. 1. Serum hormone levels before (D0), one day (D1), nine days (D9), and 14 days (D14) after the testosterone treatment in the control (white columns) and test males (gray columns); Fig. 1a: testosterone; Fig. 1b: corticosterone. The median is indicated by the horizontal bar inside the box, the first and third quartiles by the box itself, outliers are shown as black dots.

3.2. Reproductive status and body mass

Test males did not differ from control males in body mass before and during the testosterone treatment (D0: 28.92 ± 3.47 g vs. 27.94 ± 3.08 g; N = 9, V = 25, p = 0.82; D1: 31.47 ± 3.53 g vs. 30.28 ± 3.07 g; N = 9, V = 31, p = 0.34; D9: 34.67 ± 2.34 g vs. 34.89 ± 2.76 g; N = 9, V = 20, p = 0.82; D14: 34.03 ± 3.16 g vs. 34.60 ± 3.17 g; N = 8, V = 14, p = 0.64). The test males did not differ from the control males in reproductive status before and during the testosterone treatment, as most males were scrotal already on D0 (89% vs. 78%; Fisher's Exact Test: N = 9, p > 0.99), D1 (89% vs. 89%; Fisher's Exact Test: N = 9, p > 0.99), D9 (100% vs. 89%; Fisher's Exact Test: N = 9, p > 0.99), and all the test and control males were scrotal on D14.

3.3. Breeder aggression

We never observed the breeding males and females biting either the test or control males. Fighting occurred in one out of nine families and did not differ significantly between the test and control males (0.2 ± 0.2 vs. 0; N = 9; V = 1; p > 0.99). Chasing occurred in three out of nine families and did not differ significantly between the test and control males (2.7 ± 2.7 vs. 0.8 ± 0.5; N = 9; V = 3; p > 0.99).

3.4. Alloparental care

The percentage of time that males showed alloparental care did not differ between the test males and the control males (15.40 ± 5.77% vs. 5.92 ± 2.26%; N = 9; V = 34; p = 0.20; Fig. 2). The test males tended to huddle the pups longer than the control males (12.43 ± 5.23% vs. 3.90 ± 2.30%; N = 9; V = 38.5; p = 0.07) while there was no difference for the percentage of licking (0.29 ± 0.14% vs. 0.12 ± 0.06%; N = 9; V = 15; p = 0.40) and no difference for the percentage of time spent inside the nest (2.69 ± 1.43% vs. 1.89 ± 0.87%; N = 9; V = 19; p = 0.94).

3.5. Boldness and activity (open field test)

The test males tended to spend more time away from the wall than that of the control males (52.25 ± 14.77 s vs. 26.00 ± 9.61 s; N = 8; V = 31; p = 0.08; Fig. 3a). The test males were significantly more active than that of control males (61.25 ± 10.80% vs. 38.75 ±

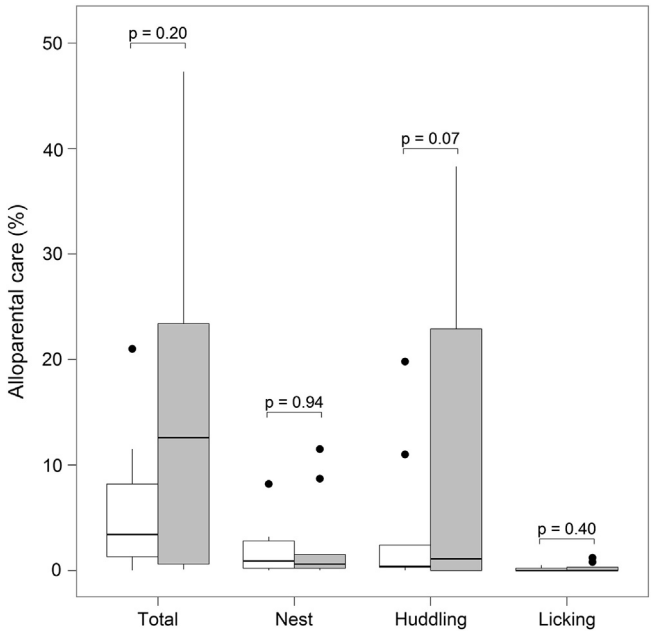

Fig. 2. Percentage of time per day that the alloparental care was shown by the control (white columns) and test males (gray columns): Total = huddling + licking + Nest; Nest = time spent inside the nest with the pups. The median is indicated by the horizontal bar inside the box, the first and third quartiles by the box itself, outliers are shown as black dots.

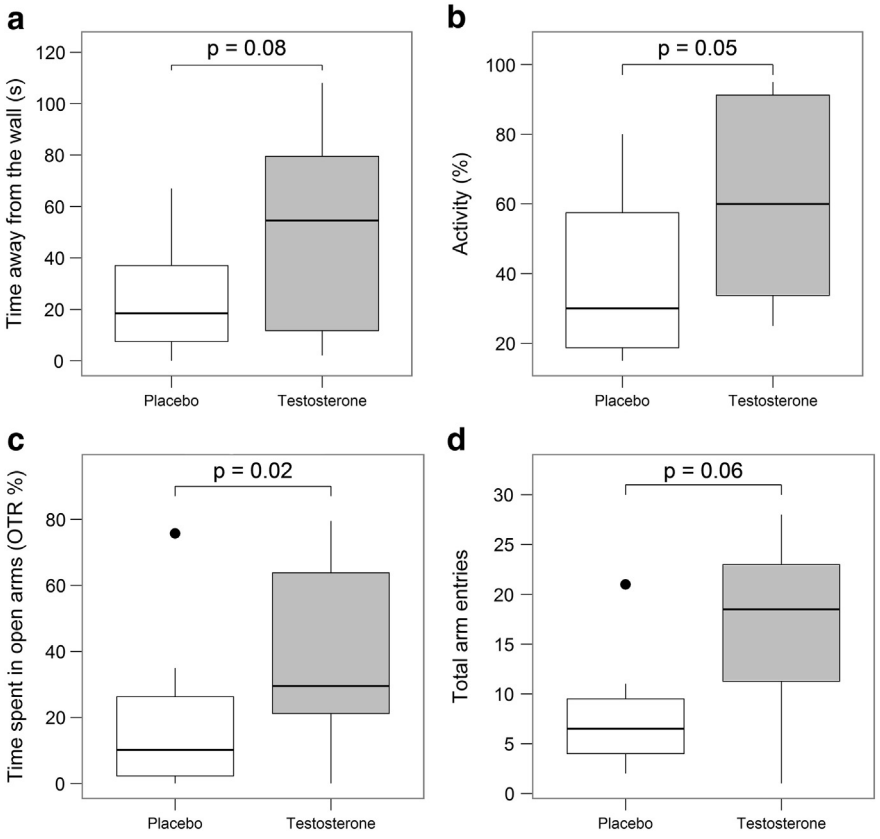

Fig. 3. Fig. 3a: time (s) away from the wall during the open field test; Fig. 3b: activity (%) of the test and control males during the open field test; Fig. 3c: time spent in the open arm (OTR; %) during the elevated plus maze test; Fig. 3d: total number of arm entries during the elevated plus maze test. The median is indicated by the horizontal bar inside the box, the first and third quartiles by the box itself, outliers are shown as black dots. Placebo (white columns) = control males; Testosterone (gray columns) = test males.

8.80%; N = 9; V = 32.5; p = 0.05; Fig. 3b). Both for the test and control males, the time spent away from the wall was significantly positively correlated with activity (test males: $r_s = 0.92$, p = 0.001; control males: $r_s = 0.88$, p = 0.004).

3.6. Exploration (novel object test)

Neither the latency to approach the fixed nor to approach the mobile object differed between the test and control males (fixed object: 101.13 ± 34.05 s vs. 148.13 ± 45.02 s; N = 8; V = 8; p = 0.35; mobile object: 118.88 ± 33.97 s vs. 147.75 ± 46.07 s; N = 8; V = 10; p = 0.31). The test males did neither sniff the fixed nor the mobile object more often than that of the control males did (fixed object: 8.50 ± 2.49 vs. 4.50 ± 1.89; N = 8; V = 17; p = 0.21; mobile object: 7.38 ± 1.80 vs. 4.75 ± 1.57; N = 8; V = 29; p = 0.14).

3.7. Aggression (dyadic encounter)

The frequency of aggressive behaviors by the test and control males toward the stimulus animal did not differ significantly (2.00 ± 0.82 vs. 1.63 ± 0.84; N = 8; V = 10; p = 0.59). The time spent in body contact with the stimulus animal did not differ significantly between the test and control males (42.63 ± 34.83 s vs. 27.63 ± 20.09 s; N = 8; V = 17; p = 0.94). There were no significant differences between the test and control males for the frequency of sniffing (8.50 ± 2.35 vs. 7.50 ± 2.99; N = 8; V = 17; p = 0.94) and for the frequency of grooming the stimulus animal (0.88 ± 0.52 vs. 0.63 ± 0.38; N = 8; V = 6; p = 0.86).

3.8. Anxiety (elevated plus maze)

The test males spent more time in the open arm (OTR) than the control males (39.82 ± 10.34% vs. 19.78 ± 9.06%; N = 8; V = 28; p = 0.02; Fig. 3c). The open entry ratio (OER) did not differ significantly between the test and control males (36.85 ± 7.58% vs. 32.72 ± 7.26%; N = 8; V = 16; p = 0.29). The test males tended to be more active (total arm entries) than the control males (16.88 ± 3.24 vs. 8.00 ± 2.12 N = 8; V = 32; p = 0.06; Fig. 3d). The OTR was significantly positively correlated with the total arm entries for the control males ($r_s = 0.83$, p = 0.01) but not for the test males ($r_s = 0.19$, p = 0.66).

4. Discussion

The up-regulation of testosterone can decrease the expression of alloparental care [24] and may facilitate dispersal [20]. In many cooperatively breeding species, juvenile males reaching puberty can either stay in their natal groups as helpers showing the alloparental care, low androgen, and glucocorticoid levels, or they can disperse, which are usually positively correlated with an increase of testosterone levels [31]. However, the extent to which testosterone influences these behavioral changes is still debated [46,47]. In the current study, we demonstrated that an experimental increase of testosterone levels in the philopatric group-living males increases activity, boldness, and decrease anxiety, i.e. traits that may facilitate dispersal [12]. However, we found no evidence that increased testosterone levels enhance aggressive behavior or decrease the expression of alloparental care.

The 3.5 mg testosterone pellets increased serum testosterone levels above the maximum physiological levels measured in the field samples (15 ng/ml; Schradin unpublished data). This was unexpected as in other rodent species of similar body mass and age, 5 mg testosterone doses resulted in a much lower increase of testosterone levels (in Syrian hamsters: increase from 1 ng/ml to 4 ng/ml [48]; in castrated adult male Rockland–Swiss albino mice: increase to 4.5 ng/ml [49]). One hypothesis might be species differences in the metabolism of testosterone, i.e. a higher conversion rate of testosterone into its metabolites in

the Syrian hamsters and wild house mice than in the African striped mice. As we induced supra-physiological testosterone levels, our results have to be discussed cautiously. A physiological increase of testosterone levels might have had different effects, which could explain why we did not find an effect on the aggression and alloparental care. Importantly, we found the expected effects on the dispersal related behaviors, indicating that our supra-physiological testosterone levels did not induced unresponsiveness to testosterone, for example via reduced androgen or estrogen receptor expression. However, the aggression, alloparental care, and dispersal related behaviors are regulated by different brain areas that might respond differently to very high testosterone levels. In conclusion, while the very high testosterone levels only influenced dispersal-like behaviors, it is currently unknown what effect physiologically relevant levels would have had on the aggression and on the alloparental care.

In the African striped mice, both paternal group-living territorial breeders and non-paternal solitary-living roamers display high testosterone levels [26,27] but differ in prolactin levels [50], indicating that prolactin may mediate paternal care [51,52]. However, the African striped mouse philopatric males show low prolactin levels which indicate that alloparenting is mediated by different mechanisms [50]. Our results show that the test males did not show significantly less alloparenting than the control males. Interestingly, the test males even tended to huddle pups more often than the control males. Our results are surprising because free-ranging dispersing males showing high testosterone levels are more aggressive toward pups than non-dispersing philopatrics males showing low testosterone levels [30,31]. As exogenous testosterone alters alloparenting in other mammal species, for instance, in prairie voles [24], our results support the hypothesis that testosterone effects on parental care are species specific in mammals [23].

The breeding males and females showed very few aggressive behaviors toward male helpers, and they did not show more aggression toward test than the control males. This suggests that the behavior of breeders did not influence the display of alloparental care in our study. Our results also suggest that the test males might be insensitive to the increased testosterone levels during the experiment. Similarly, exogenous testosterone did not reduce paternal care in the Puerto Rican frogs, *Eleutherodactylus coqui* [53] and in the Chestnut-collared longspurs, *Calcarius ornatus* [54]. The behavioral insensitivity to testosterone may also explain why the test and control males did not differ in aggressive behavior. Thus, our results might be either due to the non-readiness of the brain to respond to testosterone signals [46] or to the adverse effects of testosterone supra-physiological levels produced by 3.5 mg testosterone pellets, for example, by downloading androgen receptors [55] or estrogen receptor α, which are important in the regulation of social behavior [56].

While we found no significant effect of testosterone on the alloparental care and aggression, we observed significant changes in the activity, boldness, and anxiety-like behaviors. The test males tended to be bolder and also significantly more active than the control males during the open-field tests. While boldness was significantly and positively correlated with activity, the test males also spent significantly more time in the open arms of the elevated plus maze regardless of their levels of activity (i.e. total arm entries). Thus, increased testosterone levels increase boldness and this indicates a significant anxiolytic effect of testosterone in the captive philopatric male African striped mice. These results contrast with the non-conclusive effects of testosterone on the alloparenting and aggression. Activation of androgen receptors in the hypothalamus is necessary to reduce anxiety in mice and rats [57,58]. If the supra-physiological levels of testosterone had down-regulated androgen receptors, reducing brain responsiveness to testosterone, we would expect no significant difference in anxiety-like behavior between the test and control males.

The test males showed significantly lower corticosterone levels than the control males. Our result indicates that an increase of testosterone decreases the basal corticosterone levels of the philopatric males. Interestingly, the test males also showed lower anxiety-related responses during the elevated plus maze than the control males. In laboratory mice, it is well known that lower basal corticosterone levels decrease the reactivity of the hypothalamus-pituitary-adrenal (HPA) axis [59]. In the African striped mice, the lower basal corticosterone levels induced by the increase of testosterone levels might be related to a decreased stress response, for example during the open-field test and elevated plus maze test. In other words, changes in serum testosterone levels might mediate the reactivity of the HPA axis, which could influence how the philopatric males cope with stressful situations such as dispersal [12].

Important questions are whether it is the changes in hormones that drive behavioral changes to disperse in free ranging individuals, or whether it is the decision to disperse that drives the changes in hormone levels, or a combination of both. At present, we cannot fully answer these questions. An experimental field study demonstrated that males showed an increase of testosterone levels and a decrease of corticosterone levels after they dispersed, but it was not known whether these hormonal changes occurred before or after dispersal [31]. However, males that had dispersed in this experimental field study were already scrotal before dispersal [30], while males that remained philopatric were often non-scrotal, indicating that physiological changes might have been initiated before dispersal [31]. In laboratory conditions, the testes of philopatric males are as good in producing testosterone as those of solitary males, indicating that philopatric males could quickly increase testosterone levels [36]. Our present study gives support to the hypothesis that changes in hormone levels induce behavioral changes, as an experimental increase of testosterone levels increased dispersal-like behaviors, which would increase the likelihood of dispersal in the field. Under field conditions, philopatric males with experimentally increased testosterone levels responded similarly, by decreasing their corticosterone levels and by increasing their home ranges, suggesting that they explored possibilities for the dispersal [18]. Yet, we would expect a combination: hormonal changes induce behavioral changes and these, in a kind of positive feedback, may then increase hormonal differences.

5. Conclusion

Our results support the role of testosterone in important behavioral and physiological changes associated with male dispersal. The up-regulation of testosterone seems to facilitate the dispersal-like behavior either through direct testosterone effects or, perhaps, through the reduction of the reactivity of the HPA axis when the philopatric males are coping with dispersing opportunities. These two hypotheses are not mutually exclusive and both involve testosterone actions. We previously demonstrated that an experimental increase of testosterone levels caused juvenile philopatric group-living males to expand their home ranges in the African striped mice [18]. However, the decision to disperse relies on other factors; population density and reproductive competition are critical predictors of the dispersal in male African striped mice [8,19]. Thus, ecological factors may regulate testosterone signals which facilitate behavioral, physiological, and morphological changes needed to disperse [18] supporting the role of testosterone in dispersal.

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References

[1] Lott DF. Intraspecific variation in the social system of wild vertebrates. New York: Cambridge University Press; 1991.
[2] Gross MR. Alternative reproductive strategies and tactics: diversity within sexes. Trends Ecol Evol 1996;11:92–8.
[3] Oliveira RF, Ros AFH, Goncalves DM. Intra-sexual variation in male reproduction in teleost fish: a comparative approach. Horm Behav 2005;48:430–9.
[4] Taborsky M. Bourgeois and parasitic tactics: do we need collective, functional terms for alternative reproductive behaviours? Behav Ecol Sociobiol 1997;41:361–2.
[5] Oliveira RF, Canário AVM, Ros AFH. Hormones and alternative reproductive tactics in vertebrates. In: Oliveira RF, Taborsky M, Brockmann HJ, editors. Alternative Reproductive Tactics. Cambridge: Cambridge University Press; 2008. p. 132–73.
[6] Schradin C, Lindholm AK, Johannesen J, Schoepf I, Yuen C-H, Koenig B, et al. Social flexibility and social evolution in mammals: a case study of the African striped mouse (Rhabdomys pumilio). Mol Ecol 2012;21:541–53.
[7] Schradin C, Lindholm AK. Relative fitness of alternative male reproductive tactics in a mammal varies between years. J Anim Ecol 2011;80:908–17.
[8] Schoepf I, Schradin C. Better off alone! Reproductive competition and ecological constraints determine sociality in the African striped mouse (*Rhabdomys pumilio*). J Anim Ecol 2012;81:649–56.
[9] Moore MC. Application of organizational activation theory to alternative male reproductive strategies — a review. Horm Behav 1991;25:154–79.
[10] Moore MC, Hews DK, Knapp R. Hormonal control and evolution of alternative male phenotypes: generalizations of models for sexual differentiation. Am Zool 1998;38:133–51.
[11] Nelson RJ. An introduction to behavioral endocrinology. Third ed. Sunderland: Sinauer Associates, INC.; 2005.
[12] Holekamp KE, Smale L, Simpson HB, Holekamp NA. Hormonal influences on natal dispersal in free-living belding ground-squirrels (spermophilus-beldingi). Horm Behav 1984;18:465–83.
[13] Nunes S, Duniec TR, Schweppe SA, Holekamp KE. Energetic and endocrine mediation of natal dispersal behavior in Belding's ground squirrels. Horm Behav 1999;35:113–24.
[14] Metzgar LH. An experimental comparison of screech owl predation on resident and transient white-footed mice (*Peromyscus Leucopus*). J Mammal 1967;48:387–91.
[15] Solomon NG. A reexamination of factors influencing philopatry in rodents. J Mammal 2003;84:1182–97.
[16] Wolf JO. More on juvenile dispersal in mammals. Oikos 1994;71:349–52.
[17] Aikey JL, Nyby JG, Anmuth DM, James PJ. Testosterone rapidly reduces anxiety in male house mice (Mus musculus). Horm Behav 2002;42:448–60.
[18] Raynaud J, Müller K, Schradin C. Experimental increase of testosterone levels in free-ranging juvenile male African striped mice (*Rhabdomys pumilio*) induces physiological, morphological, and behavioral changes. Gen Comp Endocrinol 2012;178:108–15.
[19] Schradin C, König B, Pillay N. Reproductive competition favours solitary living while ecological constraints impose group-living in African striped mice. J Anim Ecol 2010;79:515–21.
[20] Schoech SJ, Reynolds SJ, Boughton RK. Endocrinology. In: Koenig W, Dickinson J, editors. Ecology and Evolution of Cooperative Breeding in Birds. Cambridge: Cambridge University Press; 2004.
[21] Wingfield JC, Hegner RE, Dufty AM, Ball GF. The challenge hypothesis — theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. Am Nat 1990;136:829–46.
[22] Hirschenhauser K, Oliveira RF. Social modulation of androgens in male vertebrates: meta-analyses of the challenge hypothesis. Anim Behav 2006;71:265–77.
[23] Storey AE, Delahunty KM, McKay DW, Walsh CJ, Wilhelm SI. Social and hormonal bases of individual differences in the parental behaviour of birds and mammals. Can J Exp Psychol 2006;60:237–45.
[24] Roberts RL, Zullo A, Gustafson EA, Carter CS. Perinatal steroid treatments alter alloparental and affiliative behavior in prairie voles. Horm Behav 1996;30:576–82.
[25] Young AJ, Carlson AA, Clutton-Brock T. Trade-offs between extraterritorial prospecting and helping in a cooperative mammal. Anim Behav 2005;70:829–37.
[26] Schradin C, Pillay N. Paternal care in the social and diurnal striped mouse (*Rhabdomys pumilio*): laboratory and field evidence. J Comp Psychol 2003;117:317–24.
[27] Schradin C, Scantlebury M, Pillay N, Koenig B. Testosterone levels in dominant sociable males are lower than in solitary roamers: physiological differences between three male reproductive tactics in a sociably flexible mammal. Am Nat 2009;173:376–88.
[28] Schradin C, Pillay N. Intraspecific variation in the spatial and social organization of the African striped mouse. J Mammal 2005;86:99–107.
[29] Schradin C, Yuen C-H. Hormone levels of male African striped mice change as they switch between alternative reproductive tactics. Horm Behav 2011;60:676–80.
[30] Schoepf I, Schradin C. Flexibility in social behaviour and predispositions to change reproductive tactics in African striped mice (*Rhabdomys pumilio*). Anim Behav 2012;84:1159–67.
[31] Schoepf I, Schradin C. Endocrinology of sociality: comparisons between sociable and solitary individuals within the same population of African striped mice. Horm Behav 2013;64:89–94.
[32] Wilson DS, Clark AB, Coleman K, Dearstyne T. Shyness and boldness in humans and other animals. Trends Ecol Evol 1994;9:442–6.
[33] Powell SB, Geyer MA, Gallagher D, Paulus MP. The balance between approach and avoidance behaviors in a novel object exploration paradigm in mice. Behav Brain Res 2004;152:341–9.
[34] Pellow S, Chopin P, File SE, Briley M. Validation of open — closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149–67.
[35] Schradin C, Schneider C, Yuen CH. Age at puberty in male African striped mice: the impact of food, population density and the presence of the father. Funct Ecol 2009;23:1004–13.
[36] Schradin C, Eder S, Müller K. Differential investment into testes and sperm production in alternative male reproductive tactics of the African striped mouse (*Rhabdomys pumilio*). Horm Behav 2012;61:686–95.
[37] Schradin C, William R, Krackow S, Carter S. Staying put or leaving home: endocrine, neuroendocrine and behavioral consequences in male African striped mice. Horm Behav 2013;63:136–43.
[38] Heimann M. Development and validation of the method of sublingual blood sampling in mice and other small rodents. (PhD thesis) University of Zurich; 2006.
[39] Ottoni EB. EthoLog 2.2: a tool for the transcription and timing of behavior observation sessions. Behav Res Methods Instrum Comput 2000;32:446–9.
[40] Sneddon LU. The bold and the shy: individual differences in rainbow trout. J Fish Biol 2003;62:971–5.
[41] Schradin C. Territorial defense in a group living solitary forager: who, where, against whom? Behav Ecol Sociobiol 2004;55:439–46.
[42] Schradin C, Schneider C, Lindholm AK. The nasty neighbour in the striped mouse (*Rhabdomys pumilio*) steals paternity and elicits aggression. Front Zool 2010;7:19.
[43] Handley SL, Mithani S. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of fear-motivated behavior. Naunyn Schmiedebergs Arch Pharmacol 1984;327:1–5.
[44] Schradin C. Seasonal changes in testosterone and corticosterone levels in four social classes of a desert dwelling sociable rodent. Horm Behav 2008;53:573–9.
[45] R Development Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2012 [ISBN 3-900051-07-0, URL http://www.R-project.org/].
[46] Lynn SE. Behavioral insensitivity to testosterone: why and how does testosterone alter paternal and aggressive behavior in some avian species but not others? Gen Comp Endocrinol 2008;157:233–40.
[47] Gleason ED, Fuxjager MJ, Oyegbile TO, Marler CA. Testosterone release and social context: when it occurs and why. Front Neuroendocrinol 2009;30:460–9.
[48] Romeo RD, Schulz KM, Nelson AL, Menard TA, Sisk CL. Testosterone, puberty, and the pattern of male aggression in Syrian hamsters. Dev Psychobiol 2003;43:102–8.
[49] Barkley MS, Goldman BD. Effects of castration and silastic implants of testosterone on intermale aggression in mouse. Horm Behav 1977;9:32–48.
[50] Schradin C. Differences in prolactin levels between three alternative male reproductive tactics in striped mice (*Rhabdomys pumilio*). Proc R Soc Lond B Biol Sci 2008;275:1047–52.
[51] Wynne-Edwards KE, Timonin ME. Paternal care in rodents: weakening support for hormonal regulation of the transition to behavioral fatherhood in rodent animal models of biparental care. Horm Behav 2007;52:114–21.
[52] Schradin C. Comments to K.E. Wynne-Edwards and M.E. Timonin 2007. Paternal care in rodents: weakening support of hormonal regulation of the transition to behavioral fatherhood in rodent animal models of biparental care, Horm & Behav 52:114–121. Horm Behav 2007;52:557–9.
[53] Townsend DS, Palmer B, Guillette LJ. The lack of influence of exogenous testosterone on male parental behavior in a neotropical frog (eleutherodactylus) — a field experiment. Horm Behav 1991;25:313–22.
[54] Lynn SE, Hayward LS, Benowitz-Fredericks ZM, Wingfield JC. Behavioural insensitivity to supplementary testosterone during the parental phase in the chestnut-collared longspur, — *Calcarius ornatus*. Anim Behav 2002;63:795–803.
[55] Handa RJ, Burgess LH, Kerr JE, Okeefe JA. Gonadal-steroid hormone receptors and sex-differences in the hypothalamo-pituitary-adrenal axis. Horm Behav 1994;28:464–76.
[56] Cushing BS, Perry A, Musatov S, Ogawa S, Papademetriou E. Estrogen receptors in the medial amygdala inhibit the expression of male prosocial behavior. J Neurosci 2008;28:10399–403.
[57] Zuloaga DG, Morris JA, Jordan CL, Breedlove SM. Mice with the testicular feminization mutation demonstrate a role for androgen receptors in the regulation of anxiety-related behaviors and the hypothalamic-pituitary-adrenal axis. Horm Behav 2008;54:758–66.
[58] Zuloaga DG, Poort JE, Jordan CL, Breedlove SM. Male rats with the testicular feminization mutation of the androgen receptor display elevated anxiety-related behavior and corticosterone response to mild stress. Horm Behav 2011;60:380–8.
[59] Touma C, Bunck M, Glasl L, Nussbaumer M, Palme R, Stein H, et al. Mice selected for high versus low stress reactivity: a new animal model for affective disorders. Psychoneuroendocrinology 2008;33:839–62.