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Paternal and infanticidal behavior in the Mongolian gerbil (*Meriones unguiculatus*): An approach to neuroendocrine regulation

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ABSTRACT

This study aimed to provide evidence on estrogen and androgen pathways regulating the Mongolian gerbil's paternal and infanticidal behaviors (Meriones unguiculatus). We analyzed estrogen receptor alpha (ERα) and androgen receptor (AR) distribution in the medial preoptic area (mPOA), the bed nucleus of stria terminalis (BNST), as well as the anterior hypothalamic nucleus (AHN), the ventromedial hypothalamus nucleus (VMH), and the periaqueductal gray area (PAG) nuclei activated when males interact paternally or aggressively with the pups, respectively. Twenty aggressive males towards the pups and 10 paternal were selected through a screen paternal behavior test. Three groups of 10 males each were formed: paternal males (PAT), males with testosterone (T)-induced paternal behavior (T-PAT), and aggressive males (AGG). Male gerbils could interact with a pup for a few minutes, and their brains were removed and dissected for ERα and AR immunoreactivity (ir). The results showed that in T-PAT and PAT males, the number of ERα-ir and AR-ir cells in the mPOA/BNST was significantly higher than in AGG males. In AGG males, the number of ERα-ir and AR-ir cells in the AHN/VMH/PAG was significantly higher than PAT and T-PAT males. This difference in the presence of ERα and AR in nuclei activated in paternal interactions in the Mongolian gerbil supports the idea that these receptors participate in regulating paternal behavior. Also, these results suggest, for the first time, that they could be involved in the infanticidal behavior in this rodent.

1. Introduction

In mammals, paternal behavior is uncommon, only occurring between 9–10 % of the mammalian genera [1]. Paternal behavior is defined as any activity carried out by the male for the benefit of the offspring and that increases their survival [2]. Regardless, some male rodents are known to crouch over the pups, perform pup retrieval, provide grooming, and socialize with their offspring [2].

Depending on the species, a proportion of virgin males display paternal or infanticidal behavior. In dwarf hamster (Phodopus campbelli), 73.34 % of virgin males are spontaneously paternal [3], while in the Mongolian gerbil (Meriones unguiculatus), 87.5 % of virgin males display infanticidal behavior. However, in the Mongolian gerbil these proportions vary according to age since older males are more likely to exhibit aggression towards unfamiliar pups of their species than younger adults [4]. For example, most of the virgin gerbils from 79 to 90 days of age do not attack the pups of their species [5]. Likewise, it is less likely that gerbils attack the young when the birth of their pups is near [6]. In addition, virgin males of the Mongolian gerbil that are aggressive with the pups display paternal behavior after they copulate [7]. There is some evidence that hormonal changes facilitate the transition from infanticidal to paternal. Hormones implicated include vasopressin, oxytocin, as well as testosterone (T), and its metabolites estradiol and dihydrotestosterone [3,4,8–12]. Among these hormones, T and its metabolites have been the most studied.

In the California mouse (Peromyscus californicus) and the Mexican volcano mouse (Neotomodon alstoni), T promotes paternal cares in castrated males [9–11]. In the dwarf hamster, males that were

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aggressive to pups transitioned to paternal after being treated with estradiol [3]. In the Mongolian gerbil, it was initially reported that castration increases paternal behavior and T replacement decreases this behavior [13]. However, our research group has shown that virgin males that displayed aggression towards the pups transitioned to paternal after being castrated and received T, estradiol, or dihydrotestosterone replacement [4].

In the laboratory rat, when virgin females maternally interact with unfamiliar pups of their species, the medial preoptic area (mPOA) and bed nucleus of stria terminalis (BNST) are significantly activated [14,15]. Vast experimental evidence has shown that these nuclei, mainly MPOA, have an essential function in the regulation of maternal behavior [16,17]. In males of the California mouse, electrolyte lesions in the mPOA, nucleus accumbens, and MeA cause a decrease in the time invested in retrieving, crouching, and grooming [18,19]. On the other hand, when aggressive virgin female laboratory rat interacts with unfamiliar pups of their species, anterior hypothalamic nucleus (AHN), ventromedial hypothalamus nucleus (VMH), and periaqueductal gray area (PAG) become activated [14,15,20]. The AHN, VMH, and PAG are components of the neural circuit that mediates aversive behavior towards pups in female laboratory rats [14,21,22]. Excitotoxic lesions of the AHN and VMH stimulate the display of maternal care in nulliparous rats treated with estradiol and progesterone [23]. Recently, we showed that in the Mongolian gerbil, using c-Fos as a neural marker, mPOA and BNST are significantly activated when virgin paternal males give paternal cares to unfamiliar pups. Meanwhile, aggressive interactions with pups activate the AHN, VMH, and PAG [24]. These results suggest that the neural regulation of paternal behavior in the Mongolian gerbil includes positive and negative mechanisms as in maternal behavior.

As mentioned above, in the Mongolian gerbil, virgin males aggressive with unfamiliar pups of their species display paternal behavior when primed with T, estradiol, or dihydrotestosterone. These results strongly suggest that T regulates paternal behavior through estrogenic and androgenic pathways [4]. In addition, Martínez et al. [7], reported that paternal gerbils and fathers have a significantly higher presence of estrogen receptor alpha (ERα) and androgen receptor (AR) in the mPOA than non-paternal gerbils. However, in this study paternal gerbils interacted with the pups shortly after mating. Therefore, mating instead of paternal behavior could be the cause of the increase in ERα and AR in mPOA.

In our laboratory, previous observations showed that aggressive virgin males of Mongolian gerbil have a high number of ERα and AR in AHN, VMH/PAG after interactions with the pups. The presence of these receptors suggests that they could be involved in regulating aggressive behavior towards pups. Furthermore, under the paradigm that T participates in the control of most male rodents' social behaviors, by its conversion to estradiol or dihydrotestosterone [25], it could also participate in regulating the infanticidal behavior through the estrogenic and androgenic pathways. This study aimed to provide evidence that estrogen and androgen pathways participate in the regulation of paternal and infanticidal behaviors through the presence of ERα and AR in cells at nuclei that become activated when males are paternal (mPOA and BNST) and when the males interacted aggressively with pups (AHN, VMN, and PAG).

2. Material and methods

2.1. Animals

We used virgin male Mongolian gerbils 180–210 days old. Virgin male gerbils were used to avoid the effect of sexual experience. Gerbils were weaned at 25 through 28 days of age. The animals were obtained from a breeding colony kept at the Facultad de Estudios Superiores Iztacala, UNAM. They were kept under an inverted photoperiod of 12:12 h (light-dark cycle; onset of light at 1800 h) at an ambient temperature between 17 and 21 °C. Gerbils were fed pellets of Lab Chow 5001 (Nutrimentos Purina, México) and tap water *ad libitum*. Throughout the study, two or three gerbil's siblings of the same sex were housed in a polycarbonate cage (37 × 27 × 15 cm) with sawdust bedding. The paternal behavior tests the gerbils were individually removed from their cage and placed in the test cage during the paternal. At the end of the experimental procedure (paternal behavior tests or extraction of blood samples) the gerbils were returned to their cage, except the five males from each group that were used for immunohistochemistry. The gerbils that sharing the same cage were subjected to experimental procedure the same day.

During the paternal behavior test, each male was placed in a polycarbonate cage (37 × 27 × 15 cm) with clean sawdust bedding. After 10 min, two 1- to 3-day-old pups were placed in the cage. Paternal males smell and touch young with their nose and groom and crouch beside them. The male-pup paternal interactions lasted 60 min. Sniffing is a behavior that is observed in both paternal and aggressive males, so to determine whether a gerbil is aggressive with the pups, attack behavior was used. The pups were quickly withdrawn whether they were attacked by the male. Forty-eight virgin males were subject to screen test of paternal behavior, resulting in 38 aggressive males and 10 paternal males, of these we only use ten paternal males and 20 aggressive. These gerbils were organized into three groups with ten animals each: the first group included paternal males (PAT). The second group included males that were initially aggressive but primed with T for inducing paternal behavior after the screening test of paternal behavior (T-PAT). The third group included males that were aggressive towards pups, these animals are referred to as "aggressive males" (AGG). Aggressive males were randomly assigned to the T-PAT and AGG Groups. The induction of paternal behavior in the Mongolian gerbil is a routine procedure in our laboratory. Previous studies have shown that T administration to aggressive virgin males triggers the onset of paternal behavior [4,14]. We included the T-PAT group because males of this group have higher T concentrations than spontaneously paternal males due to treatment with this hormone, so we associate T concentrations with the number of ERα and AR immunoreactive cells in mPOA and BNST.

Between 15 and 20 days after the screening test of paternal behavior, males of the PAT, T-PAT, and AGG group were subjected to second paternal behavior test. During these tests, the males of the PAT and T-PAT groups had paternal interactions with the pups, while that the males of the AGG aversively interacted with the pups.

All the experiments were performed under the ethical guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023) and the ethical guidelines and technical specifications of the Mexican Official Norm for the Production, Care and Use of Laboratory Animals [26].

2.2. Induction of paternal behavior in aggressive males

Aggressive gerbils (T-PAT group) were primed with a T solution (1 mg/10 mL sesame oil, Sigma-Aldrich, St. Louis, MO, USA) [4,13]. Each gerbil received a subcutaneous injection of 0.1 mL of T solution in the dorsal region after asepsis of the zone with benzalkonium chloride. Paternal and aggressive interactions (second test of paternal behavior) were carried out 24 h after T administration, the time needed for the animals to display paternal behavior [4,13,27,28].

2.3. Interactions with pups

Paternal and aggressive interactions were evaluated following the method described above for screening tests of paternal behavior. Two pups were used in each test, these pups were used only once, and returned to their parents. If a male was aggressive, the test was stopped as soon as the attack started. Five pups were slightly bitten, and their wounds were treated with gentian violet (1%), used since this antiseptic has no odor. In aggressive interactions, no behavior was recorded because the pups were quickly removed from the male's cage when they

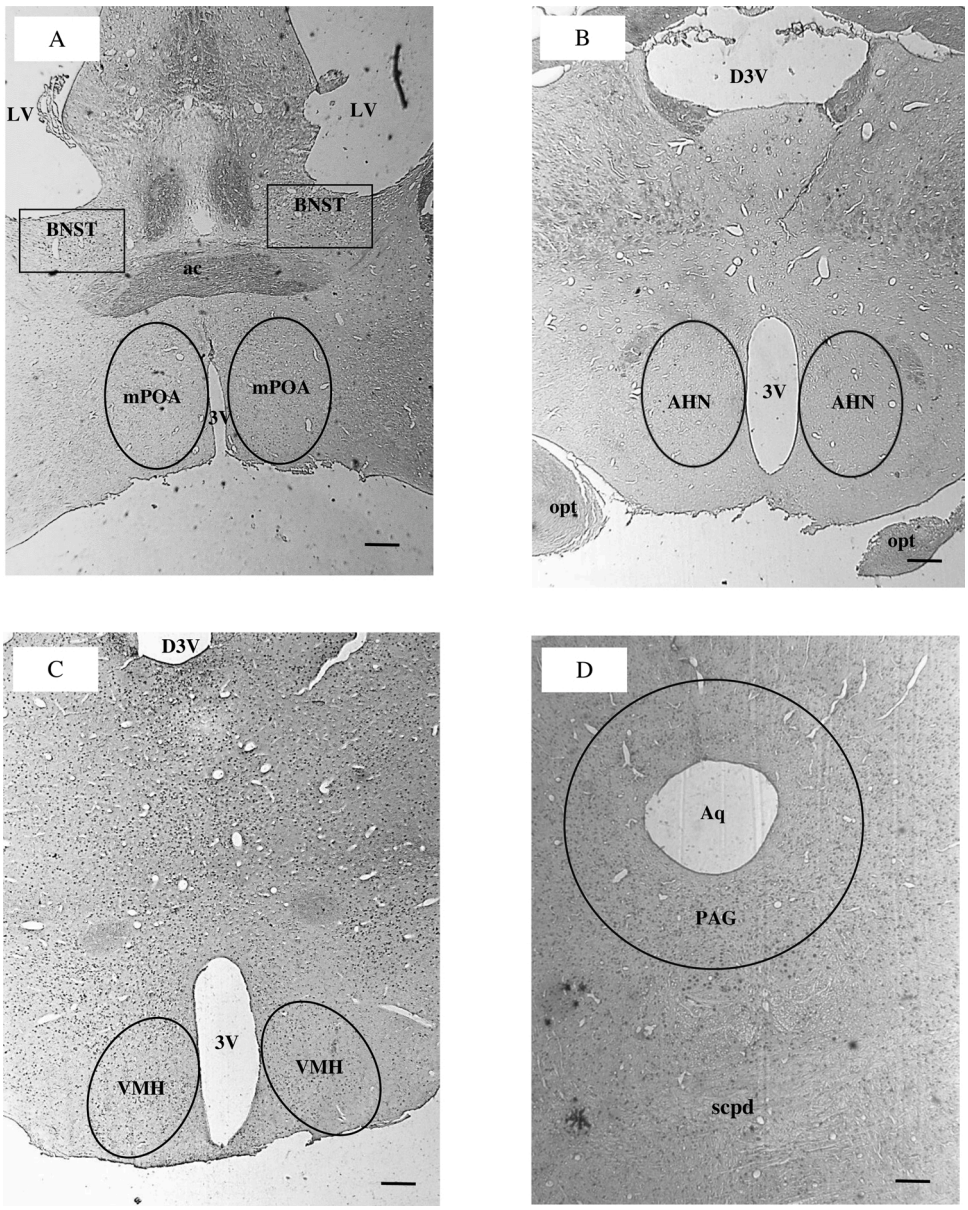

Fig. 1. Representative photomicrographs showing the location of neural áreas: A) medial preoptic area (mPOA), A) bed nucleus of stria terminalis (BNST), B) anterior hypotalamic nucleus (AHN), C) ventromedial hypothalamic nucleus (VMH) and D) periaqueductal gray (PAG). 3 V = third ventricle, LV = lateral ventricle, ac = anterior commissure, D3 V = dorsal third ventricle, opt = optic tract, scpd = superior cerebellar peduncle, Aq = aqueduct. Coronal sections, scale bars = 100 μm.

were attacked. The male-pup paternal interactions were videotaped for 60 min with a high-definition infrared camera (IR Bullet camera, 2.1 megapixels). Latencies of the onset of paternal behavior (the length of time until the male contacted the pups) and the time invested in crouching and grooming were noted. Behavioral observations were recorded in the dark of the inverted photoperiod under red light illumination, corresponding to between 11:00 and 14:00 h.

2.4. Hormone assay

Immediately after the male-pup interaction test, blood samples (250 μL) were collected from the retro-orbital sinus of all males of the three groups. Previously, the gerbils were lightly anesthetized (5 mg/kg xylazine and 60 mg/kg ketamine, i.p.). Blood samples were taken with heparinized tubes. It took less than a minute to sample each animal. Sampling was performed between 11:00 and 14:00 h. Plasma was separated by centrifugation and stored at −70 °C.). Hormonal analysis was performed by ELISA. T level was measured with a DRG commercial kit (Diagnostics, Frauenbergstr 18, D-35,039 Marburg, Germany) with a sensitivity of 0.083 ng/mL. The intra- and inter-assay coefficients of variation were 3.9 % and 4.6 %, respectively. The T assay was validated, and a good correlation between Mongolian gerbils serum dilutions and the standard curve was found. The recovery rate for T was 95.0 % (r = 1.0). The plate was read in a plate reader (Thermo Electron Corporation, model Multiskan Ascent V1.25, with a 450-nm filter).

2.5. ERα and AR immunohistochemistry

After taking the blood sample, five gerbils from each group were randomly chosen for perfusion. They were deeply anesthetized with an i. p. dose of 10 mg/kg xylazine and 90 mg/kg ketamine. The animals were perfused with physiological saline (0.9 %) through the heart, followed by a 2% paraformaldehyde solution in sodium phosphate buffer 0.1 M pH 7.6. The brain was then removed and post-fixed for 18 h in the same

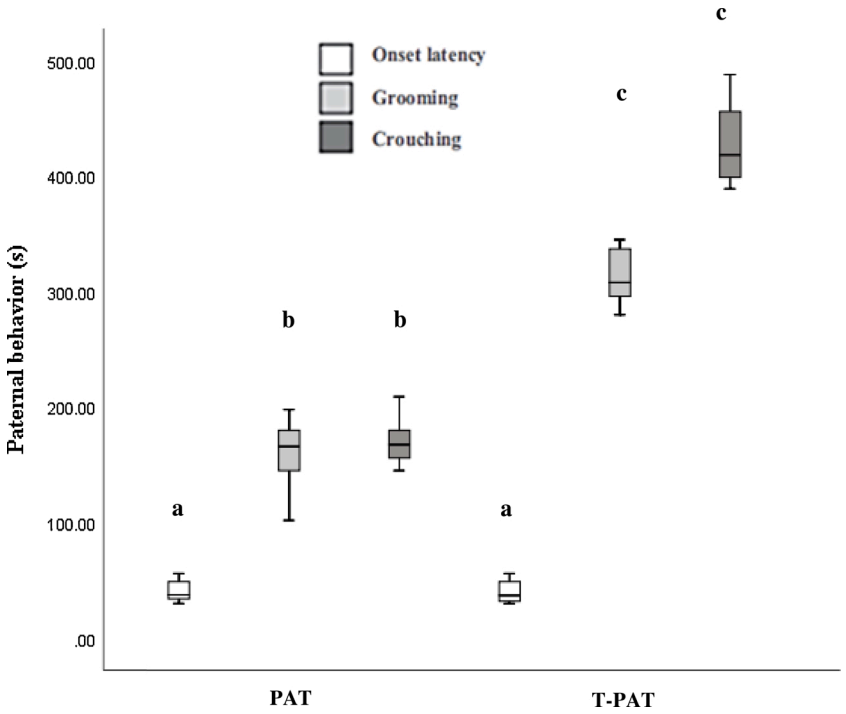

Fig. 2. Onset latency of paternal behavior of the males of the PAT and T-PAT was not significantly different. The males of the T-PAT spent more time in crouching and grooming than males of the PAT ($P < 0.05$). Data are presented as the median. Letters indicate significant differences.

fixative solution. Subsequently, this tissue was processed and cut into 30-μm-thick coronal sections with a microtome. The neural areas were located by comparison with the stereotaxic atlas of the Mongolian gerbil [29], including the mPOA and BNST (both −0.1 mm, image 520, the same section, Fig. 1), AHN (both −0.8 mm, image 590, the same section, Fig. 1), VMH (−1.3 mm, image 640, Fig. 1) and PAG (−3.5 mm, image 860, Fig. 1). The sections were placed on gelatinized slides (Nutrient Gelatin, 70151-500G-F, Sigma-Aldrich, CA, USA). After each of the following steps, sections received a rinse in PBS for 5 m in. 1) 10-min incubation in 3% H_2O_2 in PBS, 2) 20-min incubation in 5% normal goat serum (Vector Laboratories, Vectastain ABC kit, PK-4000) in PBS, and 3) 16-h incubation at 25 °C with a 1:300 dilution of a rabbit ERα antibody epitope located in the C-terminus of the human estrogen receptor, or alpha protein (Thermo Fisher Scientific, PA5-16440), or AR antibody epitope located in the N-terminus of the rat AR (Thermo Fisher Scientific, PA5-16750) in PBS. After the last 5-min rinse in PBS, the sections were incubated with a biotinylated goat anti-rabbit antibody (Vector Laboratories, Vectastain ABC kit, PK-6102) in PBS for 90 min and rinsed twice in PBS. The sections were then incubated with an avidin-biotin complex (Vector Laboratories, Vectastain ABC kit, PK-6100) for 30 min, followed by two more PBS rinses. Finally, binding was visualized using diaminobenzidine as the chromogen (Vector Laboratories, DAB Peroxidase Substrate, SK-4100). The slides were dehydrated and then cover-slipped. Negative controls were obtained by omitting the primary antibody incubation step. The same assay kit was used to process all neural tissues. The ERα antibody was validated utilizing oviduct and kidney as positive and negative controls, respectively. The specificity of the AR antibody was validated utilizing epididymis and kidney as the positive and negative controls. Both antibodies have already been used in brain tissue in the Mongolian gerbil [24].

2.6. Image analysis

The number of cells immunoreactive (ir) to ERα or AR was determined in microphotographs with an area of 180 μm^2 (18 × 10 μm). Microphotographs were taken with a Motic camera (10 megapixels) attached to a Leica microscope. Three sections of the same area for each animal were bilaterally analyzed for ERα-ir or AR-ir.

2.7. Statistical analysis

The latency of the onset of paternal behavior between males of the PAT and T-PAT was compared using the nonparametric Mann-Whitney *U* test due to the non-normality of the data (Anderson-Darling test, $P < 0.05$). The time spent in grooming and sniffing was contrasted with the same test.

The numbers of ERα or AR ir-cells in the neural areas of males of the PAT, T-PAT, and AGG were compared using a nonparametric Kruskal-Wallis test due to the non-normality of the data (Anderson-Darling test, $P < 0.05$). Pairwise comparisons were performed using Mann-Whitney *U* test and Bonferroni correction.

The T levels of males of the PAT, T-PAT, and AGG were analyzed with the Kruskal-Wallis test due to the non-normality of the data (Anderson-Darling test, $P < 0.05$). Pairwise comparisons were performed using Mann-Whitney *U* test and Bonferroni correction.

Correlations between T levels and numbers of ERα-ir and AR-ir cells in the mPOA/BNST and AHN/VMH/PAG were performed using Spearman correlational analyses.

Statistical analyzes were carried out using SPSS version 21.0 (IBM SPSS, Armonk, NY).

3. Results

3.1. Behavior

In the second test of paternal behavior, males of the PAT and T-PAT groups had no significant differences in onset latency of paternal behavior ($U = 64.5$, $P > 0.05$, Fig. 2). In contrast, males of the T-PAT spent more time in crouching pups ($U = 55.0$, $P < 0.01$, Fig. 2) and grooming ($U = 57.0$, $P < 0.05$, Fig. 2) than males of the PAT group.

3.2. ERα and AR immunoreactivity in mPOA/BNST

The number of ERα-ir cells in the mPOA varied significantly between

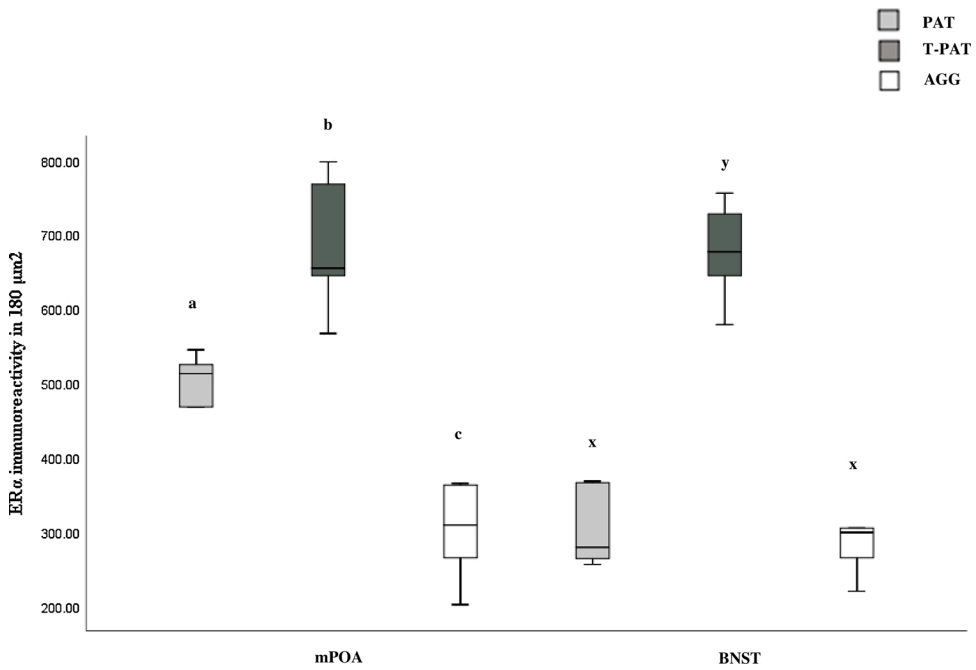

Fig. 3. Paternal males (PAT and T-PAT) had higher ERα-ir in the mPOA than aggressive males (AGG). However, in the BNST, only paternal males of the T-PAT had higher ERα than males aggressive with the pups (AGG). Data are presented as medians and quartiles. Letters indicate significant differences.

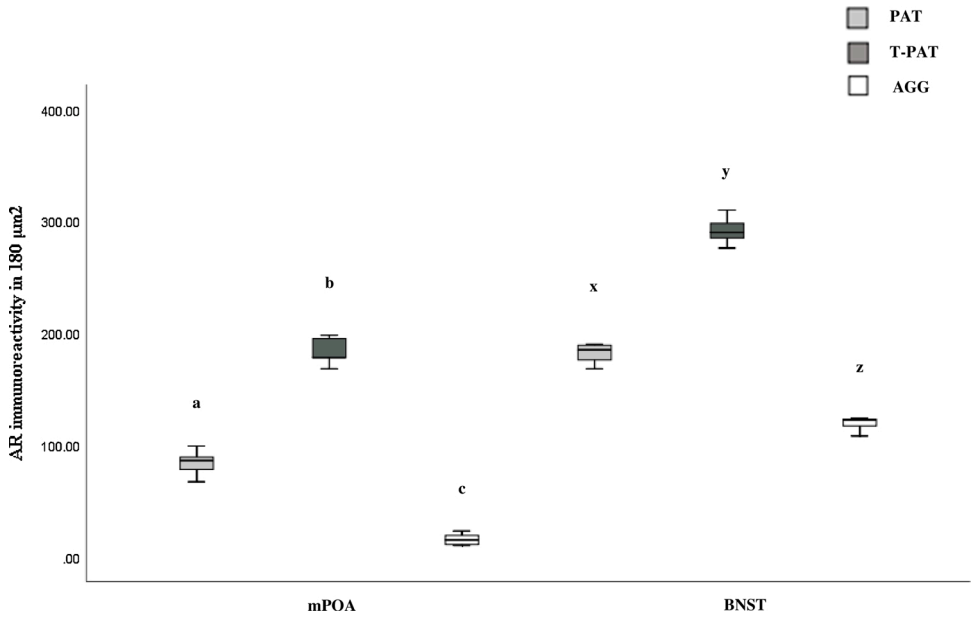

Fig. 4. Paternal males (PAT and T-PAT) had higher AR-ir in mPOA/BNST than aggressive males with the pups (AGG). Data are presented as medians and quartiles. Letters indicate significant differences.

the males of the PAT, T-PAT, and AGG ($H = 8.62$, $df = 2$, $P < 0.05$; Figs. 3 and 5). Post hoc analysis indicated that males of the PAT and the T-PAT had significantly higher ERα-ir levels than males of the AGG (PAT *vs* AGG: $U = 35$, $P = 0.012$; T-PAT *vs* AGG: $U = 36$, $P = 0.004$). Males of the T-PAT had significantly higher ERα-ir than males of the PAT ($U = 19$, $P = 0.009$). In BNST, the presence of ERα-ir in males of the PAT, T-PAT, and AGG varied significantly ($H = 9.89$, $df = 2$, $P < 0.05$; Figs. 3 and 5). Males of the PAT and T-PAT had higher ERα-ir than males of the AGG (PAT *vs* AGG: $U = 40$, $P = 0.012$; T-PAT *vs* AGG: $U = 36$, $P = 0.004$). The number of ERα-ir cells in BNST between males of the PAT and T-PAT was not significantly different ($U = 40$, $P = 0.35$) (Bonferroni adjusted $P = 0.016$).

The number of AR-ir cells in mPOA was significantly different between males of the PAT, T-PAT, and AGG ($H = 12.5$, $df = 2$, $P < 0.05$; Figs. 4 and 5). Post hoc analysis indicated that males of the T-PAT had significantly higher AR-ir in this neural area than males of the PAT ($U = 15$, $P = 0.012$) and males of the AGG ($U = 40$, $P = 0.012$). In BNST, the presence of the AR-ir varied significantly between males of the PAT, T-PAT, and AGG ($H = 12.5$, $df = 2$, $P < 0.05$; Figs. 4 and 5). Pairwise comparison showed that the AR-ir in BNST in males of the T-PAT was significantly higher than in males AGG ($U = 40$, $P = 0.012$). There were no significant differences between males of the PAT and males of the T-PAT ($U = 40$, $P = 0.347$) (Bonferroni adjusted $P = 0.016$).

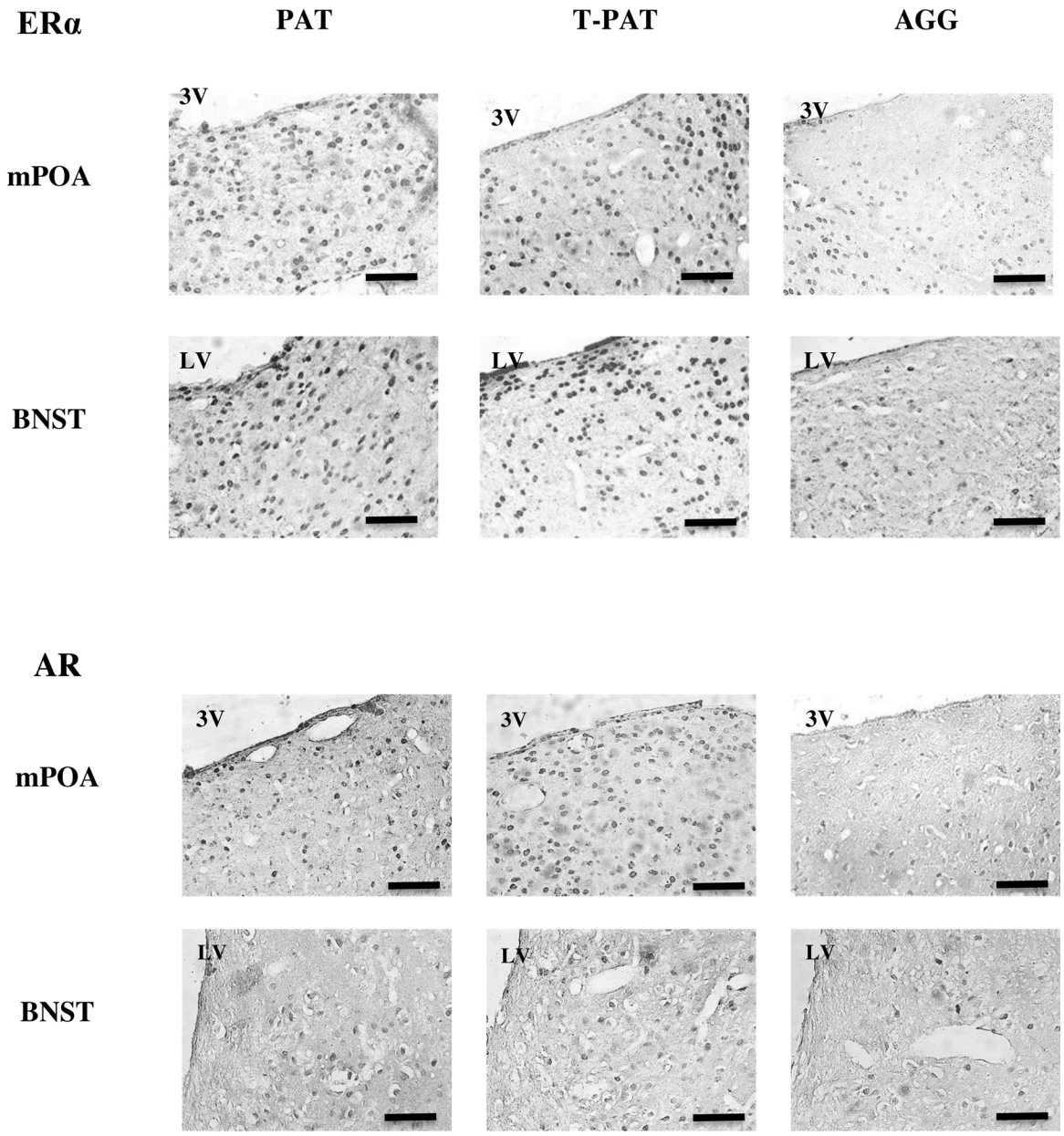

Fig. 5. Representative photomicrographs showing ERα-ir and AR-ir cells in the mPOA and BNST of paternal males (AGG and T-PAT) and males that were aggressive with the pups (AGG). 3 V = third ventricle, LV = lateral ventricle. Coronal sections, scale bars = 100 μm.

3.3. ERα and AR immunoreactivity in AHN/VMH/PAG

The numbers of ERα-ir cells in males of the AGG, PAT, and T-PAT were significantly different in the AHN ($H = 8.90$, $df = 2$, $P < 0.05$; Figs. 6 and 8), VMH ($H = 9.62$, $df = 2$, $P < 0.05$; Figs. 6 and 8) and PAG ($H = 7.34$, $df = 2$, $P < 0.05$; Figs. 6 and 8). The ERα-ir level in the AHN in males of the AGG was significantly higher than in males of the PAT ($U = 39$, $P = 0.012$) and males of the T-PAT ($U = 40$, $P = 0.012$). In the VMH, the males of the AGG had a significantly higher number of ERα-ir cells than males of the PAT ($U = 15$, $P = 0.012$) and T-PAT ($U = 40$, $P = 0.012$). Likewise, the number of ERα-positive cells in the PAG of males of the AGG was also significantly higher than in males of the PAT ($U = 37$, $P = 0.012$) and males of the T-PAT ($U = 40$, $P = 0.012$) (Bonferroni adjusted $P = 0.016$).

AR-ir levels in males of the AGG, PAT and T-PAT were significantly different in the AHN ($H = 12.5$, $df = 2$, $P < 0.05$; Figs. 7 and 8), VMH ($H = 12.5$, $df = 2$, $P < 0.05$; Figs. 7 and 8) and PAG ($H = 12.5$, $df = 2$, $P < 0.05$; Figs. 7 and 8). Post hoc analysis indicated that males of the AGG had significantly higher AR-ir levels in the AHN than males of the PAT ($U = 40$, $P = 0.012$) and males of the T-PAT ($U = 15$, $P = 0.012$), but there was no significant difference between males of the PAT and those of the T-PAT ($U = 39$, $P = 0.022$). Males of the AGG also had higher AR-ir levels in the VMH than males of the PAT ($U = 15$, $P = 0.012$) and males of the T-PAT ($U = 15$, $P = 0.012$), but there was no significant difference between males of the PAT and males of the T-PAT ($U = 31$, $P = 0.53$). In the PAG, AR-ir was significantly higher in males of the AGG than in males of the PAT ($U = 40$, $P = 0.012$) and males of the T-PAT ($U = 15$, $P = 0.012$). We found no differences between males of the PAT and males of the T-PAT ($U = 40$, $P = 0.53$) (Bonferroni adjusted $P = 0.16$).

3.4. Testosterone levels

The concentrations of T in plasma between males of the PAT, T-PAT, and AGG were significantly different ($H = 25.81$, $df = 2$, $P < 0.05$, Fig. 9). Pairwise comparison indicated that males of the T-PAT had significantly higher T concentrations in plasma than males of the PAT (U

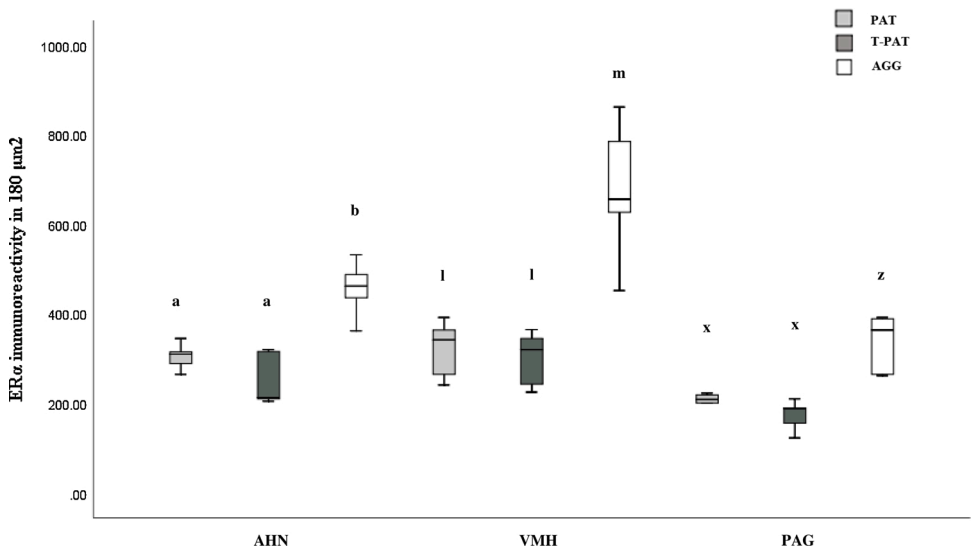

Fig. 6. Aggressive males with the pups (AGG) had higher ERα-ir in the AHN/VMH/PAG than paternal males (PAT and T-PAT). Data are presented as medians and quartiles. Letters indicate significant differences.

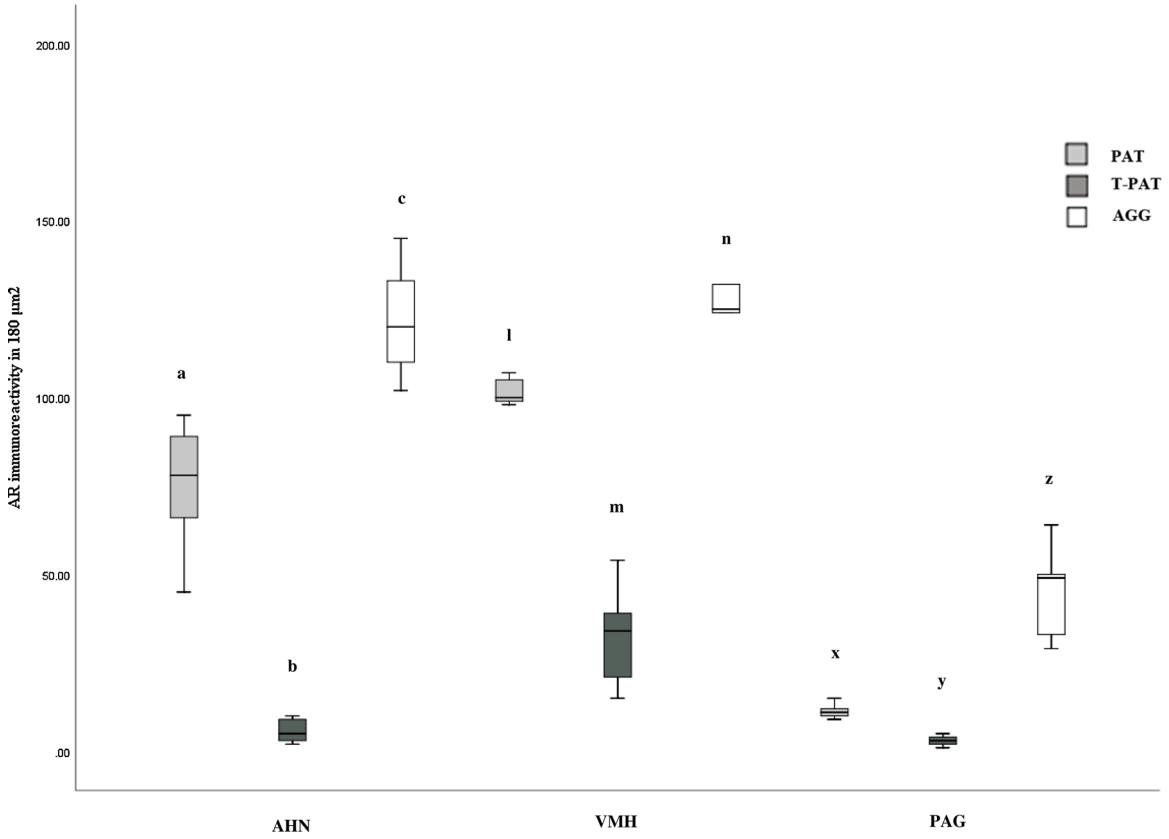

Fig. 7. Aggressive males with the pups (AGG) had higher AR-ir in AHN/ VMH/ PAG than paternal males (PAT and T-PAT). Data are presented as medians and quartiles. Letters indicate significant differences.

= 55, P = 0.01) and AGG (U = 55, P = 0.01). Males of the PAT had higher concentrations of T in plasma than males of the AGG (U = 155, P = 0.01) (Bonferroni adjusted P = 0.02).

We did not find a significant correlation between T levels in plasma and the number of ERα-ir cells in males of the PAT in the mPOA (r = −0.14, P > 0.05) and the BNST (r = 0.27, P > 0.05). There was also no correlation between the levels of this hormone and the number of AR-ir cells in the mPOA (r = −0.076, P > 0.05) and the BNST (r = 0.12, P > 0.05). Moreover, correlations were not found between the T levels in plasma and the numbers of ERα-ir cells in the mPOA (r = −0.02, P > 0.05) and the BNST (r = 0.19, P > 0.05) and AR-ir cells in the mPOA (r = 0.00, P > 0.05) and BNST (r = 0.20, P > 0.05) in males of the T-PAT.

We did not observe significant correlations in males of the AGG between T levels in plasma and the number of ERα-ir cells in the AHN (r = −0.43, P > 0.05), the VMH (r = 0.19, P > 0.05) and the PAG (r = 0.19, P > 0.05) and AR-ir cells in the AHN (r = -0.03, P > 0.05), the VMH (r =

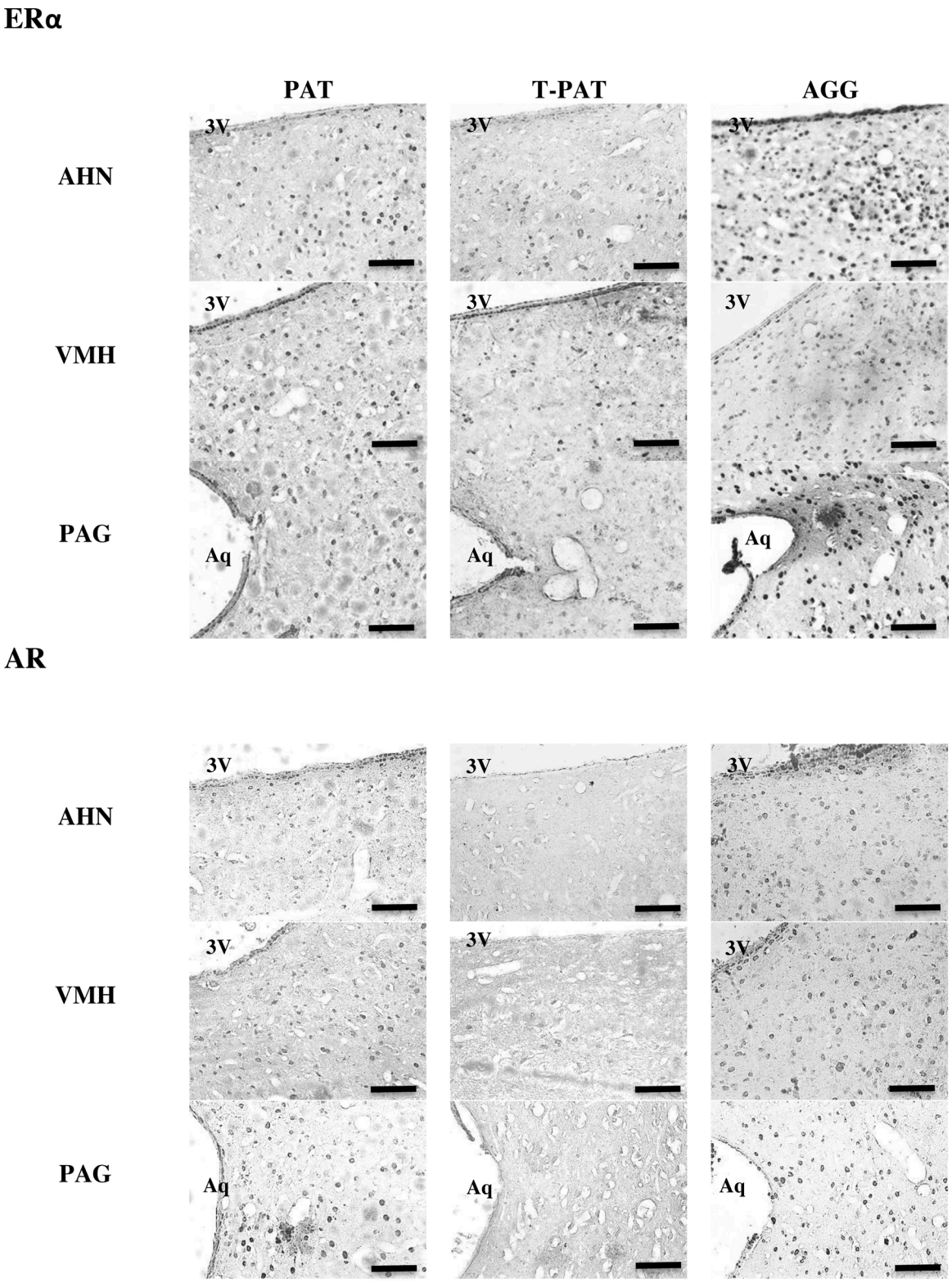

Fig. 8. Representative photomicrographs showing ERα-ir and ARα-ir cells in the AHN, VMH, and PAG of aggressive males with the pups (AGG) and paternal males (PAT and T-PAT). 3 V = third ventricle, Aq = aqueduct. Coronal sections, scale bars = 100 μm.

0.47, $P > 0.05$) and the PAG ($r = 0.51$, $P > 0.05$).

4. Discussion

The number of ERα-ir and AR-ir cells in the mPOA and BNST in paternal males (PAT and T-PAT) was significantly higher than in aggressive males (AGG). Different to Martinez et al. [7], in the present study, ERα y AR in mPOA and BNST can be associated with paternal interactions and not with mating. Therefore, these results suggest that ERα and AR are involved in the neuroendocrine regulation of paternal behavior in the Mongolian gerbil. ERα has already been found to be associated with paternal behavior in rodents; in male Mandarin voles

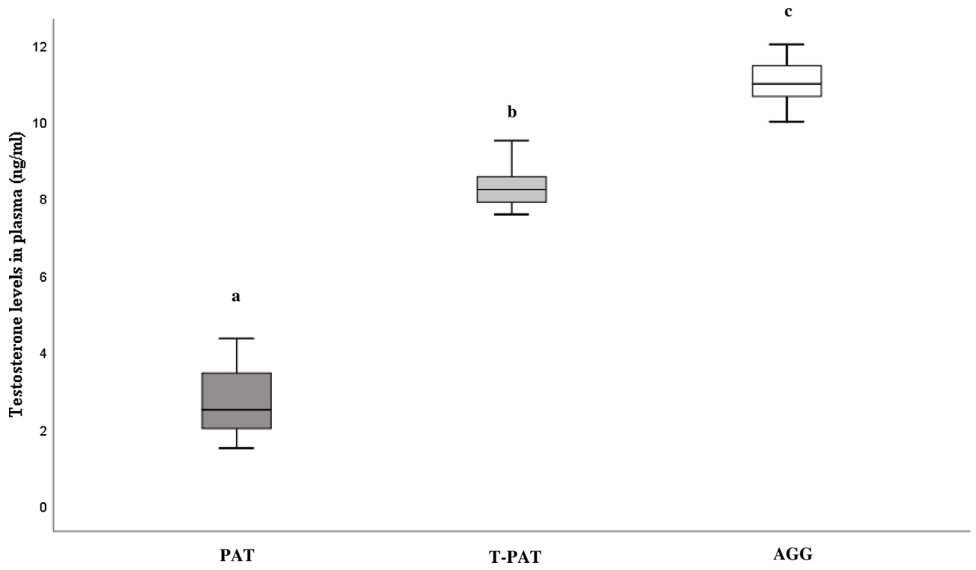

Fig. 9. Paternal males (PAT and T-PAT) had significantly higher T levels than aggressive males with pups (AGG). Data are presented as medians and quartiles. Letters indicate significant differences.

(*Microtus mandarinus*), paternity experience increases the ERα-ir cell count in the arcuate nucleus of the hypothalamus [30]. In this same rodent, males with high paternal responsiveness had a higher number of ERα-ir cells in the mPOA and BNST than males with a low one [31]. In females, many studies in the laboratory rat have established that estradiol acts at the level of the mPOA to stimulate a rapid onset of maternal behavior by binding to the alpha receptor [20,32,33]. Other studies have also found that estradiol actions are mediated through ERα since mice with deletions of this receptor display maternal care deficits [34]. Female mice with more licking and grooming to pups had increased ERα expression in the mPOA [35]. ERα-ir and AR-ir cells are present in mPOA of the laboratory rat; in this neural area, some sub-nuclei are activated during parental, sexual, and aggressive behaviors [36–38].

Recent results in the mouse support the assertion that ERα and AR are involved in neuroendocrine mechanisms regulating paternal behavior; Wu et al. [39] located a subset of galanin-expressing neurons activated when males display paternal behavior. Interestingly, 70–90 % of galanin-expressing neurons are positive for AR and ERα [40].

In the AHN/VMH/PAG, the presence of ERα-ir and AR-ir cells was significantly higher in aggressive males (AGG) than in paternal ones (T-PAT and PAT); this result suggests that estrogen and androgen pathways could be involved in the regulation of aversive behavior of males towards the pups in the Mongolian gerbil. We suggest that low concentrations of T could reduce the inhibition of AHN/VMH/PAG [41]. However, more studies are needed to corroborate this exciting finding.

This study showed that paternal male Mongolian gerbils presented significantly higher peripheral T concentrations than aggressive ones, suggesting that high levels of T may activate mPOA and BNST for the display of parental care. In contrast, low concentrations of this steroid hormone could reduce the inhibition or activation of AHN, VMH, and PAG, nuclei that are activated when virgin male gerbils show aversion towards the pups. Our research group has transformed virgin infanticidal males to paternal by increasing T concentrations [4]. However, virgin paternal males do not turn infanticidal when T concentrations decline due to bilateral castration at least 20 days after this surgery (unpublished data). Martínez et al. [7] reported that aggressive behavior changes to paternal after copulation. This transition is associated with an increase in T levels. However, around ten days after copulation, they display infanticidal behavior again. The display of aggression towards pups coincided with a significant decrease in T and ERα and AR in the mPOA. Gerbils of the T-PAT spent significantly more time crouching and grooming pups than the PAT group's gerbils. Males of the T-PAT also had high concentrations of T and a high presence of ERα and AR in the mPOA and BNST. This increase in the number of ERα and AR receptors was probably caused by high concentrations of T, which can up-regulate the expression of these receptors [42]. However, no correlation was found between T concentrations and the number of ERα-ir and AR-ir cells in these nuclei. Possibly, high T levels could enhance parental behavior through an increase in ERα and AR in neural nuclei activated when the Mongolian gerbil has paternal interactions with his species' pups. Hypogonadal mice have a minimal presence of AR-ir in neural regions implicated in regulating sexual behavior (*e.g.*, mPOA, lateral ventral septum). However, when they are treated with T, the number of neurons bearing AR is similar to that recorded in normal males, indicating that the presence of AR in neural regions that regulate sexual behavior is controlled by circulating androgens [43].

Our findings suggest that plasticity mechanisms are involved in the regulation of paternal behavior in the Mongolia gerbil. High T concentrations could be the cause of the increase in ERα and AR in nuclei activated in paternal gerbils. While that low levels of this hormone, as observed in aggressive males, could be the cause of the increase in the presence of ERα and AR in the nuclei that are activated in the gerbil when they interact aversively with the pups. In the virgin mandarin vole, males who provide little care have higher ERα-ir in VMH than males that provide more licking and grooming to pups [31].

In the Mongolian gerbil, this is the first time that ERα and AR in the mPOA/BNST and AHN/VMH/PAG have been associated with paternal and infanticidal behavior, respectively. However, a high density of ERα and AR has been observed in the mPOA, BNST, AHN, lateral septum, and MeA of this rodent [7,44]. In other laboratory rodents, ERα and AR are implicated in the neuroendocrine mechanisms that regulate social behaviors, such as sexual and aggressive behavior [45–47]. Thus, it is possible to expect that ERα and AR are also involved in regulating infanticidal behavior, besides participating in parental care. In support of this last assumption, we mention that Numan and Insel [14] hypothesized a standard central network that regulates all social behaviors in mammals. These social behaviors also seem to share mechanisms of neural regulation [48].

This study showed that in the Mongolian gerbil, the display of paternal behavior associates with high T concentrations and an increase of ERα and AR in nuclei activated when males of this rodent have a paternal response (mPOA/BNST). Also, this study showed that the display of aggressive behavior towards unfamiliar pups associates with

low T concentrations and an increase of ERα and AR in nuclei that are activated when males display an aggressive response towards pups (AHN/VMH/PAG). These findings suggest that the responsiveness of neural networks that regulate paternal and infanticidal behaviors in this rodent is dependent on peripheral concentrations of T, which could modulate by differential expression of ERα and AR in nuclei that activated in paternal and aggressive interactions.

Future studies should be focused on testing whether mouse neural markers such as galanin, *neurotensin*, and tyrosine hydroxylase [39,49,50] can be used in the Mongolian gerbil to analyze paternal and infanticidal behavior by locating specific subsets of neurons in these regions.

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