Sex differences in testosterone reactivity and sensitivity in a non-model gerbil

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\textbf{ARTICLE INFO}

\textbf{Keywords:} Androgen receptor, GnRH challenge, Testosterone implants

\textbf{ABSTRACT}

Although testosterone (T) is a key regulator in vertebrate development, physiology, and behaviour in both sexes, studies suggest that its regulation may be sex-specific. We measured circulating T levels in Baluchistan gerbils (Gerbillus nanus) in the field and in the lab all year round and found no significant sex differences. However, we observed sex differences in circulating T levels following gonadotropin-releasing hormone (GnRH) challenge and T implants in this non-model species. Whereas only males elevated T following a GnRH challenge, females had significantly higher concentrations in males during bird migration, as well as in human puberty, responsivity to other mechanisms, such as calcium influx and activation of the mitogen-activated protein kinases (MAPK) pathway (Rahman and Christian, 2007).

1. Introduction

The androgen testosterone (T) is involved in numerous physiological processes, including neuronal growth and function, muscle and bone development, immune function, and spermatogenesis in males (Ketterson et al., 2005; Staub and DeBeer, 1997). T is secreted following hypothalamic-pituitary–gonadal (HPG) axis activation (Kapra and Huhtaniemi, 2018). It is produced mostly in the gonads following luteinizing hormone (LH) secretion in the pituitary, which is initiated by gonadotropin-releasing hormone (GnRH) release in the hypothalamus. In the vast majority of mammalian species, there are sex differences in circulating T, with significantly higher concentrations in males (Torjesen and Sandnes, 2004), even in ‘masculinized” species females can be dominant to males, such as spotted hyenas (Crocuta crocuta; Goymann et al., 2001), fossa (Cryptoprocta ferox; Hawkins et al., 2002) and ring-tailed lemurs (Lemur catta; Grebe et al., 2019). In a small proportion of species, no sex differences are found (Koren et al., 2006). The classical T signaling pathway involves the binding to androgen receptors (AR), and facilitation of AR-induced transcription (Hunter et al., 2018). However, T action may be exerted by other mechanisms, such as calcium influx and activation of the mitogen-activated protein kinases (MAPK) pathway (Rahman and Christian, 2007).

Although studies on the HPG axis mostly involved either males or females, data from studies that included both sexes suggested that the regulation of T production is sex-specific (e.g., Covino et al., 2018; Potau et al., 1999), and that there is a sex-specific response to T manipulation via a GnRH challenge (Adkins-Regan, 2005). For example, during bird migration, as well as in human puberty, responsivity to

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GnRH in females was lower than in males (Covino et al., 2018; Covino et al., 2015; Greives et al., 2016; Potau et al., 1999). Sex-biased responses to exogenous T derivatives were documented in hepatic enzyme activity, motor neuron development, sexual behavior, and in the cloacal gland area (Balthazart et al., 1983; Berg and Gustafsson, 1973; Holmes et al., 2009). However, the involvement of differential receptor regulation along the HPG axis in these systems was not examined, and most species that were studied showed stereotypical (i.e., human; with males > females) sex differences in circulating T, or often T was not measured in females (Holmes et al., 2009; Potau et al., 1999).

In this study, we measured circulating T levels of *Gerbillus nanus* in the field throughout the year and found no sex differences. Thus, we proceeded in two directions. First, we questioned whether T concentrations following GnRH challenge, and exogenous T implants, are regulated in a sex-specific manner. We predicted that despite the similar concentrations of endogenous T in the blood stream, T response is sex-specific. Secondly, we examined AR expression levels in several brain regions. We predicted that the expression levels of the single T receptor, AR, will be affected by sex as well as by social and mating environment (i.e., gerbils housed alone vs. those that experienced housing as a mixed-sex couple). Since the *G. nanus* genome is not sequenced and annotated, we first identified and analyzed AR sequences in other Gerbillinae subfamily members, and then utilized them for primer design in *G. nanus*. The unique opportunity to study the HPG axis of a non-model species with similar circulating T concentrations in males and females, is expected to open new avenues of understanding the HPG axis.

### 2. Materials and methods

#### 2.1. Study species and animal maintenance

*Gerbillus nanus* is a granivorous desert rodent (Kam et al., 2011). It is a seasonal breeder with a gestation period of 22 days and an average litter size of 5.8 (SD 1.78; N = 177; unpublished data). We have been studying *G. nanus* in the field, in the Sheizaf Nature Reserve (30°45′N, 35°15′E) for the past decade (Kam et al., 2010). Our data shows that the breeding period, which contains several reproductive cycles (including courting, mating, birthing, and nursing), is from February–August (unpublished data). Preliminary molecular data analyzing paternity suggests a polygynandrous mating system (1–3 males per male and female; unpublished data). Over four years (2014–2017), blood samples (N = 650) were collected monthly from the infraorbital sinus of *G. nanus*. Our data shows that the infraorbital sinus of sexually mature gerbils using a capillary tube, then centrifuged at 3000 g for 20 min. Serum was kept at −20 °C until analysis (see below).

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#### 2.2. GnRH challenge

GnRH (A2S technologies ltd, Item no. A1147-5) was stored at −80 °C and dissolved in a phosphate buffer saline (PBS) to prepare two doses of GnRH, 50 ng and 500 ng. Each gerbil was injected subcutaneously (16 mm, 25 G needle) with a single dose (N = 40; 10 males and 10 females per dose) with a total volume of 200 µL to compensate for blood loss due to sampling. The control group (N = 5 males and 5 females) received 200 µL PBS. All treatments and blood sample collections from individual gerbils were carried out in a separate isolated room to minimize stress. We collected blood samples from each individual at 4 time points from the infraorbital sinus using a capillary tube. All gerbils were sampled at 0 (baseline), and 3 other time points, aiming at 60, 90, and 120 min post-GnRH injection, and spanning 35–122 min post-GnRH injection. Blood was centrifuged at 3000 g for 20 min, and serum was kept at −20 °C until analysis. The GnRH experiments were approved by the Bar-Ilan University Animal Ethics Committee (permit number 4–01-2016).

#### 2.3. T implants

To elevate T levels, gerbils were inserted with subcutaneous T implants prepared by mixing T (Sigma, UK, item no. 86500) and beeswax (Quispe et al., 2015). Beeswax was chosen as a vehicle because it purportedly allows a more controlled release, and it biodegrades, preventing an additional invasive procedure to remove the implant. To ensure that gerbils were not allergic or hypersensitive to the beeswax implants, 5 gerbils were implanted with pure beeswax for a week. No clinical symptoms were observed. Gerbils were then divided into four treatment groups: empty implants (10 mm; N = 8; 4 of each sex); 4.95 mg T implants (8 mm; N = 18; 9 of each sex); 6.6 mg T implants (10 mm; N = 18; 9 of each sex); and 8.25 mg T implants (12 mm; N = 18; 9 of each sex). Blood was collected at the time of implantation, and then weekly for 8 weeks. Ethical approval was obtained from the Bar-Ilan University Animal Ethics Committee (permit number 55-09-2016).

#### 2.4. T quantitation

T immunoreactivity was quantitated in individual samples in duplicates, using a commercial enzyme-linked immunosorbent assay (ELISA; item. no. ADI-901-065, Enzo Biochem, USA), following the manufacturer’s instructions. Serial dilutions of pooled gerbil serum from 10 individuals showed parallelism with the provided kit standards (univariate analysis of variance in SPSS; P = 0.266). Linearity was demonstrated between 2.5 and 20 µL of serum. Accordingly, 10 µL (middle of the linear range) was chosen. According to the manufacturer, antibody cross-reactivity is 14.6% with 19-hydroxytestosterone, 7.2% with androstenedione and less than 0.72% for all other steroids. The lowest concentration that we detected using the assay was 0.37 ng/mL. For the implant experiments, intra-assay variability was 6.25% (N = 3); inter-assay repeatability was 9.33% (N = 15); and the calculated recovery was 103% by the addition of a known amount of testosterone standard to the serum pool. For the GnRH challenge experiments, intra-assay repeatability was 9.6% (N = 5); inter-assay variability was 18.6% (N = 4); and the calculated recovery was 90.4%.

#### 2.5. Sequence analysis for primer design

Assembled genomic sequences of *Psammomys obesus* (NESSX01) and *Meriones unguiculatus* (GCF_002204375.1) were downloaded from NCBI, and used to create two BLAST databases. Using tBLASTx (Camacho et al., 2009), *Rattus norvegicus* AR (NM_012502.1) and β-actin (NM_031144.3) genes were used as a query to identify the sequence of the corresponding Gerbillinea ortholog. The best hits, in terms of both coverage and similarity, were selected. Sequent from the EMBOSs package (Rice et al., 2000) was used to extract the corresponding genomic loci. Although BLAST identifies exons, it does not include the non-aligning intronic segments in the alignment and lacks precision at splice junctions. To ensure precise identification of the
exon-intron structure of these genes in the *P. obesus* and *M. unguiculatus* genomes, GENSCAN (Burge and Karlin, 1998) and Splign (Kapustin et al., 2008) were run on this genomic loci. GENSCAN is a hidden Markov model-based program that can be used to identify complete gene structures in genomic DNA. Splign, the NCBI spliced-alignment tool, combines local and global alignment algorithms and accurately tracks splice junctions. First, the mRNA sequence was predicted using GENSCAN, and then Splign was run using the predicted mRNA sequence and the corresponding genomic loci to ascertain the exact exon-intron splice sites. Identified *P. obesus* and *M. unguiculatus* protein sequences were analyzed by multiple sequence alignment (MSA) with other homologues using CLUSTAL Omega algorithm. Similarity and coverage were determined by NCBI blast. Identification of known domains was carried out by NCBI Search for conserved domains within a protein.

### 2.6. Tissue preparation

Brain regions for mRNA expression analysis were selected based on mouse brain studies (Mahfouz et al., 2016). Expression analysis was conducted on adrenal tissue as well. Single and gerbils with a pairing experience were sacrificed for gene expression analysis by decapitation. The brain regions (prefrontal cortex, hypothalamus, hippocampus, - striatum, pituitary, olfactory bulbs) and adrenals were collected, placed in cryogenic tubes, frozen immediately in liquid nitrogen, and stored at −80 °C until analysis. The experiments were conducted in compliance with the NIH/USDA guidelines, with the approval of the Bar-Ilan University Animal Ethics Committee (permit number 50-09-2016).

### 2.7. RNA extraction and reverse transcription

Total RNA for real-time PCR (qPCR) analysis was isolated from tissues using total RNA purification micro kits (Norgenbiotek, Canada). Genomic DNA was removed from RNA samples by DNA-free™ DNA removal kits (Invitrogen, Lithuania); and reverse transcription of the purified RNA was performed using the qScript cDNA Synthesis Kit (Quantabio, MA, USA). The resulting cDNA was diluted according to a gene standard curve. First, we ran pools of brain regions and adrenals to check whether there is gene expression. Pools included multiple samples representing both sexes. Male and female sample sizes that contributed to the pooled samples were as follows: pre-frontal cortex: 14, 12; hypothalamus: 10, 10; hippocampus: 9, 3; striatum: 9, 8; pituitary: 12, 10; olfactory bulb: 9, 9; adrenal: 12, 8 (respectively).

Once we detected that all brain areas and adrenals showed AR mRNA, we analyzed individual samples from each brain region, except for the pituitary and hippocampus, which were only analyzed as pools due to low mRNA quantities. Individual samples for gene expression analysis included both samples from singles (N = 70; 7 of each sex for each region) and samples from gerbils that experienced pairing (N = 80; 8 of each sex for each region). Due to discrepancies in RNA quality and quantity, final sample size that was analyzed was lower. For pairing experience, non-related weight-matched males and females were paired in the same cage for two weeks. Then, males were removed to a separate cage for a month, while females remained in the same cage.

### 2.8. qPCR

Real-time PCR was conducted using a CFX connect (Bio-Rad Laboratories, Hercules, CA, USA), and a PerfeCTa SYBR Green FastMix, ROX qPCR Master Mix (QuantaBio, MA, USA) according to the manufacturer’s instructions. One µL of diluted cDNA was used for the qPCR reaction. The following cycling conditions were applied for 40 cycles: 95 °C for 15 s and 55 °C for 30 s. The amplification efficiency and expression levels were calculated using CFX manager software version 3.1 (Bio-Rad Laboratories, Hercules, CA, USA). Gene encoding β-actin was used as reference.

Primers were synthesized by Syntezza Bioscience Ltd (Jerusalem, Israel) from deduced exon-intron junction sequences. All primers were tested for non-specific amplification and primer-dimer formation by melting curve analysis. The primers used were as follows:

**AR**

F: 5′-ACTTTGACACTGAGCTGTTT-3′
R: 5′-AAGTGCCTCTACCCACACA-3′

**β-actin**

F: 5′-TAGGCAACGGGCTGAT-3′
R: 5′-TACGGTCCAGGATGCTCT-3′

### 2.9. Statistics

General linear mixed models (GLMM) were used for all tests of the effects on gerbil circulating T concentrations. Since T did not distribute normally (Shapiro test), we calculated the probabilities of the effects and interactions using randomization. Gerbil ID was used as a random effect. Total effect of each predictor was assessed as an independent uniform input. The model effects for wild gerbil circulating T concentrations in the field were year, month, body mass, and sex. For the GnRH challenge, we tested whether there were differences in T concentrations due to sex, body mass, GnRH dose or post-injection time (minutes; modeled non-linearly by entering it as a second-degree polynomial), and their interaction. To test whether T concentrations were affected by the treatment of T implants, variables were sex, time post-insertion (in weeks; modeled non-linearly by entering it as a second-degree polynomial), treatment level, and their interactions. To test whether sex or social conditions (i.e., solitary vs. paired) influence gene expression levels in a specific brain area, model effects were sex and social conditions as well as their interaction. All analyses were done in JMP Pro (v. 15, SAS Inc.).

### 3. Results

#### 3.1. Measurement of circulating T concentrations in free ranging G. nanus

We measured circulating T concentrations in the field monthly over 4 years and found no significant sex differences. Both month and year had significant effects on circulating T levels (Table 1; Fig. 1). Differences between years are common and significant in nature, as ecological parameters vary (e.g., temperature, precipitation, extreme flooding events). Thus, year had the strongest effect on T concentrations, followed by month. Tukey post-hoc analysis revealed that January T concentrations were significantly higher than the rest of the months.

#### 3.2. GnRH challenge

Only males elevated circulating T concentrations following GnRH challenge (Table 2; Fig. 2). The time in minutes past GnRH injection, and gerbil body mass were also significant, and there was a significant interaction between post-injection time and sex (Table 2). Gerbil body mass was the strongest predictor of T concentrations, followed by sex and time post-injection.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Predictors of circulating T concentrations in free ranging G. nanus (2014–2017), and their total effect. Significant probabilities are in bold.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects</td>
<td>F (df)</td>
</tr>
<tr>
<td>Year</td>
<td>29.22 (3,600)</td>
</tr>
<tr>
<td>Month</td>
<td>6.97 (11,610)</td>
</tr>
<tr>
<td>Sex</td>
<td>0.04 (2,482)</td>
</tr>
<tr>
<td>Body mass</td>
<td>0.64 (1,548)</td>
</tr>
</tbody>
</table>
3.3. T implants

Time, in weeks, post-insertion had the strongest effect on circulating T concentrations, driven by the elevated levels in the first and second week post-insertion. Implant size (i.e., treatment level) also affected circulating T concentrations, and there was a significant interaction between sex and treatment level, as females reacted to the implants was stronger (Table 3; Fig. 3).

3.4. Identification of AR sequences in G. nanus orthologs

We identified sequences for AR and β-actin in P. obesus and M. unguiculatus and published sequences from rats, mice, and humans, also show a remarkably high similarity and coverage (Table 4, SI Fig. 1S).

![Fig. 1. Average circulating testosterone concentrations in male and female gerbils throughout the months. Sample size is indicated above standard error bars.](image1)

![Fig. 2. Testosterone changes with time (in minutes) after injection of saline, 50 ng or 500 ng GnRH in females (A) and males (B). Lines were derived using the lambda smoothing parameter in JMP.](image2)

Table 2

<table>
<thead>
<tr>
<th>Effects</th>
<th>Estimate</th>
<th>F (df)</th>
<th>P</th>
<th>Total Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH dose</td>
<td>−0.0003</td>
<td>0.27 (1,32)</td>
<td>0.612</td>
<td>0.021</td>
</tr>
<tr>
<td>Sex</td>
<td>−0.3320</td>
<td>5.97 (1,44)</td>
<td>0.0146</td>
<td>0.317</td>
</tr>
<tr>
<td>Min</td>
<td>−0.0033</td>
<td>7.84 (1,107)</td>
<td>0.0076</td>
<td>0.22</td>
</tr>
<tr>
<td>Body mass</td>
<td>0.055</td>
<td>6.73 (1,32)</td>
<td>0.0172</td>
<td>0.494</td>
</tr>
<tr>
<td>Sex*GnRH dose</td>
<td>−0.0003</td>
<td>0.24 (1,32)</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>Sex*Min</td>
<td>0.0012</td>
<td>0.97 (1,07)</td>
<td>0.336</td>
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</tr>
<tr>
<td>Sex*Body mass</td>
<td>0.0024</td>
<td>0.01 (1,32)</td>
<td>0.9902</td>
<td></td>
</tr>
<tr>
<td>Min²</td>
<td>−0.0002</td>
<td>23.02 (1,07)</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Sex*Min²</td>
<td>0</td>
<td>4.06 (1,07)</td>
<td>0.0412</td>
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</tr>
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</table>

Table 3

<table>
<thead>
<tr>
<th>Effects</th>
<th>Estimate</th>
<th>F (df)</th>
<th>P</th>
<th>Total Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>0.386</td>
<td>2.26 (1,115)</td>
<td>0.13</td>
<td>0.119</td>
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<tr>
<td>Treatment level</td>
<td>0.201</td>
<td>5.05 (1,59)</td>
<td>0.0296</td>
<td>0.319</td>
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<tr>
<td>Week</td>
<td>−0.504</td>
<td>92.43 (1,373)</td>
<td>&lt; 0.0001</td>
<td>0.682</td>
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<tr>
<td>Sex*Treatment level</td>
<td>0.183</td>
<td>4.18 (1,59)</td>
<td>0.037</td>
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<tr>
<td>Sex*Week</td>
<td>−0.103</td>
<td>3.88 (1,373)</td>
<td>0.0504</td>
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</tr>
<tr>
<td>Week²</td>
<td>0.056</td>
<td>5.90 (1,369)</td>
<td>0.0152</td>
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<tr>
<td>Sex*Week²</td>
<td>−0.001</td>
<td>0.00 (1,369)</td>
<td>0.974</td>
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</table>

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3.5. Expression analysis of AR in G. nanus brain regions and adrenals

We first checked whether AR mRNA expression can be detected in pools of different brain regions and in adrenals. AR mRNA expression was observed in all brain region pools that were examined, with the highest expression in the pituitary of both sexes (Fig. 5). However, only one pooled sample per region was ran, preventing statistical analysis.

We then examined the AR mRNA expression in each brain region in individual males and females, single and paired. We found that overall the variability in expression between the sexes was similar, and found weak sex differences only in the striatum (Fig. 6A). AR expression levels in the striatum were slightly greater in males than in females ($F_1 = 4.519; P = 0.0476$), and singles had marginally higher expression levels than gerbils that experienced pairing ($F_1 = 4.030; P = 0.0599$). In the adrenal gland, we found that single gerbils had higher AR expression than gerbils that experienced pairing ($F_1 = 5.196; P = 0.035$; Fig. 6B). However, when corrected for multiple comparisons, none of the differences were statistically significant.

4. Discussion

In this study, we found no significant sex differences in the endogenous circulating T concentrations of intact free-ranging G. nanus. However, GnRH and T responsiveness were different between the sexes in this non-model gerbil species. While only males showed elevated T following a GnRH challenge, females reacted with higher T concentrations following exogenous T administration. Our results may suggest that males are more sensitive to the negative feedback, and may be more efficient in T clearance, supporting Saez et al.'s (1972) report of higher T clearance rate in men than in women. The sex-biased response to T implants was observed mainly a week post-T implantation. As this could be attributed to accelerated male clearance, it can also be due to rapid degradation of the beeswax implant. Although the beeswax was expected to produce more controlled release than silastic tube implants (Quispe et al., 2015), it did not show the prolonged elevated T profile expected from a slow release pellet, and may rather be considered similar to a single T injection. Unfortunately, studies that employed T implants usually examined either males or females, not both (e.g., Chen and Yu, 2018; Shevchouk et al., 2019). Moreover, in most studies, circulating T levels were not measured following exogenous T supplementation in both sexes (e.g., Berg and Gustafsson, 1973; Holmes et al., 2009). In the present study, female T levels did not respond to the GnRH challenges, suggesting an upstream regulatory mechanism, which prevents HPG axis activation by clearing excess GnRH. Such a mechanism may be highly important as accumulated evidence suggests that higher T in females may be maladaptive, associated with the development of polycystic ovary syndrome (PCOS; Marouliss and Triantafillidis, 2006; Walters, 2016), metabolic syndrome and diabetes (Kim and Halter, 2014), and breast cancer (Secreto et al., 2018). Understanding the mechanisms behind female gerbils’ lack of responsiveness to exogenous GnRH may improve our grasp of GnRH excess-related diseases.

In order to investigate the processes associated with female exogenous T elevation and GnRH response shutdown, we identified and characterized AR sequence in the Gerbillinae subfamily members, P. obesus and M. unguiculatus. This is the first report to identify homologue sequences for AR and β-actin in these species, and show that their amino acid sequences are highly conserved. The remarkable homology of these sequences across species enabled tool design for analyzing their expression in G. nanus brains and adrenals. We also found that the
expression profile of the AR in G. nanus brains resembles the documented expression sites of AR (Mahfouz et al., 2016). Initial qualitative analysis showed that AR was highly expressed in the pituitary of both males and females, which may be the closest and fastest regulation point for T other than the gonads that produce it. Due to sample size limitation, comparative expression analysis of different brain regions was conducted with pools containing multiple individuals. Thus, our results may only point to brain regions where AR is expressed and exclude a quantitative between regions comparison.

Quantitative gene expression analysis in individuals showed mRNA expression variability in both sexes. Mean AR mRNA expression in the striatum was lower in females than in males, but it was not significant once multiple comparisons were considered. This sex difference was also not observed in the initial qualitative analysis. However, as mentioned above, the results from the pools preclude statistics. Lower female striatum AR mRNA expression may be involved in the mechanism that allows females to elevate circulating T as a response to exogenous T administration. Sexual dimorphism in AR expression in several brain regions has been reported for a number of species, including an amphibian Pelophylax esculentus (Santillo et al., 2017), rodents Rattus norvegicus (Gustafsson et al., 1976; Roselli, 1991; Simerly et al., 1990) and Mus musculus (Brock et al., 2015; Lu et al., 1998; Shah et al., 2004), as well as several birds species (MacManes et al., 2017; Voigt et al., 2009). Results have been equivocal, as lower (Brock et al., 2015; Simerly et al., 1990) and higher (MacManes et al., 2017) AR mRNA levels in females than in males were reported. Higher male striatum AR expression was demonstrated in multiple species (reviewed in Tobiansky et al., 2018). The striatum harbors the nucleus accumbens, which associates mating with reward (Bamford and Bamford, 2019), and a critical signaling pathway for the reward response is triggered by dopamine secretion, which is modulated by T (Tobiansky et al., 2018). Thus, although we observed higher AR in male striatum, in the future we will specifically target the nucleus accumbens.

Expression of genes along the HPG axis may vary according to factors such as the stage of the estrous cycle (Levine, 2015), life-history stage (Schmidt and Soma, 2008), and season (Soma et al., 1999b). Likewise, seasonal variation of androgen and estrogen receptor expression in the brain was observed in several species (e.g., Santillo et al., 2017; Soma et al., 1999a; Zhang et al., 2016; Zhang et al., 2017). In birds, for example, long days were related to decreased GnRH expression in the brain was observed in several species (e.g., Santillo et al., 2017; Soma et al., 1999a; Zhang et al., 2016; Zhang et al., 2017). In birds, for example, long days were related to decreased GnRH expression (Dawson et al., 1985). When T is low, the adrenal androgen precursor dehydroepiandrosterone (DHEA) can be metabolized into active steroids in the brain (Schlinger et al., 2008; Schmidt et al., 2008).

All of the gerbils in our experiments had intact reproductive organs and were not pregnant. However, since the gerbils used for the gene expression analysis were kept in artificially long daylight, their...
reproduction could have been affected. We also did not examine the stage of the estrous cycle in which the females were sampled, which could have affected the results (Levine, 2015).

In this study, we found that T peaked a month before the breeding season in the field. Studies on vertebrate males demonstrated higher T during the mating periods and decreased T during parenting (supporting the Challenge Hypothesis, reviewed in Archer, 2006; Wingfield, 2017), in addition to AR expression upregulation in the brain during the mating period (Lea et al., 2001; Swift-Gallant et al., 2015), and downregulation during parenting (Lea et al., 2001). We had no data on mating behavior, and gerbils were only paired briefly for two weeks. However, the finding that gerbils that experienced pairing had higher AR mRNA levels in the adrenals than single individuals support the notion that AR expression is upregulated when there is an increase in mating potential.

The sexually different responses to exogenous T and GnRH in gerbils may result from sex differences downstream, for example in the amount of aromatase, 5α- or 5β-reductase activities. Several studies have shown sex differences in the expression (Patil and Gunasekera, 2008) or activity (Roselli, 1991) of brain and ovarian aromatase. Brain aromatase, 5α- and 5β-reductase change seasonally (Soma et al., 2003). We attempted to quantify aromatase mRNA in this study, but calibration results pointed to very low expression levels that could not be reliably quantified. It is possible that aromatase could have been detected in the ovaries since GnRH, followed by follicle-stimulating hormone (FSH) and LH elevation, stimulates the expression of aromatase in the ovaries, which might also lead to T reduction. Characterization of the aromatase sequence in this species may provide the necessary tools for analyzing its expression. Additionally, higher resolution analysis of more specific brain regions within the examined areas may allow better expression detection.

Our results suggest different points of regulation along the HPG axis in male and female gerbils. The GnRH challenge experiment, along with the lower expression of AR in the female striatum support the idea that females may control the HPG axis upstream to conserve energy and ensure reproduction. This mechanism may also prevent testosterone-induced behaviors, such as risk-taking and aggression, which may jeopardize fitness. In males, the regulation of the HPG axis may be downstream via testosterone sensitivity. This would enable males to respond to mating-related stimuli when conditions are optimal. Understanding the mechanisms involved with sex differences among various junctions on the HPG axis, and examining their implications in non-model species, may also open new therapeutic avenues for human diseases.

5. Disclosure summary

I certify that neither I nor my co-authors have a conflict of interest as described above that is relevant to the subject matter or materials included in this Work.

Author contributions

LK designed the study, coordinated the study, carried out the analysis, and helped draft the manuscript; DM carried out the molecular lab work and drafted the manuscript, SS conducted the GnRH challenge, MA carried out the testosterone implants experiments and assisted with brain extractions, TD carried out the bioinformatics, ISK and BRK maintained the lab gerbil population, MK and AAD helped with the experimental design, and GY punched out the brain regions.

Acknowledgements

The authors wish to thank Jennifer Israel Cohen and Eli Geffen for statistical advice, Yael Laure for English editing, Madlin Abbas for help during the implant experiments, Coral Levi for help with the GnRH challenge studies, Anat Shenbloo and Ruth Fishman for their help in brains and adrenal dissections, Noa Kinor and Sarit Lampert for help with the qPCR, Hiba Waldman Ben-Asher for assistance in the homology analysis, Galit Shohat-Ophir, Aron Weller, Greg Ball, Elana Chloes, and two anonymous reviewers for helpful discussions and comments.

Disclosure statement

The authors have no conflicts of interest to declare.

Data sharing

The data that support the findings of this study are available from Mendeley Data.

Funding sources

This study was supported by the National Institute for Psychobiology in Israel – Funded by The Charles E. Smith Family grant number 122-15-16 (to LK), and the Israel Scientific Foundation (ISF) grant number 927/13 (to AAD).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2020.113418.
References


