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Steroid 5α-reductase 2 deficiency leads to reduced dominance-related and impulse-control behaviors

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HIGHLIGHTS

- 5α-reductase 2 (5αR2) catalyzes the conversion of testosterone into DHT
- We found that 5αR2 knockout (KO) mice have reduced dominance-related behaviors
- These deficits are accompanied by lower novelty-seeking and risk-taking
- 5αR2 KO mice exhibit reduced D_2-like receptor binding in the nucleus accumbens

ABSTRACT

The enzyme steroid 5α-reductase 2 (5αR2) catalyzes the conversion of testosterone into the potent androgen 5α-dihydrotestosterone. Previous investigations showed that 5αR2 is expressed in key brain areas for emotional and socio-affective reactivity, yet the role of this enzyme in behavioral regulation remains mostly unknown. Here, we profiled the behavioral characteristics of 5αR2 heterozygous (HZ) and knockout (KO) mice, as compared with their wild-type (WT) littermates. While male 5αR2 KO mice displayed no overt alterations in motoric, sensory, information-processing and anxiety-related behaviors, they exhibited deficits in neurobehavioral correlates of dominance (including aggression against intruders, mating, and tube dominance) as well as novelty-seeking and risk-taking responses. Furthermore, male 5αR2 KO mice exhibited reduced D_2-like dopamine receptor binding in the shell of the nucleus accumbens - a well-recognized molecular signature of social dominance. Collectively, these results suggest that 5αR2 is involved in the establishment of social dominance and its behavioral manifestations. Further studies are warranted to understand how the metabolic actions of 5αR2 on steroid profile may be implicated in social ranking, impulse control, and the modulation of dopamine receptor expression in the nucleus accumbens.

Abbreviations: WT, wildtype; HZ, 5αR2 heterozygous mice; KO, 5αR2 knockout mice.

Keywords: 5α-reductase; transgenic mice; dominance; aggression; novelty seeking; risk taking; dopamine

INTRODUCTION

The steroid 5α-reductase (5αR) family includes several enzymes catalyzing the saturation of the 4,5-double bond of the A ring of several 3-ketosteroids (Paba et al., 2011; Russell and Wilson, 1994); in particular, 5αRs convert testosterone and progesterone into 5α-dihydroprogesterone (DHP) and 5α-dihydrotestosterone (DHT); these products are further metabolized into neuroactive steroids that play key roles in behavioral regulation, such as 3α,5α-tetrahydroprogesterone (allopregnanolone; AP) and 5α-androstan-3α,17β-diol (3α-diol), respectively (Frye et al., 2001; Martini et al., 1996; Pinna et al., 2003). In addition, 5αRs serve the degradation of glucocorticoids, such as corticosterone and cortisol, into their 5α-reduced derivatives (Carlstedt-Duke et al., 1977).

The two best-characterized members of the 5αR family, type 1 (5αR1) and 2 (5αR2), differ by anatomical distribution and substrate specificity. While 5αR1 is abundantly expressed in the CNS throughout all developmental stages, 5αR2 is the predominant type in the prostate and male accessory sex glands (Paba et al., 2011; Thigpen et al., 1993). In addition, 5αR2 plays a primary role in the conversion of testosterone into the potent androgen DHT (Paba et al., 2011). The brain distribution of 5αR2 was initially reported to be mainly limited to perinatal periods (Poletti et al., 1998). Recent investigations, however, have shown that, in adult rats, 5αR2 is expressed in the output neurons of brain regions involved in emotional and sensorimotor regulation, including the prefrontal and somatosensory cortices, striatum, thalamus, amygdala, hippocampus and cerebellum (Castelli et al., 2013). Furthermore, unlike 5αR1, 5αR2 is not expressed in small neurons and glial cells, pointing to cell-specific patterns in the expression of this enzyme throughout the brain (Aumuller et al., 1996; Eicheler et al., 1994).

This neuroanatomical distribution raises critical questions about the role of 5αR2 in behavioral regulation. A useful experimental approach to grapple with this issue is afforded by the characterization of the neurobehavioral phenotypes associated with the congenital deficiency of this enzyme. In men, non-functional mutations of the gene encoding 5αR2 (*SRD5A2*) result in Imperato-McGinley syndrome, a rare disorder characterized by a dramatic reduction in DHT synthesis, which leads to ambiguous genitalia at birth (Imperato-McGinley et al., 1974). The affected individuals are often raised as girls, but experience virilization at puberty, with testicular descent, hirsutism and

enlargement of the clitoris (Imperato-McGinley and Zhu, 2002). In C57BL/6 mice, the lack of 5αR2 leads to a large reduction of plasma DHT levels, as well as a reduction in prostate size and mating efficiency; however, this mutation does not affect the formation of internal and external genitalia (Mahendroo et al., 2001).

To the best of our knowledge, although 5αR2-deficient individuals do not exhibit any major psychiatric disturbance (Imperato-McGinley et al., 1974), the behavioral and brain-functional changes associated with this mutation have not been fully characterized. Thus, the present study aimed at the investigation of the behavioral repertoire of 5αR2 knockout (KO) mice - in comparison with their heterozygous (HZ) and wild-type (WT) littermates - as well as its neurochemical underpinnings. Given the role of 5αR2 in the conversion of testosterone into the more potent androgen agonist DHT, we speculated that the lack of DHT in 5αR2-deficient mice may compromise some of the behavioral paradigms affected by testosterone and DHT through the activation of androgen receptors. Our studies were particularly focused on behaviors that have been related to testosterone levels and androgen receptor activation, including aggression, dominance, sexual behavior and sensation-seeking (Batrinos, 2012; Campbell et al., 2010; Coccaro et al., 2007; Cunningham et al., 2012; Williamson et al., 2017). Furthermore, since previous work has shown that social dominance is associated with increased D_2-D_3 receptor binding in the nucleus accumbens (Jupp et al., 2016; Morgan et al., 2002; Nader et al., 2012), we also analyzed the levels of dopamine and dopamine receptor binding in this region.

MATERIALS AND METHODS

Animals. The experiments included in this study were performed on adult (3-5-month old), experimentally naïve male 5αR2KO, HZ and WT mice (strain: C57BL/6), obtained from breeding colonies at the Universities of Kansas and Utah. All mice were generated from HZ x HZ crosses. Progenitors were obtained by Dr. Mala Mahendroo (Southwestern University). Unless stated otherwise for specific experimental purposes, all mice were housed in groups of 4-5/cage, with at least 1 mouse/genotype, and had *ad libitum* access to food and water. Housing facilities were maintained at 22ºC with a reverse light/dark cycle (lights off at 08:00 AM hours and on at 08:00 PM). Whenever the same mice were used for multiple behavioral paradigms, the order of animals in each test was counterbalanced throughout the study. Tests were arranged from least to most stressful (separated by at least one week) to minimize carry-over stress. Experimental manipulations were carried out in the animals' dark cycle between 10:00 AM and 6:00 PM. All handling and experimental procedures were performed in compliance with the National Institute of Health guidelines and approved by the local Institutional Animal Care and Use Committees.

Genotyping. Mouse genotyping was performed by PCR. Samples of genomic DNA were extracted from tail biopsies acquired from mice at weaning (postnatal day 21). The following primers were used to identify 5αR2 KO mice: 1) GAT GAC CTC TCC GGG CTT CC 2) GAA TGT TCC AAG TCA CAG GC 3) CGC TTC TGA GGA GAG AAC TGA CTG A. Reaction conditions were: 94°C x 2 min, 94°C x 40s, 55°C x 40s, 72°C x 5 min, 4°C x ∞, as previously described (Mahendroo *et al*., 2001).

Behavioral testing. Mice were tested in an extensive array of behavioral tasks, aimed at evaluating motoric, sensory, and anxiety-related responses. Except when specifically stated, all tests were performed in group-caged animals. All tests and analyses were consistently performed by personnel blinded to genotypes, using littermates from all three genotypes. Thus, none of the genotypes underwent more testing than the other groups. Furthermore, no animal was tested in more than 3 paradigms.

*Locomotor Activity***.** Locomotor behaviors were measured in a square force-plate actometer as previously described (Godar et al., 2015). The apparatus consisted of a white load plate (28 x 28 cm) surrounded on all four sides and covered by a clear Plexiglas box (30 cm tall). Four force transducers placed at the corners of each load plate were sampled 100 times s^{-1}, yielding a 0.01s temporal resolution, a 0.2-g force resolution and a 2-mm spatial resolution. Custom software directed the timing and data-logging

processes via a LabMaster interface (Scientific Solutions Inc., Mentor, OH, USA). Additional algorithms were used to extract macrobehavioral variables, such as distance travelled. Overall distance was calculated as the sum of the distances between coordinates of the location of center of force recorded every 0.50 s over the recording session. Low-mobility bouts were defined as periods of 5 s during which mice confined their movements to a 15-mm radius virtual circle. Velocity was defined as the distance coved by a run divided by the duration of that run and expressed as mm/s (equivalent to the product of stride length and stride rate). Distance to the nearest wall was used as an index of thigmotaxis. Mice (n=13/genotype) were placed in the center and their behavior was monitored for 30 min.

Hot Plate. Pain sensitivity was measured in the hot plate task, as previously described (Bortolato et al., 2013a). Mice (n=12/genotype) were individually exposed to a hot plate (IITC Life Science, Woodland Hills, CA) at 47.5°C, 50°C and 52.5°C, and the latency to lick their paws was measured. The cut-off time was set at 40 s.

*Sticky Tape Removal Test***.** Sensorimotor integration was tested using the sticky tape test as previously described elsewhere (Bortolato et al., 2013a). Mice (n=12/genotype) were briefly restrained and a circular piece of tape was placed on each forepaw. The latency to remove both pieces of tape was recorded.

*Olfactory Discrimination***.** Olfactory discrimination was tested as previously described (Wyatt et al., 2013). Mice (n=13/genotype) underwent five training trials of 5-minute exposure to two identical objects of the same scent. The objects were cylinders wrapped in tape and evenly scented with diluted almond or lemon oil. On the subsequent (sixth) test trial, one of the cylinders was replaced with an identical one, sprayed with a novel scent, in counterbalanced order. Testing was performed in dim light (5 lux) and the behavior was video-recorded and olfactory discrimination was measured as a novel exploration index (NEI), calculated as the ratio of the time spent sniffing the object imbued with the novel scent and the total time spent sniffing both the novel and the familiar scent.

*Acoustic Startle Reflex and Prepulse Inhibition (PPI) of the Startle***.** Acoustic startle reflex and PPI were tested as previously described (Mosher et al., 2017). We used six startle reflex detection systems (San Diego Instruments, San Diego, CA), each consisting of one standard cage placed in sound-attenuated chambers with fan ventilation. Each cage featured a Plexiglas cylinder of 3 cm diameter, mounted on a piezoelectric accelerometric platform connected to an analog-digital converter. Background noise and acoustic bursts were conveyed by two separate speakers, each one properly placed to produce a variation of sound within 1 dB across the startle cage. Both speakers and startle cages were connected to a main personal computer, which detected and analyzed all chamber variables with specific software. Before each testing session, acoustic stimuli were calibrated via specific devices (San Diego Instruments). Mice (n=8/genotype) were placed in a cage for a 5-min acclimatization period with a 70-dB white noise background, which continued for the remainder of the session. Each session consisted of three consecutive sequences of trials (periods). Unlike the first and the third period - during which mice were presented with only five pulse-alone trials of 115 dB - the second period consisted of a pseudorandom sequence of 40 trials, including 12 pulse-alone trials and 30 trials of pulse preceded by 73, 76 and 82 dB pre-pulses (respectively defined as PP3, PP6 and PP12; 10 for each level of pre-pulse loudness). Percent PPI was calculated as 100 - (mean startle amplitude for pre-pulse trials/mean startle amplitude for pulse-alone trials) x 100.

*Resident-Intruder Aggression***.** Testing was conducted as previously described (Bortolato et al., 2011a). Two separate cohorts of mice (n=9-12/genotype) were isolated for 4 and 8 weeks. After this period, they were exposed to age- and weight-matched WT males from different litters, for 10 min within the resident's home cage. The test was performed with light levels maintained at 10 lux. Measures included the number and duration of fighting bouts. Other aggressive behaviors scored included biting, tail rattling, and chasing.

Tube Dominance Test. Testing was performed as previously described (Spencer et al., 2005). The apparatus consisted of clear PVS tubing (3 cm in diameter and 45 cm long), anchored to a solid base. All testing was performed at 30 lux. In the first experiment mice were consistently paired with cage mates carrying a different genotype. All tests within a social group were randomized across cage mates so that a given mouse did not encounter the same animal twice in succession. The submissive mouse was identified as

that animal which first withdrew from the tube. If no animal exited the tube, the trial was coded as a tie. In a subsequent experiment with a new cohort of mice, males kept in isolation for 4 weeks (during adulthood) were tested against non-cage mates (n=16-19/genotype pairing).

*Mating responses***.** To analyze mating behaviors, males (n=8/genotype) were isolated for 24h. WT female mice were placed in the male's cage for 15 minutes and the behaviors were video-recorded. All females were in estrus, as assessed by the cell morphology of vaginal smears (Caligioni, 2009). Mounting responses were monitored as previously described (Ogawa et al., 1997).

*Novel-Object Exploration***.** Novel-object exploration was tested in an experiment modified from (Bortolato et al., 2009). Mice (n=12/genotype) were individually exposed to two identical novel objects, placed equidistant in their home cage after a 2-day isolation. Light was maintained at 10 lux. Mice were placed in the center of the cage between the two objects, facing away from the objects. The behavior of each mouse was recorded for 15 minutes to attenuate any potential confounds related to neophobia. Sniffing behavior was scored as number of approaches and duration of exploration with the novel object. Test sessions were also video recorded and the behavior scored as the novel object frequency and duration.

Wire-beam bridge test. The wire-beam bridge test was conducted with slight modification as previously described (Bortolato et al., 2009). The apparatus consisted of a 30-cm high Plexiglas platform and a 50-cm high Plexiglas wall, oppositely placed at 30 cm distance. The edge of the platform and the wall were connected by a horizontal, unrailed bridge (1.25 × 30 cm), made in black aluminum wire. The bridge consisted of 2 parallel beams (0.01 cm thick) perpendicularly connected by 24 equally distanced cross-ties (1.25 cm long). It was modestly flexible, with a downward deflection of 1 cm per 100-g load at the center point. Mice (N = 8/genotype) were placed on the end of the wire-beam bridge by the Plexiglas wall and their behavior was recorded for 5 min. Behavioral measures included the latency for the mouse to cross the bridge to the safe platform. Testing was conducted in dim (10 lux) light conditions.

*Light-Dark Box***.** Testing was performed as previously described (Bortolato et al., 2011b). The apparatus consisted of a Plexiglas cage (20 x 30 x 20 cm) comprising of a dark area

(20 x 10 x 20 cm) and an adjacent brightly lit compartment (20 x 20 x 20 cm; illumination: 250 lux). The two compartments were separated by a Plexiglas divider, providing a 7 x 4 cm opening. Briefly, mice (N = 11/genotype) were individually placed in the corner of the light area, and allowed to freely explore either compartment for 10 minutes. Mice were video-recorded, and the latency to exit the light compartment as well as the number and total duration of light compartment-entries were scored.

*Marble Burying***.** Marble burying was conducted as previously described (Bortolato et al., 2009). Briefly, mice (N = 6-7/genotype) were individually placed in a dimly lit (10 lux) cage (35 × 28 cm), with 5 cm of fine sawdust, for a 30-min acclimatization period. Subsequently, mice were briefly removed and 20 marbles (1 cm diameter) were placed in the cage, on top of the sawdust, arranged in 5 equidistant rows (each consisting of 4 marbles). Mice were then returned to the cages, and their behavior was video recorded for the following 30 min. A marble was considered buried if at least two thirds of its surface area was covered in sawdust.

*Saccharin Consumption and Preference***.** Mice (N = 12-13/genotype) were isolated for 2 weeks, and given *ad libitum* access to food throughout the remainder of this test. They were then given free access to two water bottles, containing either water or saccharin (at one of the following concentrations: 0.025%; 0.05%, 0.1%) for 48 h, as described by Tordoff et al. (2002). The overall consumption and preference (calculated as the ratio of saccharin solution/ total liquid consumed by each mouse) were measured.

Biochemical testing. *Striatal biogenic amines determination***.** The left striata were dissected at coordinates anterior to Bregma -0.10 mm according to the mouse brain atlas of Franklin and Paxinos (Paxinos and Franklin, 2012). Tissues were homogenized at 4°C in 250 µl of 0.1 N $HClO_4$ and centrifuged at 13,200xg for 20 min to precipitate proteins. The supernatants were stored at -80°C in small polyethylene tubes until they were assayed. The pellet was dissolved in 250 µl of 1 M NaOH for the determination of proteins content using a Micro BCA Protein Assay kit (Thermo Scientific, Rockford, IL). The content of dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 3-methoxytyramine (3-MT) were measured by high performance liquid chromatography (HPLC) with electrochemical detection (D'Astous et

al., 2004). Supernatants of striatal tissue were directly injected into the chromatograph consisting of a Waters 717 plus autosampler automatic injector, a Waters 515 pump equipped with a C-18 column (Waters Nova-Pak C18, 3 µm, 3.9 mm × 150 cm), a BAS LC-4C electrochemical detector and a glassy carbon electrode. The mobile phase consisted of 0.025 M citric acid, 1.7 mM 1-heptane-sulfonic acid, and 10% methanol, in filtered distilled water, delivered at a flow rate of 0.8 ml/min. The final pH of 4.0 was obtained by addition of NaOH. The electrochemical potential was set at 0.8 V with respect to an Ag/AgCl reference electrode. Results were expressed in ng of amine per mg of protein.

[^{3}H] SCH 23390 and [^{3}H] spiperone autoradiography. Right hemispheres were immersed in ShandonTM M-1 embedding matrix (Thermo Scientific, Rockford, IL) at -20°C, mounted on cryostat chucks and cut into 12-µm-thick coronal slices for autoradiography at three rostro-caudal levels (anterior: bregma 1.70 at 1.34 mm; medial: 0.98 at 0.38 mm; posterior: 0.02 at -0.94 mm). Slices were thaw-mounted on superfrost pre-cleaned slides (Thermo Scientific). Slices and dissections were kept at -80 °C until use for assays. D_1- and D_2- like receptor antagonist sites were labeled using the radioligands [^{3}H] SCH 23390 (81.9 Ci/mmol; PerkinElmer, MA, USA) and [^{3}H]spiperone (81.2 Ci/mmol; PerkinElmer, MA, USA) respectively (Morin et al., 2014). Two slides containing each 3 to 6 mounted coronal brain sections per animal for each level for D1 and D2 receptor binding were preincubated 15 min at room temperature in sodium phosphate buffer (PBS, 100 mM, pH 7.4) containing $MgCl_2$ 2 nM and in a Tris[hydroxymethyl]aminomethane (TRIS) buffer solution (Tris-HCl 50 mM containing KCl 5 mM, $CaCl_2$ 2 mM, $MgCl_2$ 2 mM, pH 7.4) respectively. Sections were then incubated for 60 min at room temperature in their respective buffer containing either 1 nM of [^{3}H] SCH 23390 and 50 nM ketanserin (to block 5-HT_{2A} receptors) or 1 nM of [^{3}H] spiperone and 50 nM ketanserin. Non-specific binding was defined in the presence of SKF-38393 1 µM and (+)-butaclamol 1 µM for D_1 and D_2 receptors respectively. After two 5-min washes in respective buffer at room temperature, sections were then rinsed briefly (10 s) in ice-cold distilled water. Finally, the slide-mounted tissue sections were exposed to BioMax MR films (Kodak, Rochester, NY) along with tritium standards [^{3}H]-microscales (Amersham, Arlington Heights, IL) for 16

and 29 days at room temperature for D_1- and D_2-like receptor binding experiments respectively. Films were developed and quantification of autoradiograms was performed on a Power Mac G4 computer connected to a video camera (XC-77, Sony, Tokyo, Japan) with a constant illumination light table using computerized densitometry and the public domain Image J processing software from NIH (v. 1.63). Optical gray densities were transformed into nCi/mg of tissue equivalent by using a [^{3}H] standard curve and then converted into fmol/mg of tissue using the specific activity of the radioligand.

For densitometric measurements of striatal D_1-like receptors, the optical density of the total area of the brain structure was measured since it was previously reported that specific binding was evenly distributed in the striatum (Morissette et al., 1992). For D_2-like receptors, a striatal lateral/medial gradient was observed with higher specific binding in the lateral versus the medial part; hence, these subregions were quantified separately (Falardeau and Di Paolo, 1987; Morissette et al., 1992). The nucleus accumbens is differentiated into at least two anatomically and functionally distinct regions, the core and the shell (Voorn et al., 2004). Thus, densitometric measurements of D_1- and D_2-like receptors were measured separately in these two nucleus accumbens subregions. Analyses were conducted by personnel blinded to group genotypes.

Statistical Analyses. Data distributed binomially (such as the results of the tube test) were analyzed by a binomial test. Continuously distributed data were tested for normality and homoscedasticity by the Kolmogorov-Smirnov and Bartlett's test. Based on these results, parametric and non-parametric statistical analyses were performed by a one-way ANOVA and Kruskal-Wallis test, followed by Neuman-Keuls' or Nemenyi's tests for post-hoc comparisons, respectively. Significance was set at $P = 0.05$.

RESULTS

Behavioral characterization of 5αR2 mutant mice. Neither 5αR2 KO nor HZ mice exhibited any overt abnormality in physical appearance and body weight (both across development and in adulthood), as compared with WT littermates. Similarly, the analysis of locomotor activity did not point to differences in any index, including total distance travelled (Fig. 1A), number of low-mobility bouts (Fig. 1B), maximum velocity (Fig. 1C), mean velocity of top ten runs (Fig 1D), or average distance from the walls (Fig 1E), an index of thigmotaxis. 5αR2 KO mice failed to exhibit any deficit in thermic pain responsiveness and sensorimotor integration, as measured by the latencies to lick the paws in the hot-plate test (Fig. 1F) and to remove sticky tape from the paws (Fig. 1G). Finally, no alterations were found in olfactory discrimination (Fig. 1H), acoustic startle response (Fig. 1I), and PPI (Fig. 1J).

We then proceeded to assess whether 5αR2 genotype may affect aggressive and dominance-related behaviors. Resident 5αR2 KO and HZ mice were less aggressive than WT littermates toward WT intruders after 4 weeks of social isolation (Fig. 2A-C). Indeed, both genotypes were found to engage in aggressive behavior for a significantly lower duration [Fig. 2A; H (2), = 14.09, $P < 0.001$; multiple comparisons: WT vs HZ, $P < 0.01$; WT vs KO, $P < 0.05$]. Mutants also displayed a lower number of aggressive bouts [Fig. 2B; H (2) = 13.76, $P < 0.01$; multiple comparisons: WT vs HZ, $P < 0.01$; WT vs KO, $P < 0.05$] and a greater latency to engage in aggression [Fig. 2C; H (2) = 14.50, $P < 0.001$; multiple comparisons: WT vs HZ, $P < 0.01$; WT vs KO, $P < 0.05$]. After 8 weeks of isolation, different cohorts of both HZ and KO mice showed significant reductions in the duration of aggressive behaviors [Fig. 2D; $F(2,24) = 8.99$, $P < 0.01$; *post hoc* comparisons: WT vs HZ, $P < 0.01$; WT vs KO, $P < 0.01$] as well as in the number of aggressive behaviors [Fig. 2E; $F(2,24) = 11.55$, $P < 0.001$; *post hoc* comparisons: WT vs HZ $P < 0.01$; WT vs KO $P < 0.001$]; furthermore, the latency to the first aggressive interaction was longer than those exhibited by WT [Fig. 2F; H(2) = 18.09, $P < 0.001$; multiple comparisons: WT vs HZ, $P < 0.01$; WT vs KO, $P < 0.001$]. Throughout both experiments, only a small percentage of KO mice (22.2% after 4 weeks of isolation and 33.3% after 8 weeks of isolation) initiated occasional, brief attacks, which were not conducive to victories (i.e., the opponents did not assume a subordinate posture after fighting).

Building on this finding, we tested 5αR2KO mice for potential changes in behavioral domains relevant to social dominance. First, we tested the behavior of 5αR2-deficient mice in the tube dominance test. 5αR2KO mice consistently retreated when confronted with WT cage mates (Fig. 2G; $P < 0.001$). To verify whether this behavior was indeed related to social ranking with respect to cage mates, we studied whether the tendency to retreat in the tube was ablated in confrontations among isolated mice. Indeed, KO mice did not exhibit any significant differences in tube dominance against non-littermates after a 4-week social isolation (Fig. 2H). We next measured the mounting behavior of 5αR2-deficient mice towards estrous WT females (Fig. 3A-B); in comparison with WT males, 5αR2 KO mice exhibited a trend toward a reduction in the frequency of mounting bouts [$H(2) = 8.422$, $P < 0.05$; multiple comparisons: WT vs KO = 0.08, HZ vs KO < 0.05] and a significant overall duration of active mounting [$H(2) = 8.67$, $P < 0.05$; multiple comparisons: WT vs KO < 0.05; HZ vs KO < 0.05]. Females were equally receptive to males of all genotypes.

Given that androgens have been described to be associated with higher novelty-seeking and risk-taking proclivity (Zuckerman et al., 1980), we verified whether 5αR2 deficiency could affect these impulsivity constructs using a novel-object exploration task and a wire-beam bridge paradigm, respectively. In comparison with WT mice, 5αR2 KO littermates explored novel objects for significantly less time [Fig. 3C; $F(2,33) = 3.37$, $P < 0.05$; *post hoc* comparisons: WT vs KO, $P < 0.05$], and with a reduced number of approaches [Fig. 3D; $F(2,33) = 8.55$, $P < 0.01$; *post hoc* comparisons: WT vs HZ, $P < 0.05$; WT vs KO, $P < 0.001$]. Finally, both 5αR2 KO and HZ mice crossed a wire-beam suspended bridge with a significantly increased latency [Fig. 3E; $F(2, 21) = 6.25$, $P < 0.01$; *post hoc* comparisons: WT vs HZ, $P < 0.01$; WT vs KO, $P < 0.05$].

To verify whether these changes were reflective of changes in anxiety- and reward-related responses we next assessed the reactivity of 5αR2-deficient mice to environmental stimuli. 5αR2KO mice failed to exhibit anxiety-like behaviors in the light-dark box (Fig. 4A-C) and in the marble-burying task (Fig. 4D), Furthermore, 5αR2-deficient mice displayed no overt alterations in saccharin preference and consumption (irrespective of the concentration) over 48 h (Supplementary Fig. 1).

DAergic neurotransmission. Given that dominance is characterized by increased binding of D2-like receptors in the nucleus accumbens, we performed these analyses in 5αR2 mutant mice, as compared with WT littermates (Supplementary Fig. 2). 5αR2 KO mice exhibited a significant reduction in the binding of D_2-like receptors in the rostral shell of this region (between 1.70 mm and 1.10 mm from the bregma) [Fig 5A; $F(2,15) = 4.53$, $P < 0.05$; *post hoc* comparisons: WT vs KO, $P < 0.01$]. No significant difference was found in either the rostral shell or the core of the nucleus accumbens (Fig 5B-D). Finally, no significant changes in D_1-like receptor binding were identified (Fig. 5E-H), although a statistical trend toward a decrease in D_1 receptor binding in the 5αR2 HZ and KO mice was observed in the caudal portion of the core [Fig. 5H; $F(2, 15) = 3.56$; $P = 0.051$]. Finally, 5αR2 HZ, but not KO mice, exhibited decreased levels of DA in the striatum [Fig 6A; $F(2,21) = 6.11$, $P < 0.01$; *post hoc* comparisons: WT vs HZ, $P < 0.05$; KO vs HZ, $P < 0.01$], DOPAC [Fig 6B; $F(2,21) = 5.55$, $P < 0.05$; *post hoc* comparisons: WT vs HZ, $P < 0.05$; KO vs HZ, $P < 0.05$], HVA [Fig 6C; $F(2,21) = 5.73$, $P < 0.05$; *post hoc* comparisons: WT vs HZ, $P < 0.01$; KO vs HZ, $P < 0.05$], and 3-MT [Fig. 6D; $F(2,21)=3.5$, $P < 0.05$; *post hoc* comparisons: WT vs HZ, $P < 0.05$]. However, there were no alterations in DA metabolism as evidenced by the ratios between DOPAC/DA (Fig 6E), HVA/DA (Fig. 6F) or 3-MT/DA (Fig. 6G).

DISCUSSION

The main results of this study show that 5αR2-deficient mice exhibit a reduction in dominance-related behavioral phenotypes, including aggression against intruders, tube-dominance and mating with receptive females. These changes are not accompanied by sensorimotor deficits or abnormalities in anxiety- or reward-related responses, pointing to a specific importance of 5αR2 in dominance-related behaviors.

Confrontations between WT and 5αR2 KO mice in the tube-dominance test resulted in the consistent predominance of the former. This paradigm (Lindzey et al.,1961; Messeri et al., 1975) is often used to study social dominance hierarchies and their genetic underpinnings (van den Berg et al., 2015), and has been recently validated as a reliable paradigm to investigate social dynamics (Wang et al., 2011). Although the relation between social dominance and tube dominance is not linear (Lindzey et al., 1966; Benton et al., 1980), the latter index has been shown to be consistently associated with multiple components of social dominance, including the Dalila barbering effect (Strozik and Festing, 1981), urine marking (Desjardin et al., 1973) and emission of ultrasonic vocalizations during courtship (de Catanzaro et al., 1983). Notably, the reduction in tube dominance in 5αR2 KO mice was only observed in group-housed mutants, indicating that this phenotype is not intrinsically due to the genotype - as shown by the different results of the encounters after a 4-week isolation period - but rather to the effects of the genetic mutation on social interaction with cage mates. Extensive evidence has shown that, in mice, social isolation enhances aggression and other dominance-related behaviors (Brain, 1975; Benton and Brain, 1979). These results indicate that, while 5αR2 contributes to shaping social hierarchies, its influence on the escalation to dominance is mediated by interactions with environmental factors (such as the exposure to social interactions). From this perspective, it is likely that 5αR2-deficient mice may exhibit alterations in dominance following social housing. Future studies will be necessary to examine the impact of these and other environmental variables (including stress and progressive habituation to social contexts) on 5αR2-deficient mice.

Another key result of our study was that the reduction in dominance-related behaviors in 5αR2-deficient mice was accompanied by lower impulsivity-related responses, such as

novel-object exploration and risk-taking (measured as the proclivity to cross a suspended wire-beam bridge). These behavioral domains are instrumental to the allocation of environmental resources and, ultimately, the development of social stratification (Sapolsky, 2005; Wilson and Daly, 1985), and, thus, may be related to social dominance. Accordingly, previous work has shown that dominant rodents have higher risk-taking propensity (Davis et al., 2009); furthermore, high novelty-seeking has been shown to serve as a robust predictor of aggression (Kerman et al., 2012).

Rich evidence has shown that the activation of androgen receptors by testosterone influences the formation of hierarchical ranks, as well as the ontogeny of aggression, mating, risk-taking, and novelty-seeking behaviors, in men and other animals (Breuer et al., 2001; Campbell et al., 2010; Damassa et al., 1977; Ehrenkranz et al., 1974; Lumia et al., 1994; Mazur and Booth, 1998; Slatcher et al., 2011; Stanton et al., 2011). Given that 5αR2-deficient males exhibit normal to high plasma testosterone (Mahendroo et al., 2001; Wilson et al., 1993), our findings suggest that the conversion of testosterone into DHT is instrumental in the acquisition of dominance. In line with this notion, prior research has documented that the relationship between testosterone and dominance and aggression is not direct, but rather modulated by environmental factors, including the presence of social stressors or the uncertainty of resources (Mazur and Booth, 1998). Several findings suggest that 5αR2 may mediate this environmental influence on the behavioral effects of testosterone on social dominance. First, 5αR2 synthesis in the brain is dependent on stress exposure; specifically, the brain expression of this enzyme is increased by acute or short-term stress (Frau et al., 2017; Sánchez et al., 2008), and reduced by chronic, inescapable stress (Bortolato et al., 2011b). Second, the conversion of testosterone into DHT by 5αR2 amplifies androgen signaling, given that DHT activates androgen receptors more potently than its precursor (Kovacs et al., 1984; Wilbert et al., 1983). Third, DHT promotes the synthesis of 5αR2 by a unique feed-forward mechanism (George et al., 1991), which may be instrumental for the behavioral escalation to dominance. In fact, androgens have been posited to increase aggressive proclivity following a successful confrontation with a competitor, and this process is finalized to the acquisition of a higher status in social hierarchy (Carré and McCormick, 2008; Mazur and Booth, 1998; Mehta and Josephs, 2006; Oyegbile and Marler, 2005).

In addition to the reduction in dominance-related and impulse-control behaviors, 5αR2 KO mice displayed a significant reduction in D_2/D_3 DA receptor binding in the rostral shell of the nucleus accumbens. Reductions in D_2/D_3 receptor binding have been associated with social subordinate status in rats (Jupp et al., 2016) and non-human primates (Morgan et al., 2002; Nader et al., 2012). Furthermore, D_2-like receptor availability has been associated with trait extraversion in humans (Depue and Collins, 1999) which is believed to reflect aspects of social dominance (Wiggins and Trapnell, 1996). Accordingly, PET studies have shown lower uptake of D_2/D_3 receptor ligands in the striatum of subordinate cynomolgus monkeys, as compared with dominant ones (Grant et al., 1998; Morgan et al., 2002). These behavioral deficits were not accompanied by changes in the levels of DA or its receptors; however, 5αR2 KO mice showed a reduction in D_2/D_3 receptor binding in the rostral shell of the nucleus accumbens. Taken together, these results strongly suggest that 5αR2 is an important substrate for the regulation of dominance through the modulation of DAergic signaling in the mesolimbic system.

Our studies did not include direct mechanistic experiments to probe the mechanisms underlying the reduction in D_2-like receptor binding in the rostral shell of the nucleus accumbens; thus, it remains unclear whether this phenotype is a direct consequence of 5αR2 deficiency, or rather a mere epiphenomenon of reduced dominance. While further research is needed to explore this critical issue, it is worth noting that previous data from our group point to a link between the functions of 5αR2 and the synthesis/regulation of DA receptors. For example, we found that, the 5αR2 inhibitor finasteride curbed the risk-taking effects of pramipexole (Bortolato et al., 2013), a dopaminergic agonist with high affinity for D_3 receptors (Mierau et al., 1995). Furthermore, we showed that finasteride suppresses behavioral responses mediated by D_1 and D_3 DA receptors (Frau et al, 2013; Frau et al., 2015) through its effects in the nucleus accumbens (Devoto et al., 2012).

Although the involvement of DA in the behavioral profile of 5αR2-deficient mice remains unclear, it is tempting to speculate that changes in DA receptor binding in the nucleus accumbens may contribute to the reduced aggression, mating, sensation seeking and risk-taking observed in these mutants, in view of the well-documented role of dopamine in these behavioral domains (Aragona et al., 2003; Dalley et al., 2007; Gjedde et al., 2010; Mai et al., 2015; van Erp and Miczek, 2000).

The complexity of the relationship between DA receptor availability and these domains of behavioral disinhibition may be related to the balance between presynaptic and postsynaptic receptors. Overexpression of postsynaptic D_2-like receptors, for example, increases motivation without altering consummatory behavior (Trifilieff et al., 2013). This connection may be mediated by the reduction in DHT synthesis. Indeed, DHT increases DA synthesis and stimulates the transcription of DA D_2 receptors, as well as molecules involved in the signaling pathway in the nigrostriatal pathway, including DA and vesicular monoamine transporter (Purves-Tyson et al., 2012; Purves-Tyson et al., 2014). The possibility that 5αR2 controls DAergic responses is also in line with our findings on the behavioral effects of finasteride. This drug is currently approved for the therapy of conditions linked to excess DHT, such as benign prostatic hyperplasia and androgenetic alopecia. In addition to these applications, finasteride may have therapeutic properties for several neuropsychiatric conditions characterized by poor impulse control and externalizing manifestations (Paba et al., 2011). Studies in rat models suggest that these effects may be underpinned by anti-DAergic properties (Bortolato et al., 2008; Frau et al., 2013). Taken together, these results suggest that 5αR2 may play a key role in the organization of behavioral responses related to DA.

In summary, the results of this study document that 5αR2 deficiency in mice results in reduced social dominance and related behavioral traits, ranging from aggression and mating to sensation seeking and risk taking. These results complement previous findings on the role of androgens and novelty-seeking personality, suggesting that the changes observed in 5αR2 KO mice are reflective of changes in androgen profile. Nevertheless, the interpretation that 5αR2 deficiency may have similar effect in humans should be considered with caution, in view of potential differences in the role of this enzyme between mice and humans. Additional limitations should be acknowledged. First, we did not identify the steroid mechanisms responsible for these changes. Previous studies have documented that plasma of 5αR2 KO mice displays no detectable DHT levels, and slightly increased levels of testosterone. Understanding whether alterations of the brain steroid profile may contribute to the observed phenotypes is extremely important; unfortunately, previous analyses of brain-regional changes in DHT have proven unsatisfactory, given the detection limits posed by current techniques and the small size of mouse brain

regions. To overcome this barrier, we are in the process of developing a novel line of 5αR2 KO rats. The analysis of steroid levels in these animals will also enable to confirm whether DHT replenishment (either in adulthood or in earlier developmental stages) may restore the ability to achieve a dominant status and normalize the related behavioral traits, including aggressiveness, mating efficiency, risk-taking and novelty seeking. Given that 5αR2 KO mice are characterized by low DHT levels, it is possible that DHT replenishment (either in adulthood or in earlier developmental stages) may restore the ability to achieve a dominant status and normalize the related behavioral traits, including aggressiveness, mating efficiency, risk-taking and novelty seeking. Second, our studies were only limited to male rodents; it is possible that, in consideration of the contributions of androgens to the synthesis of 5αR2, there may be sex differences in the expression of these behaviors. Irrespective of these potential limitations, these findings suggest the role of 5αR2 in the achievement of social dominance, and in the regulation of impulse control, possibly through the modulation of dopaminergic mesolimbic signaling. These results warrant further ethological investigations on the function of this enzyme in social dominance and hierarchical ranking, as well as with endophenotypes related to sensation seeking and risk taking (such as delay and probability discounting). Finally, future studies will be needed to understand the mechanisms whereby steroids can influence dopaminergic signaling in the nucleus accumbens.

Conflict of Interest

The authors declare no conflict of interest.

Contributors

LM monitored data collection, analyzed behavioral data, performed statistical analyses and wrote the first draft of the manuscript. SG and KM performed behavioral tests and performed statistical analyses. MM and SS performed biochemical testing and related statistical analyses. CG, SF and TDP designed the experiments, analyzed data and discussed the paper. MB designed the experiments, supervised the experimental execution, monitored data collection, wrote and revised the manuscript.

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REFERENCES

Aragona, B.J., Liu, Y., Curtis, J.T., Stephan, F.K., Wang, Z., 2003. A critical role for nucleus accumbens dopamine in partner-preference formation in male prairie voles. J. Neurosci. 23, 3483–3490. doi:23/8/3483 [pii]

Aumuller, G., Eicheler, W., Renneberg, H., Adermann, K., Vilja, P., Forssmann, W.G., 1996. Immunocytochemical evidence for differential subcellular localization of 5 alpha-reductase isoenzymes in human tissues. Acta Anat 156, 241–252.\

Batrinos, M.L., 2012. Testosterone and aggressive behavior in man. Int. J. Endocrinol. Metab. 10, 563-568. doi: 10.5812/ijem.3661.

Benton, D., Brain, P.F., 1979. Behavioural comparisons of isolated, dominant and subordinate mice. Behav. Processes. 4, 211-219. doi: 10.1016/0376-6357(79)90002-0.

Benton, D., Dalrymple-Alford, J.C. and Brain, P.F. (1980). Comparisons of measures of dominance in the laboratory mouse. Anim. Behav. 28, 1274-1279.

Bortolato, M., Cannas, A., Solla, P., Bini, V., Puligheddu, M., Marrosu, F., 2012. Finasteride attenuates pathological gambling in patients with Parkinson disease. J Clin Psychopharmacol. 32, 424-425. doi: 10.1097/JCP.0b013e3182549c2a.

Bortolato, M., Chen, K., Godar, S.C., Chen, G., Wu, W., Rebrin, I., Farrell, M.R., Scott, A.L., Wellman, C.L., Shih, J.C., 2011a. Social Deficits and Perseverative Behaviors, but not Overt Aggression, in MAO-A Hypomorphic Mice. Neuropsychopharmacology 36, 2674–2688. doi:10.1038/npp.2011.157

Bortolato, M., Devoto, P., Roncada, P., Frau, R., Flore, G., Saba, P., Pistritto, G., Soggiu, A., Pisanu, S., Zappala, A., Ristaldi, M.S., Tattoli, M., Cuomo, V., Marrosu, F., Barbaccia, M.L., 2011b. Isolation rearing-induced reduction of brain 5α-reductase expression: Relevance to dopaminergic impairments. Neuropharmacology 60, 1301–1308. doi:10.1016/j.neuropharm.2011.01.013

Bortolato, M., Frau, R., Orru, M., Bourov, Y., Marrosu, F., Mereu, G., Devoto, P., Gessa, G.L., 2008. Antipsychotic-like properties of 5-alpha-reductase inhibitors. Neuropsychopharmacology 33, 3146–3156. doi:10.1038/npp.2008.39

Bortolato, M., Godar, S.C., Alzghoul, L., Zhang, J., Darling, R.D., Simpson, K.L., Bini, V., Chen, K., Wellman, C.L., Lin, R.C.S., Shih, J.C., 2013a. Monoamine oxidase A and A/B knockout mice display autistic-like features. Int. J. Neuropsychopharmacol. 16, 869–888. doi:10.1017/S1461145712000715

Bortolato, M., Godar, S.C., Davarian, S., Chen, K., Shih, J.C., 2009. Behavioral disinhibition and reduced anxiety-like behaviors in monoamine oxidase B-deficient mice. Neuropsychopharmacology 34, 2746–57. doi:10.1038/npp.2009.118

Bortolato, M., Yardley, M.M., Khoja, S., Godar, S.C., Asatryan, L., Finn, D.A., Alkana, R.L., Louie, S.G., Davies, D.L., 2013b. Pharmacological insights into the role of P2X4 receptors in behavioural regulation: lessons from ivermectin. Int. J. Neuropsychopharmacol. 16, 1059–1070. doi:10.1017/S1461145712000909

Brain, P., 1975. What does individual housing mean to a mouse? Life Sci. 16, 187-200.

Breuer, M.E., McGinnis, M.Y., Lumia, A.R., Possidente, B.P., 2001. Aggression in male rats receiving anabolic androgenic steroids: effects of social and environmental provocation. Horm. Behav. 40, 409–18. doi:10.1006/hbeh.2001.1706

Caligioni, C.S., 2009. Assessing reproductive status/stages in mice. Curr. Protoc. Neurosci. doi:10.1002/0471142301.nsa04is48

Campbell, B.C., Dreber, A., Apicella, C.L., Eisenberg, D.T.A., Gray, P.B., Little, A.C., Garcia, J.R., Zamore, R.S., Lum, J.K., 2010. Testosterone exposure, dopaminergic reward, and sensation-seeking in young men. Physiol. Behav. 99, 451–456. doi:10.1016/j.physbeh.2009.12.011

Carlstedt-Duke, J., Gustafsson, S.A., Wtange, Ö., Gustafsson, J., 1977. Interactions of Corticosterone, 5α-Dihydrocorticosterone and Dexamethasone with Proteins in Rat-Liver Cytosol. Eur. J. Biochem. 73, 231–238. doi:10.1111/j.1432-1033.1977.tb11311.x

Carré, J.M., McCormick, C.M., 2008. Aggressive behavior and change in salivary testosterone concentrations predict willingness to engage in a competitive task. Horm. Behav. 54, 403–409. doi:10.1016/j.yhbeh.2008.04.008

Castelli, M.P., Casti, A., Casu, A., Frau, R., Bortolato, M., Spiga, S., Ennas, M.G., 2013. Regional distribution of 5alpha-reductase type 2 in the adult rat brain: an immunohistochemical analysis. Psychoneuroendocrinology 38, 281–293. doi:S0306-4530(12)00223-5

Coccaro, E.F., Beresford, B., Minar, P., Kaskow, J., Geracioti, T., 2007. CSF testosterone: relationship to aggression, impulsivity, and venturesomeness in adult males with personality disorder. J Psychiatr Res. 41, 488-492.

Cunningham, R.L., Lumia, A.R., McGinnis, M.Y., 2012. Androgen receptors, sex behavior, and aggression. Neuroendocrinology. 96, 131-140. doi: 10.1159/000337663.

D'Astous, M., Gajjar, T.M., Dluzen, D.E., Di Paolo, T., 2004. Dopamine transporter as a marker of neuroprotection in methamphetamine- lesioned mice treated acutely with estradiol. Neuroendocrinology 79, 296–304. doi:10.1159/000079664

Dalley, J.W., Fryer, T.D., Brichard, L., Robinson, E.S.J., Theobald, D.E.H., Laane, K., Pena, Y., Murphy, E.R., Shah, Y., Probst, K., Abakumova, I., Aigbirhio, F.I., Richards, H.K., Hong, Y., Baron, J.-C., Everitt, B.J., Robbins, T.W., 2007. Nucleus Accumbens D2/3 Receptors Predict Trait Impulsivity and Cocaine Reinforcement. Science (80-.). 315, 1267–1270. doi:10.1126/science.1137073

Damassa, D.A., Smith, E.R., Tennent, B., Davidson, J.M., 1977. The relationship between circulating testosterone levels and male sexual behavior in rats. Horm. Behav. 8, 275–286. doi:10.1016/0018-506X(77)90002-2

Davis, J.F., Krause, E.G., Melhorn, S.J., Sakai, R.R., Benoit, S.C., 2009. Dominant rats are natural risk takers and display increased motivation for food reward. Neuroscience. 162, 23-30. doi: 10.1016/j.neuroscience.2009.04.039.

de Catanzaro, D., Ngan, E.T., 1983. Dominance in intermale encounters and subsequent sexual success in mice. J. Comp. Psychol. 97, 269-278.

Depue, R.A., Collins, P.F., 1999. Neurobiology of the structure of personality: Dopamine, facilitation of incentive motivation, and extraversion. Behav. Brain Sci. 22. doi:10.1017/S0140525X99002046

Desjardins, C., Maruniak, J.A., Bronson, F.H., 1973. Social rank in house mice: differentiation revealed by ultraviolet visualization of urinary marking patterns. Science. 182, 939-941.

Devoto, P., Frau, R., Bini, V., Pillolla, G., Saba, P., Flore, G., Corona, M., Marrosu, F., Bortolato, M., 2012. Inhibition of 5α-reductase in the nucleus accumbens counters sensorimotor gating deficits induced by dopaminergic activation. Psychoneuroendocrinology 37, 1630–1645. doi:10.1016/j.psyneuen.2011.09.018

Ehrenkranz, J., Bliss, E., Sheard, M.H., 1974. Plasma testosterone: Correlation with aggressive behavior and social dominance in man. Psychosom. Med. 36, 469–475. doi:10.1097/00006842-197411000-00002

Eicheler, W., Tuohimaa, P., Vilja, P., Adermann, K., Forssmann, W.G., Aumüller, G., 1994. Immunocytochemical localization of human 5 alpha-reductase 2 with polyclonal antibodies in androgen target and non-target human tissues. J. Histochem. Cytochem. 42, 667–675. doi:10.1177/42.5.8157936

Falardeau, P., Di Paolo, T., 1987. Regional effect of estradiol on rat caudate-putamen dopamine receptors: lateral-medial differences. Neurosci. Lett. 74, 43–48. doi:10.1016/0304-3940(87)90048-6

Frau, R., Abbiati, F., Bini, V., Casti, A., Caruso, D., Devoto, P., Bortolato, M., 2015. Targeting neurosteroid synthesis as a therapy for schizophrenia-related alterations induced by early psychosocial stress. Schizophr. Res. 168, 640–648. doi:10.1016/j.schres.2015.04.044

Frau, R., Bini, V., Soggiu, A., Scheggi, S., Pardu, A., Fanni, S., Roncada, P., Puligheddu, M., Marrosu, F., Caruso, D., Devoto, P., Bortolato, M., 2017. The Neurosteroidogenic Enzyme 5α-Reductase Mediates Psychotic-Like Complications of Sleep Deprivation. Neuropsychopharmacology 1–30. doi:10.1038/npp.2017.13

Frau, R., Pillolla, G., Bini, V., Tambaro, S., Devoto, P., Bortolato, M., 2013. Inhibition of 5alpha-reductase attenuates behavioral effects of D1-, but not D2-like receptor agonists in C57BL/6 mice. Psychoneuroendocrinology 38, 542–551. doi:10.1016/j.psyneuen.2012.07.014

Frye, C.A., Park, D., Tanaka, M., Rosellini, R., Svare, B., 2001. The testosterone metabolite and neurosteroid 3 alpha-androstanediol may mediate the effects of testosterone on conditioned place preference. Psychoneuroendocrinology 26, 731–750. doi:Doi 10.1016/S0306-4530(01)00027-0

George, F.W., Russell, D.W., Wilson, J.D., 1991. Feed-forward control of prostate growth: dihydrotestosterone induces expression of its own biosynthetic enzyme, steroid 5 alpha-

reductase. Proc. Natl. Acad. Sci. U. S. A. 88, 8044–7. doi:10.1073/pnas.88.18.8044

Gjedde, A., Kumakura, Y., Cumming, P., Linnet, J., Møller, A., 2010. Inverted-U-shaped correlation between dopamine receptor availability in striatum and sensation seeking. Proc. Natl. Acad. Sci. 107, 3870–3875. doi:10.1073/pnas.0912319107

Godar, S.C., Bortolato, M., Richards, S.E., Li, F.G., Chen, K., Wellman, C.L., Shih, J.C., 2015. Monoamine oxidase A is required for rapid dendritic remodeling in response to stress. Int. J. Neuropsychopharmacol. 18, 1–12. doi:10.1093/ijnp/pyv035

Grant, K.A., Shively, C.A., Nader, M.A., Ehrenkaufer, R.L., Line, S.W., Morton, T.E., Gage, H.D., Mach, R.H., 1998. Effect of social status on striatal dopamine D2 receptor binding characteristics in cynomolgus monkeys assessed with positron emission tomography. Synapse. 29(1), 80-83.

Imperato-McGinley, J., Guerrero, L., Gautier, T., and Peterson, R. E., 1974. Steroid 5-reductase deficiency in man: An inherited form of male pseudohermaphroditism. Science, 186, 1213-1216.

Imperato-McGinley, J., Zhu, Y.S., 2002. Androgens and male physiology the syndrome of 5α-reductase-2 deficiency, in: Molecular and Cellular Endocrinology. pp. 51–59. doi:10.1016/S0303-7207(02)00368-4

Jupp, B., Murray, J.E., Jordan, E.R., Xia, J., Fluharty, M., Shrestha, S., Robbins, T.W., Dalley, J.W., 2016. Social dominance in rats: Effects on cocaine self-administration, novelty reactivity and dopamine receptor binding and content in the striatum. Psychopharmacology (Berl). 233, 579–589. doi:10.1007/s00213-015-4122-8

Kerman, I.A., Clinton, S.M., Simpson, D.N., Bedrosian, T.A., Bernard, R., Akil, H., Watson, S.J., 2012. Inborn differences in environmental reactivity predict divergent diurnal behavioral, endocrine, and gene expression rhythms. Psychoneuroendocrinology. 37, 256-269. doi: 10.1016/j.psyneuen.2011.06.010.

Kovacs, W.J., Griffin, J.E., Weaver, D.D., Carlson, B.R., Wilson, J.D., 1984. A mutation that causes lability of the androgen receptor under conditions that normally promote transformation to the DNA-binding state. J. Clin. Invest. 73, 1095–1104.

Lindzey, G., Winston, H., Manosevitz, M., 1961. Social dominance in inbred mouse strains. Nature 191, 474–476.

Lindzey, G., Manosevitz, M., Winston, H., 1966. Social dominance in the mouse. *Psychon Sci 5*, 451-452.

Lumia, A.R., Thorner, K.M., McGinnis, M.Y., 1994. Effects of chronically high doses of the anabolic androgenic steroid, testosterone, on intermale aggression and sexual behavior in male rats. Physiol. Behav. 55, 331–335. doi:10.1016/0031-9384(94)90142-2

Mahendroo, M.S., Cala, K.M., Hess, D.L., Russell, D.W., 2001. Unexpected virilization in male mice lacking steroid 5 alpha-reductase enzymes. Endocrinology 142, 4652–4662. doi:10.1210/endo.142.11.8510

Mai, B., Sommer, S., Hauber, W., 2015. Dopamine D1/D2 receptor activity in the nucleus

accumbens core but not in the nucleus accumbens shell and orbitofrontal cortex modulates risk-based decision making. Int. J. Neuropsychopharmacol. 18. doi:10.1093/ijnp/pyv043

Martini, L., Celotti, F., Melcangi, R.C., 1996. Testosterone and progesterone metabolism in the central nervous system: Cellular localization and mechanism of control of the enzymes involved. Cell. Mol. Neurobiol. 16, 271–282. doi:10.1007/BF02088095

Matsumoto, K., Pinna, G., Puia, G., Guidotti, A., Costa, E., 2005. Social isolation stress-induced aggression in mice: a model to study the pharmacology of neurosteroidogenesis. Stress. 8 (2): 85-92.

Mazur, A., Booth, A., 1998. Testosterone and dominance in men. Behav. Brain Sci. 21, 353–397. doi:10.1017/S0140525X98001228

Mehta, P.H., Josephs, R.A., 2006. Testosterone change after losing predicts the decision to compete again. Horm. Behav. 50, 684–692. doi:10.1016/j.yhbeh.2006.07.001

Messeri, P., Eleftheriou, B.E., Oliverio, A., 1975. Dominance behavior: a phylogenetic analysis in the mouse. Physiol Behav. 14, 53-58.

Mierau, J., Schneider, F.J., Ensinger, H.A., Chio, C.L., Lajiness, M.E., Huff, R.M., 1995. Pramipexole binding and activation of cloned and expressed dopamine D_2, D_3 and D_4 receptors. Eur. J. Pharmacol. 290, 29-36.

Morgan, D., Grant, K.A., Gage, H.D., Mach, R.H., Kaplan, J.R., Prioleau, O., Nader, S.H., Buchheimer, N., Ehrenkaufer, R.L., Nader, M.A., 2002. Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. Nat. Neurosci. 5, 169–174. doi:10.1038/nn798

Morin, N., Jourdain, V.A., Morissette, M., Grégoire, L., Di Paolo, T., 2014. Long-term treatment with l-DOPA and an mGlu5 receptor antagonist prevents changes in brain basal ganglia dopamine receptors, their associated signaling proteins and neuropeptides in parkinsonian monkeys. Neuropharmacology 79, 688–706. doi:10.1016/j.neuropharm.2014.01.014

Morissette, M., Levesque, D., Di Paolo, T., 1992. Effect of chronic estradiol treatment on brain dopamine receptor reappearance after irreversible blockade: an autoradiographic study. Mol. Pharmacol. 42, 480–488.

Mosher, L.J., Godar, S.C., Nelson, M., Fowler, S.C., Pinna, G., Bortolato, M., 2017. Allopregnanolone mediates the exacerbation of Tourette-like responses by acute stress in mouse models. Sci. Rep. 7, 3348. doi:10.1038/s41598-017-03649-1

Nader, M.A., Nader, S.H., Czoty, P.W., Riddick, N. V., Gage, H.D., Gould, R.W., Blaylock, B.L., Kaplan, J.R., Garg, P.K., Davies, H.M.L., Morton, D., Garg, S., Reboussin, B.A., 2012. Social dominance in female monkeys: Dopamine receptor function and cocaine reinforcement. Biol. Psychiatry 72, 414–421. doi:10.1016/j.biopsych.2012.03.002

Ogawa, S., Lubahn, D.B., Korach, K.S., Pfaff, D.W., 1997. Behavioral effects of estrogen receptor gene disruption in male mice. Proc. Natl. Acad. Sci. U. S. A. 94, 1476–1481. doi:10.1073/pnas.94.4.1476

Oyegbile, T.O., Marler, C.A., 2005. Winning fights elevates testosterone levels in California mice and enhances future ability to win fights. Horm. Behav. 48, 259–267. doi:10.1016/j.yhbeh.2005.04.007

Paba, S., Frau, R., C. Godar, S., Devoto, P., Marrosu, F., Bortolato, M., 2011. Steroid 5α-Reductase as a Novel Therapeutic Target for Schizophrenia and Other Neuropsychiatric Disorders. Curr. Pharm. Des. 17, 151–167. doi:10.2174/138161211795049589

Paxinos, G., Franklin, K., 2012. The Mouse Brain in Stereotaxic Coordinates, Academic Press.

Pinna, G., Dong, E., Matsumoto, K., Costa, E., Guidotti, A., 2003. In socially isolated mice, the reversal of brain allopregnanolone down-regulation mediates the anti-aggressive action of fluoxetine. Proc. Natl. Acad. Sci. U. S. A. 100, 2035–2040. doi:10.1073/pnas.0337642100

Poletti, A., Coscarella, A., Negri-Cesi, P., Colciago, A., Celotti, F., Martini, L., 1998. 5 Alpha-Reductase Isozymes in the Central Nervous System. Steroids 63, 246–251. doi:S0039-128X(98)00018-X [pii]

Purves-Tyson, T.D., Handelsman, D.J., Double, K.L., Owens, S.J., Bustamante, S., Weickert, C., 2012. Testosterone regulation of sex steroid-related mRNAs and dopamine-related mRNAs in adolescent male rat substantia nigra. BMC Neurosci. 13, 95. doi:10.1186/1471-2202-13-95

Purves-Tyson, T.D., Owens, S.J., Double, K.L., Desai, R., Handelsman, D.J., Weickert, C.S., 2014. Testosterone induces molecular changes in dopamine signaling pathway molecules in the adolescent male rat nigrostriatal pathway. PLoS One 9. doi:10.1371/journal.pone.0091151

Russell, D.W., Wilson, J.D., 1994. Steroid 5 alpha-reductase: two genes/two enzymes. Annu Rev Biochem. 63, 25-61.

Sánchez, P., Torres, J.M., Gavete, P., Ortega, E., 2008. Effects of swim stress on mRNA and protein levels of steroid 5alpha-reductase isozymes in prefrontal cortex of adult male rats. Neurochem. Int. 52, 426–31. doi:10.1016/j.neuint.2007.07.019

Sapolsky, R.M., 2005. The influence of social hierarchy on primate health. Science. 308, 648-652.

Slatcher, R.B., Mehta, P.H., Josephs, R.A., 2011. Testosterone and Self-Reported Dominance Interact to Influence Human Mating Behavior. Soc. Psychol. Personal. Sci. 2, 531–539. doi:10.1177/1948550611400099

Soggiu, A., Piras, C., Greco, V., Devoto, P., Urbani, A., Calzetta, L., Bortolato, M., Roncada, P., 2016. Exploring the neural mechanisms of finasteride: a proteomic analysis in the nucleus accumbens. Psychoneuroendocrinology 74, 387–396. doi:10.1016/j.psyneuen.2016.10.001

Spencer, C.M., Alekseyenko, O., Serysheva, E., Yuva-Paylor, L.A., Paylor, R., 2005. Altered anxiety-related and social behaviors in the Fmr1 knockout mouse model of fragile X syndrome. Genes Brain Behav. 4, 420–430. doi:10.1111/j.1601-183X.2005.00123.x

Stanton, S.J., Liening, S.H., Schultheiss, O.C., 2011. Testosterone is positively associated with risk taking in the Iowa Gambling Task. Horm. Behav. 59, 252–256. doi:10.1016/j.yhbeh.2010.12.003

Strozik, E., Festing, M.F., 1981. Whisker trimming in mice. Lab. Anim. 15, 309-312.

Thigpen, A.E., Silver, R.I., Guileyardo, J.M., Casey, M.L., McConnell, J.D., Russell, D.W., 1993. Tissue distribution and ontogeny of steroid 5 alpha-reductase isozyme expression. J. Clin. Invest. 92, 903-910. doi:10.1172/jci116665

Tordoff, M.G., Pilchak, D.M., Williams, J.A., McDaniel, A.H., Bachmanov, A.A., 2002. The maintenance diets of C57BL/6J and 129X1/SvJ mice influence their taste solution preferences: implications for large-scale phenotyping projects. J Nutr. 132(8), 2288-97.

Trifilieff, P., Feng, B., Urizar, E., Winiger, V., Ward, R.D., Taylor, K.M., Martinez, D., Moore, H., Balsam, P.D., Simpson, E.H., Javitch, J.A., 2013. Increasing dopamine D2 receptor expression in the adult nucleus accumbens enhances motivation. Mol. Psychiatry 18, 1025–1033. doi:10.1038/mp.2013.57

van den Berg, W.E., Lamballais, S., Kushner, S.A., 2015. Sex-specific mechanism of social hierarchy in mice. Neuropsychopharmacology. 40, 1364-1372. doi: 10.1038/npp.2014.319.

van Erp, A.M., Miczek, K.A. 2000. Aggressive behavior, increased accumbal dopamine, and decreased cortical serotonin in rats. J Neurosci 20, 9320–9325. doi:20/24/9320 [pii]

Voorn, P., Vanderschuren, L.J., Groenewegen, H.J., Robbins, T.W., Pennartz, C.M., 2004. Putting a spin on the dorsal-ventral divide of the striatum. Trends Neurosci. doi:10.1016/j.tins.2004.06.006

Wang, F., Zhu, J., Zhu, H., Zhang, Q., Lin, Z., Hu, H., 2011. Bidirectional control of social hierarchy by synaptic efficacy in medial prefrontal cortex. Science 334, 693–7. doi:10.1126/science.1209951

Wiggins, J.S., Trapnell, P.D., 1996. A dyadic-interactional perspective on the five-factor model, in: The Five-Factor Model of Personality: Theoretical Perspectives. pp. 88–162.

Wilbert, D.M., Griffin, J.E., Wilson, J.D., 1983. Characterization of the cytosol androgen receptor of the human prostate. J Clin Endocrinol Metab 56, 113–120.

Williamson, C.M., Lee, W., Romeo, R.D., Curley, J.P., 2017. Social context-dependent relationships between mouse dominance rank and plasma hormone levels. Physiol Behav. 171:110-119. doi: 10.1016/j.physbeh.2016.12.038.

Wilson, J.D., Griffin, J.E., Russell, D.W., 1993. Steroid 5 alpha-reductase 2 deficiency. Endocr. Rev. 14, 577–593. doi:10.1210/edrv-14-5-577

Wilson, M. Daly, M., 1985. Competitiveness, risk taking, and violence: the young male syndrome, Ethol. Sociobiol. 6, 59-73.

Wyatt, L.R., Godar, S.C., Khoja, S., Jakowec, M.W., Alkana, R.L., Bortolato, M., Davies, D.L., 2013. Sociocommunicative and Sensorimotor Impairments in Male P2X4-Deficient

Mice. Neuropsychopharmacology 38, 1993–2002. doi:10.1038/npp.2013.9.

Zuckerman, M., Buchsbaum, M.S., Murphy, D.L., 1980. Sensation Seeking and its Biological Correlates. Psychol Bull. 88: 187-214.

FIGURE LEGENDS

Fig. 1 5αR2-deficient mice do not display deficits in motoric and sensory functions. Mice (n=13/genotype) tested on a force-plate actometer revealed no differences among genotypes in (A) total distance travelled, (B) number of low-mobility episodes, (C) maximum velocity, (D) mean velocity of the fastest 10 runs, and (E) average distance to the nearest wall. Similarly, no differences were found in pain sensitivity and sensorimotor integration, as revealed by the latencies to (F) lick paws in the hot-plate test (n=12/genotype) and (G) remove sticky tape from the forepaws (n=12/genotype). No alterations were found in (H) odor discrimination, as measured by % novel exploration index (%NEI) (n=13/genotype), (I) acoustic startle response, and (J) % prepulse inhibition (PPI) of the startle reflex (n=8/genotype). Data are shown as mean ± SEM. **Fig. 2** 5αR2 knockout mice display decreases in aggression and tube dominance. Aggressive behaviors were measured in the resident intruder paradigm following either a 4 week (A-C) or 8 week (D-F) isolation. The total time of aggressive behaviors (A,D), the number of aggressive bouts (B,E), and the latency to aggression (C,F) were evaluated. N=9-12/group. Dominance behaviors were measured in the tube test while the mice were (G) socially caged or (H) socially isolated. N=16-19/genotype. Data are shown as mean ± SEM. Abbreviations: WT, wildtype; HZ, 5αR2 heterozygous; KO, 5αR2 knockout. *P<0.05, **P<0.01, and ***P<0.001 compared to WT littermates.

Fig. 3 5αR2 knockout mice display decreases in mating behaviors and exploration. (A) The total time engaged in mounting behaviors and (B) the number of encounters were measured when males were exposed to WT females in estrous. N=8/genotype. (C) The duration of exploration of novel objects expressed as % of total time, and (I) the bouts of exploration were measured during the object exploration task. N=12/genotype. (J) Risk-taking behaviors were measured as the latency to traverse a wire-beam bridge. N=8/genotype. Data are shown as mean ± SEM. Abbreviations: WT, wildtype; HZ, 5αR2 heterozygous; KO, 5αR2 knockout. *P<0.05, **P<0.01, and ***P<0.001 compared to WT littermates. $^{\#}$P<0.05 compared to HZ mice.

Fig. 4 5αR2-deficient mice do not display anxiety-like manifestations. In the light-dark box (n=11/genotype), no differences were found in the (A) total duration of time (expressed

as % of total time) spent by mice in the lit compartment, (B) number of transitions between the two compartments, and (C) latency to enter the dark compartment. Similarly, no differences among genotypes were detected in either the (D) number of marbles buried in a marble-burying task (n=6-7 genotype). Data are shown as mean ± SEM. Abbreviations: WT, wildtype; HZ, 5αR2 heterozygous mice; KO, 5αR2 knockout mice.

Fig. 5 D_1- and D_2-like dopamine receptor binding in the nucleus accumbens of 5αR2-deficient mice. Binding was measured in (A; E) the rostral and (B; F) the caudal shell as well as in (C; G) the rostral and (D; H) caudal core of the nucleus accumbens. Data are shown as mean ± SEM. N=5-8/genotype. Abbreviations: WT, wildtype; HZ, 5αR2 heterozygous; KO, 5αR2 knockout. **P<0.01 compared to WT littermates.

Fig. 6 (A) Dopamine, (B) DOPAC, (C) HVA, (D) 3-MT, (E) DOPAC/DA, (F) HVA/DA, (G) 3-MT/DA levels in the striatum. N=8/genotype. Abbreviations: WT, wildtype; HZ, 5αR2 heterozygous; KO, 5αR2 knockout. *P<0.05 and **P<0.01 compared to WT littermates. $^{\#}$P<0.05 and $^{\#\#}$P<0.01 compared to HZ mice.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Fig. 1 5αR2-deficient mice do not exhibit alteration in saccharin preference. All genotypes (n=12-13/genotype) exhibited equivalent preferences for saccharin taste, at (A) 0.1%, (B) 0.05% and (C) 0.025% concentrations. Furthermore, no differences were found in total liquid consumption, irrespective of saccharin concentrations (D-F). Data are represented as mean ± SEM. Abbreviations: WT, wildtype; HZ, 5αR2 heterozygous; KO, 5αR2 knockout mice.

Supplementary Fig. 2 Representative images from (A-C) [^{3}H]SCH 23390 and (D-F) [^{3}H]spiperone autoradiography experiments on ventral and dorsal striata of wild-type (WT) and 5αR2 knockout (KO) mice.

Figr-1

Figure 1

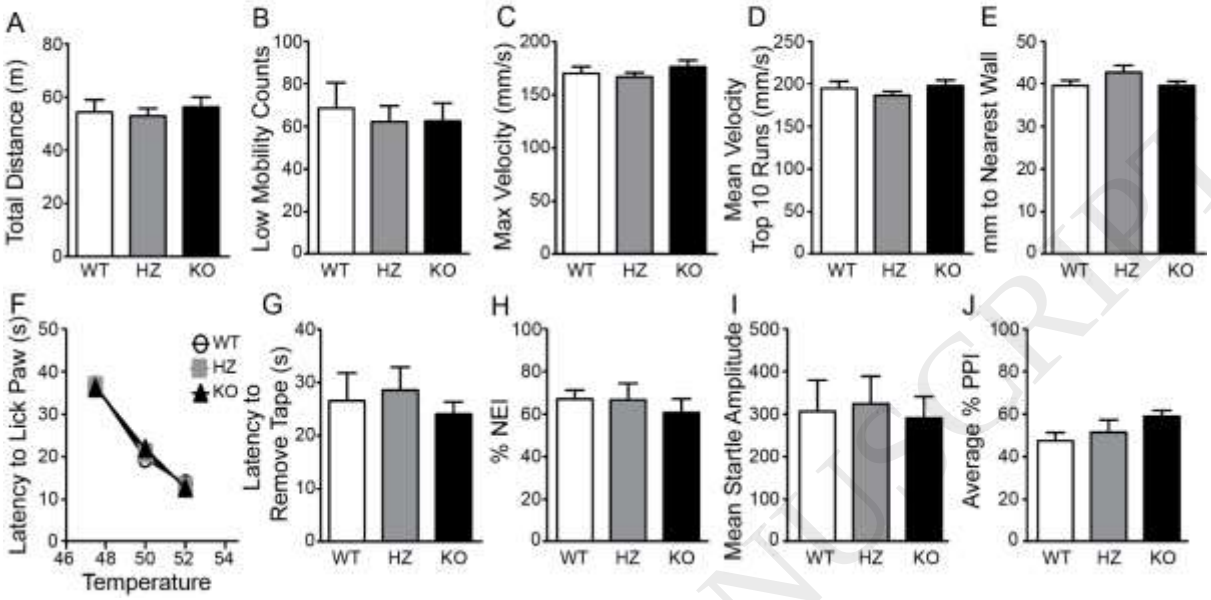
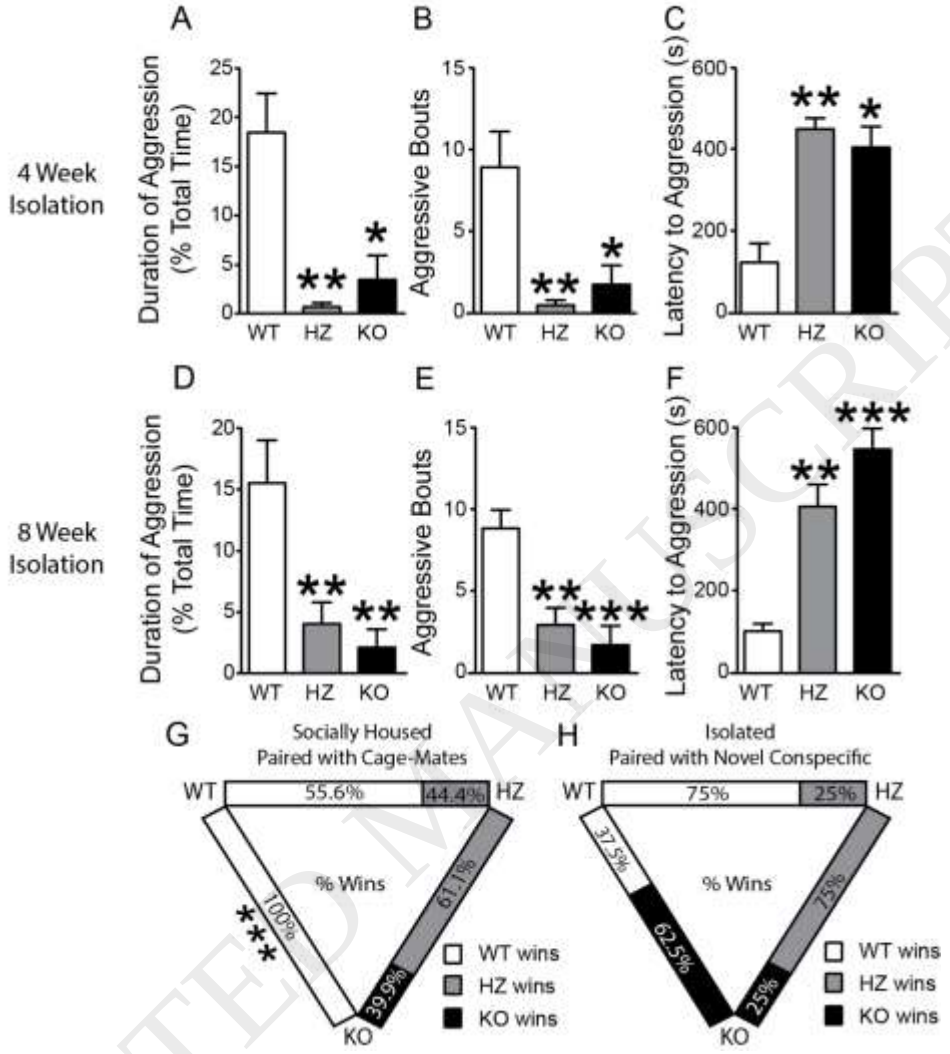


Figure 2



Figr-2

Figr-3

Figure 3

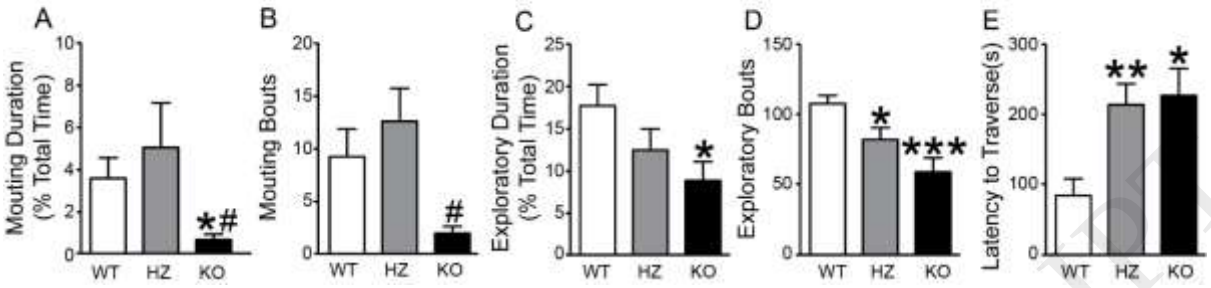
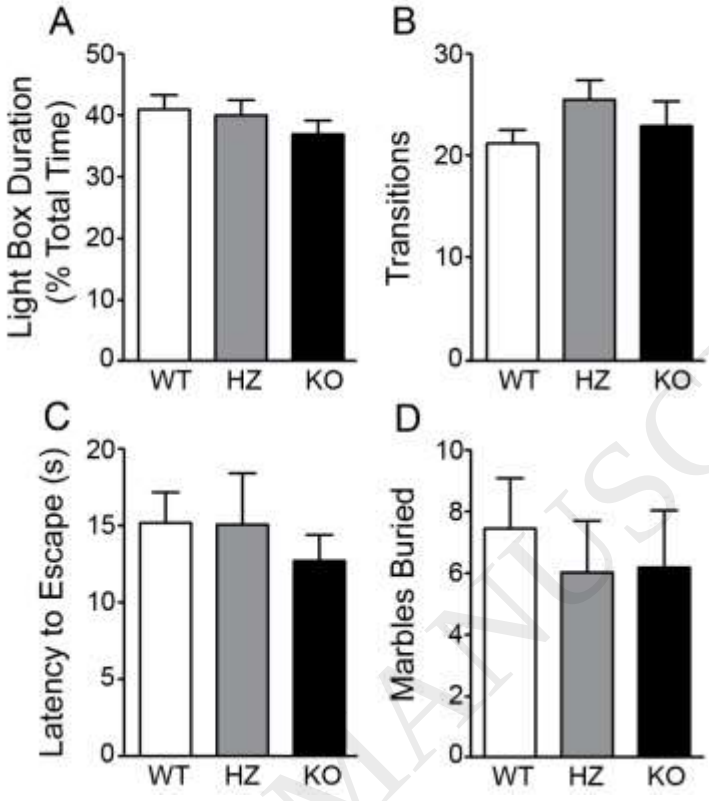
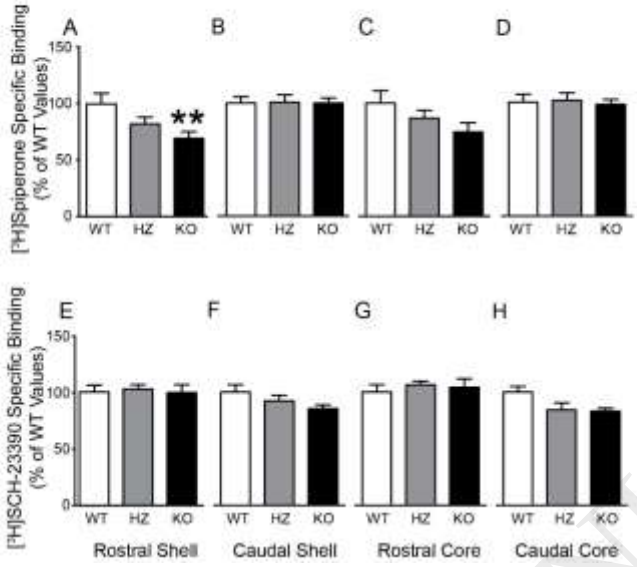


Figure 4



Figr-4

Figr-5
Figure 5



Figr-6

Figure 6