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Sex-specific effect of the anabolic steroid, 17 α -methyltestosterone, on inhibitory avoidance learning in periadolescent rats

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Abstract

The illicit use of anabolic androgenic steroids (AAS) has gained popularity among adolescents in the last decade. However, although it is known that exposure to AAS impairs cognition in adult animal models, the cognitive effects during adolescence remain undetermined. An inhibitory avoidance task (IAT) was used to assess the effect of AAS (17 α -methyltestosterone; 17 α -meT-7.5 mg/kg) in male and female periadolescent rats. A single injection of 17 α -meT immediately before the footshock produced significant impairment of inhibitory avoidance learning in males but not females. Generalized anxiety, locomotion, and risk assessment behaviors (RAB) were not affected. Our results show that exposure to a single pharmacological dose of 17 α -meT during periadolescence exerts sex-specific cognitive effects without affecting anxiety. Thus, disruption of the hormonal milieu during this early developmental period might have negative impact on learning and memory.

Keywords

anabolic androgenic steroids; anxiety; cognition; inhibitory avoidance learning; periadolescent

1. Introduction

In spite of being prohibited by law, anabolic androgenic steroids (AAS) are often taken in high doses by athletes to increase muscle mass and enhance athletic performance. In the last decades, illicit use of AAS by adolescents has generated significant concern (Castillo and Comstock, 2007; Mulcahey et al., 2010); particularly because adolescence is a developmental period characterized by an increase in sex hormones when reproductive, neural, and behavioral maturation occurs (Blakemore et al., 2010; Sisk and Foster, 2004). In addition to the well-known physiological effects of AAS (Hartgens and Kuipers, 2004),

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these compounds have been associated with multiple psychological disorders in humans, such as anxiety, aggression, or mood disorders (for review see Graham et al., 2008; Maravelias et al., 2005). In animals, anxiety and aggression-like behaviors have been shown to be affected by AAS (Oberlander and Henderson, 2012), as well as changes in sexual behavior, learning, and memory (for review see Clark and Henderson, 2003).

Studies support that gonadal hormones modulate learning and memory, but considerable debate exist regarding their effects on cognitive function. Specifically, acute (single injection following training; 1 mg/kg) or chronic (5 weeks through Silastic capsules) testosterone administration enhances cognitive performance on the inhibitory avoidance task (IAT) in adult male rats (Edinger et al., 2004; Frye et al., 2010; Frye and Seliga, 2001). On the other hand, studies have demonstrated that a single intracerebral administration of several doses of exogenous testosterone (10, 20, 40, 80, and 120 µg/0.5 µl) caused a dose-dependent impairment of spatial memory (Naghdi et al., 2001; 2003), as well as acquisition, consolidation and retrieval of inhibitory avoidance learning (Harooni et al., 2008). Similar to the endogenous compounds, studies have shown that high doses of synthetic androgens (nandrolone: 15 mg/kg), when given in daily subcutaneous injections for either 6 weeks (Kouvelas et al., 2008) or 14 days, (Magnusson et al., 2009) can also impair cognition, although a single acute injection of the same steroid (4 mg) showed cognition facilitation (Vázquez-Pereyra et al., 1995). Therefore, as reflected by the diversity of these results, it seems that the lack of consistency between androgens effects on IAT might depend on different experimental paradigms that might include class of androgens, dose, exposure duration, as well as route of administration.

To date, available data regarding AAS effects on cognitive behaviors is limited to adult animals, whereas their effects around adolescence have not been described. Prepubertal sex differences in learning and memory in rats have received limited attention (Frankola et al., 2010; Grissom, et al., 2012), although it is known that males outperform females in specific cognitive tasks and vice versa in both humans and rodents (Andreano and Cahill, 2009).

Here we tested the effect of the AAS, 17 α -methyltestosterone (17 α -meT), on the IAT using a periadolescent animal model. The periadolescent period is the age around the time of sexual maturation when age-specific behavioral and physiological changes are evident (for review see Spear, 2000). In rats it is defined as approximately, 30-42 postnatal days (PN 30-42). 17 α -meT is a Class III AAS with an alkylation at C-17 and with restricted potential of metabolizing substrates for aromatization (Penatti et al., 2009). In fact, 17 α -meT cannot be aromatize to 17 β -estradiol, the most potent form of mammalian estrogenic steroids, and the main product of aromatase. Nevertheless, it can be converted to the 17 β -isomer of estradiol (Pawlowski et al, 2004), which binds weakly to estrogen receptors and exhibits weaker estrogenic activity (Shughrue et al., 1997). Thus, the use of this drug will diminish confounding effects that might result from potent estrogenic metabolites. We choose to administer an acute AAS injection by several reasons. The most important was to establish the minimum AAS exposure required to attain cognitive negative effects, given that such adverse effects are usually associated with chronic or continuous androgen exposure. To address this question is of great significance when studying subjects undergoing developmental changes, as this is the case in adolescence. Even though scarce studies have been done that test acute AAS exposure, it has been shown that a single androgen injection is sufficient to cause behavioral changes such as increased punished licks (Bing et al, 1998), and to modulate short and long-term memory (Vázquez-Pereyra et al., 1995). In addition, acute drug exposure is more compatible for the 3-day IAT, a short behavioral test that is very convenient to determine AAS-induced cognitive effects around adolescence without having effects in adulthood. Besides acquisition on inhibitory avoidance learning, AAS effects on generalized anxiety and risk assessment behaviors (RAB) were also evaluated.

We hypothesized that a disturbance in the normal hormonal milieu by a pharmacological dose of AAS will have sex-specific effects on cognition in periadolescent rats.

2. Materials and Methods

2.1. Animals

Gonadally-intact male and female Sprague Dawley rats were purchased from Charles Rivers Labs (Wilmington, MA). Animals were received at PN-25, maintained in the vivarium for acclimation for four days after arrival, and housed on a 12:12-hour reverse light/dark cycle (lights off at 8:30 A.M.). Food and water was available *ad libitum*. The IAT and EPM were performed during the dark phase of the cycle. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Puerto Rico, Medical Sciences Campus.

2.2. Vaginal lavage and smears

For female experiments, daily vaginal lavage, starting at PN-31 was performed at 1:00 P.M. for at least two estrous cycles. Vaginal lavage was performed with a small eyedropper containing 0.25 ml of 0.9% saline. The fluid obtained after the lavage was evenly distributed onto a microscope slide for further cytology analysis under low magnification. We monitored for a normal four-day cycle, consisting of vaginal smears evaluated for the presence of leukocytes, nucleated epithelial cells, or cornified epithelial cells (Cooper et al., 1993). The vaginal smears are classified as diestrus (predominance of leukocytes and a few scattered cornified epithelial cells), proestrus (round polynucleated epithelial cells), or estrus (cornified epithelial cells). Metestrus is considered a transition out of estrus, characterized by a large number of cornified cells and infiltration of leukocytes.

2.3. Drug

In the first experiment, animals were exposed to a single (acute) dose of 17 β -methyltestosterone (17 β -meT; 7.5 mg/kg; 0.2cc/kg, i.p.). Thereafter, to determine if the effect of 17 β -meT (7.5 mg/kg) on the IAT was dose-dependent, lower AAS doses (0.075 and 0.75 mg/kg) were tested. Also, we administered 17 β -meT (7.5 mg/kg) for two weeks to determine its effect during longer exposure. The high pharmacological dose (7.5 mg/kg) reflects a medium to high dose range of abuse in humans on a milligram per kilogram basis (for review see Clark and Henderson, 2003; Blasberg et al., 1997). 17 β -meT was dissolved in 0.9% saline containing 30% cyclodextrin and this vehicle was injected to control animals. Cyclodextrin enhances drug solubility due to the affinity between the drug and the interior of the cyclodextrin host molecules (Chaudhari et al., 2007). Their water soluble aggregates are able to solubilize lipophilic water-insoluble drugs through micelle-like structures (Loftsson et al., 2004). Moreover, no behavioral changes or alteration of gonadal weight have been reported when used as vehicle for 17 β -meT or testosterone (Alexander et al., 1994; Barreto-Estrada, 2004; Packard et al., 1997). Compounds were purchased from Sigma (St. Louis, MO).

2.4. Inhibitory Avoidance Task (IAT)

The step-through inhibitory avoidance apparatus (AccuScan Instruments; Columbus, OH) consists of a two-compartment acrylic box (45 $\times$ 22 $\times$ 33 cm) with an illuminated compartment connected to a darkened one, by a movable guillotine door and a stainless steel grid floor. During this test, the rat learns to avoid entering the dark compartment once it becomes associated with an aversive stimulus (Jarvik and Kopp, 1967).

Sixty (60) females (cohort I; n=40 and cohort II; n=20) and 19 males were tested on the IAT. Males were tested on the IAT on days PN-40 (habituation), PN-41 (acquisition) and

PN-42 (retention), whereas females in each experiment were divided in two cohorts according to estrus cyclicity. Females in cohort I were tested in diestrus during IAT acquisition (day 2), whereas animals in cohort II were tested while they were in proestrus. Specifically, females were tested on habituation day (cohort I: PN-40 or cohort II: PN-43), acquisition day (cohort I: PN-41 or cohort II: PN-44) and retention (cohort I: PN-42 or cohort II: PN-45). The difference in age between female rats in the two cohorts is due to the days needed for the females to reach the proestrus stage according to observations in vaginal lavages. Statistical analysis between females in PN-40 versus females in PN-43 did not find any significant difference, thus the two cohorts were analyzed together.

In general, rats were placed in the dark compartment of the chamber during two minutes, and then transferred to the home cage for one additional minute. Thereafter, rats were placed again in the illuminated side of the chamber and the latency to enter to the dark compartment was recorded. Animals were removed from the chamber after crossing to the dark compartment. During day 2 (acquisition phase), rats were injected with either 17 β -meT or vehicle, and immediately placed in the dark compartment with the door closed. A mild electric footshock (0.3 mA; 3 sec) was delivered through the grid floor. The retention test was carried out 24 hours later. Rats were placed in the illuminated compartment with the posterior body parts facing the guillotine door. Immediately the door was opened. Rats were allowed to enter the dark compartment and crossover latency was measured as an index of avoidance learning. The maximum entry latency allowed in the retention test was 300 seconds.

2.5. Hot Plate Test

Male rats were placed on a hot plate surface that was heated to 55°C immediately after injection of either 17 β -meT (7.5 mg/kg) or vehicle. A tall glass beaker was placed around the rat to prevent it from walking or jumping off of the surface of the apparatus. Latency to lick a paw, jump up, and/or vocalize was measured with a stopwatch and was registered as a sign of discomfort. The cut-off time of the test was 30 sec per rat in order to prevent injury to the paws.

2.6. Elevated Plus Maze (EPM)

The EPM (AccuScan Instruments; Columbus, OH) has been validated in rodents to measure generalized anxiety, and was performed as previously described (Pérez-Acevedo et al., 2006). An increase in time spent or number of entries to the open arms reflects anti-anxiety behavior. RAB such as stretch attended posture (SAP), flat back approach (FBA), and head dipping (HD), as well as grooming (non-emotional) and freezing (fear) were also monitored. This paradigm was used in animals that were not tested in the IAT to avoid confounding effects due to the footshock. Males (n=20) and diestrus females (n=20) were injected with 17 β -meT (7.5 mg/kg) or vehicle at PN-41, immediately prior to the EPM.

2.7. Statistical analysis

Data are presented as mean $\pm$ standard error of the mean (SEM). Two-way Repeated Measures Analysis of Variance followed by Student-Newman-Keuls post hoc analysis was employed for the IAT experiments, while Two-way Analysis of Variance was employed to analyze the EPM and RAB. Statistical significance was established at $p < 0.05$.

3. Results

3.1. Inhibitory Avoidance Task

In control animals, a two-way ANOVA repeated measures indicated both a significant effect of testing day (Day 1 vs. Day 3; $F(1, 77) = 25.14$, $p < 0.001$), as well as sex (males vs.

females; $F(1, 77) = 15.06, p < 0.001$). In addition, there was a significant testing day $\times$ sex interaction ($F(1, 77) = 14.80, p < 0.001$) in the control groups. On Day 1 (habituation), the latency to enter the dark compartment was similar for animals that were assigned to control and AAS groups in both males (Fig. 1A) and females (Fig. 1B). Post-hoc analysis revealed that on Day 3 (retention), male control rats significantly increased the crossover latency when compared to Day 1 (Fig. 1A; white bars; $F(1, 77) = 14.80, p = 0.001$). Specifically, crossover latencies were 6.37 ± 1.21 (Day 1) and 165.80 ± 47.98 (Day 3). However, control females showed a non-significant increase in the crossover latency between day 1 (7.35 ± 1.16) and day 3 (28.09 ± 12.27 ; Fig. 1B; white bars). Since no difference was observed between females in diestrus (cohort I) or proestrus (cohort II), the presented data represents both cohorts. Even though the crossover latency did not attain a significant increase in females between days 1 and 3, a post-hoc analysis revealed a significant difference between control males and females on the crossover latency on Day 3 ($F(1, 77) = 14.77, p < 0.001$; males: 165.80 ± 47.98 ; females: 28.09 ± 12.27).

At day 3, after animals were exposed to 17 β -meT, a two-way ANOVA showed a significant main effect of both sex ($F(1, 78) = 18.94, p < 0.001$) and treatment ($F(1, 78) = 15.88, p < 0.001$), with a statistically significant sex $\times$ treatment interaction ($F(1, 78) = 7.73, p = 0.007$). Post-hoc analysis revealed that males showed a main effect of treatment during day 3, in which 17 β -meT-treated males significantly decreased the crossover latency when compared with their control counterparts (Fig. 1A; $F(1, 78) = 5.34, p < 0.001$). Particularly, crossover latencies were 165.8 ± 47.98 (control) and 35.18 ± 29.45 (AAS). Results in males are suggested to be dose-dependent since on day 3, the dose of 0.075 mg/kg was not significantly altered in the IAT, while the intermediate dose of 0.75 mg/kg produced a tendency to enhance avoidance learning (Control: 6.700 ± 1.744 ; AAS-0.075 mg/kg: 3.983 ± 0.4593 ; AAS-0.75 mg/kg: 111.8 ± 59.74 ; $F(3,22) = 0.9982, p = 0.0589$). Furthermore, the AAS-induced impairment on the IAT after an acute injection was also observed after continuous exposure of the drug, since a two-week duration treatment with 17 β -meT (7.5 mg/kg) still reduced the crossover latency on day 3 (Control: 10.03 ± 1.596 ; AAS: $5.217 \pm 0.492, p = 0.0163$). Regarding nociception in males, 17 β -meT (7.5 mg/kg) did not represent a noxious stimulus as tested in the hot-plate test (Control: 27.600 ± 3.25 ; AAS: $31.900 \pm 4.029, P = 0.414$). In contrast to males, females showed no effect on the IAT after 17 β -meT; 7.5 mg/kg (Fig. 1B; Control 28.09 ± 12.27 ; AAS 4.82 ± 6.81).

3.2. Elevated Plus Maze

Periadolescent rats were tested in the EPM to determine if 17 β -meT (7.5 mg/kg) affects generalized anxiety. Acute injection of 17 β -meT did not cause any significant changes on the time spent in the open arms in either males (Fig. 2A; control 83.0 ± 19.45 ; AAS 129.7 ± 13.79) or females (Fig. 2B; control 107.6 ± 13.53 ; AAS 110.4 ± 10.84). Likewise, the number of entries to the open arms (Fig. 2C, Males: control 5.0 ± 0.85 , AAS 4.3 ± 0.45 ; Fig. 2D, Females: control 6.8 ± 0.53 , AAS 7.0 ± 0.74) or the locomotion, as measured by total number of arm entries (Fig. 2E, Males: control 10.4 ± 1.31 , AAS 8.6 ± 0.62 ; Fig. 2F, Females: control 13.1 ± 0.87 , AAS 12.6 ± 0.65), were not altered by AAS treatment. No effect was observed in the RAB in either males or females (Table 1) after acute exposure to 17 β -meT, but significant sex-differences were found in HD (males 8.8 ± 0.77 ; females 4.4 ± 0.24 ; $F(1,13) = 15.309, p = 0.002$), and SAP (males 1.8 ± 0.35 ; females 0.4 ± 0.24 ; $F(1,13) = 6.635, p = 0.023$). Non-emotional measures and fear response such as grooming and freezing, respectively, were not affected by treatment or sex (data not shown).

4. Discussion

The present study is the first one addressing negative cognitive effects after acute exposure of AAS in periadolescent rats. Our results showed that this single exposure to 17 β -meT (7.5

mg/kg), when tested on the IAT, produced cognitive impairment in males but not females. Results from our IAT studies demonstrated that all animals in the control group increased in the crossover latency 24 hours after the footshock, suggesting acquisition of a memory associating the dark side of the chamber with the footshock. Also, as validated in our results, sexual differences have been observed in passive avoidance conditioning (Heinsbroek et al., 1988), with male rats having longer crossover latencies than females and showing stronger acute stress outcome (Andreano and Cahill, 2009). However, in males, injection of 17 -meT interfered with this learning process, as evidenced by the significant reduction of the crossover latency on day 3. This result is suggested to be dose-dependent since 0.075 mg/kg had no effect, while 0.75 mg/kg produced a tendency to increase inhibitory avoidance learning on Day 3 as relative to vehicle-treated rats. Furthermore, the anabolic steroid effect on the IAT did not depend on continuous exposure of the drug since a two-week treatment with the higher dose (7.5 mg/kg) also affects avoidance learning.

It is noteworthy that in our protocol, 7.5 mg/kg 17 -meT did not elicit pain nociception as tested in the hot-plate test. Consistently with this result, recent studies have shown that subcutaneous injections of testosterone, DHT, or stanozolol (5mg/kg; acute or chronic) did not alter pain nociception or induce chronic inflammatory pain in adult intact male rats (Tsutsui et al., 2011). However, we cannot rule out the possibility that 17 -meT elicited some kind of impulsivity effect, given that testosterone (5 mg/kg) displayed increased punished responses in the Vogel Conflict Test (Bing et al., 1998), although this measure is more associated with anxiolytic processes (Millan and Brocco, 2003) than to impulsivity. Interestingly, we found that AAS impaired performance on the IAT without having effects on anxiety. This lack of effect was observed while females were in diestrus. In the future, it would be interesting to know if anabolic steroids affect anxiety levels in females according to estrus cyclicity, since Frye et al., 2000 has shown that females in proestrus spend more time in the open arms of the EPM. However, this result might be interpreted with caution, as long as higher levels of hormones might cause an increase in general activity as reflected by an increase in total arm entries observed in the same study. Nonetheless, our results are consistent with Oberlander and Henderson (2012), who demonstrated that a single acute injection of an AAS cocktail (7.5 mg/kg) did not affect anxiety in adolescent female mice while in diestrus. This result is therefore in agreement with our study in that no anxiety effect was observed in female rats in the diestrus stage.

In adult male rats, previous reports showed that intra-amygdalar and intra-hippocampal injections of testosterone produced impairment on spatial memory (Naghdi et al., 2001; 2003), with peak effects at 120 µg/0.5 µl and 80 µg/0.5 µl of, respectively. Additionally, intra-hippocampal injections of testosterone produced impairment on IAL acquisition (1 and 80 µg/0.5 µl/side), consolidation (20 µg/0.5 µl/side), and retrieval (20 and 40 µg/0.5 µl/side) (Harooni et al., 2008). Moreover, anabolic steroids compromise learning and memory. Particularly, daily subcutaneous injections of a high dose (15 mg/kg) of nandrolone decanoate, a Class II AAS and a 19-nortestosterone derivative, elicit impairment in spatial (Magnusson et al., 2009) and social memory (Kouvelas et al., 2008). Conversely, a single intramuscular injection of a lower dose of nandrolone (4 mg) seems to improve short and long-term memory (Vázquez-Pereyra et al., 1995), although difficulties in the interpretation of the results might obscure their conclusions. In this regard, from the four nandrolone doses tested (1, 2, 4 and 6 mg), only the 4 mg was effective, suggesting that the beneficial effects of steroids on learning and memory may require optimum level of androgens. From these studies in adults, it is suggested that the diverse spectrum of AAS effects on cognitive performance depends on an optimal level of testosterone (Muller et al., 2005), where low doses enhance, and high doses impair cognition. In this respect, endogenous androgen circulating levels have been shown to be important for the appropriate memory formation,

since castration during periadolescence showed reduced contextual fear memory (McDermott et al., 2012).

However, the fact that some studies showed opposite results about steroid-enhanced memory (Schneider-Rivas et al., 2007) and resistance to extinction (Rivas-Arancibia and Vázquez-Pereyra, 1994) might be due to other factors such as footshock intensity and the cognitive demands of each task. For example, we showed memory impairment using a 0.3 mA footshock in a 3-day duration IAT, while Schneider-Rivas and colleagues (2007) reported that testosterone enanthate (20 mg) improved long term memory and delay extinction of the conditioned response in a one trial passive avoidance task using a footshock intensity of 3 mA; an intensity ten times higher than the one used in our study. Nonetheless, in studies where a low intensity (0.25 mA) footshock was used, the enhanced avoidance learning might probably occur due to the lower androgen dose (testosterone, DHT or 3 α -diol: 1 mg/kg) rather than to the intensity of the footshock (Edinger et al., 2004). Similarly, studies in which androgens were microinfused directly to the brain followed by impaired cognition after a high intensity footshock (1 mA; Harooni et al., 2008), highlight the possible correlation between high androgen levels and disruption of cognitive processes.

Concerning females, previous studies reported that adult female rats injected with 500 μ g T propionate (class I AAS) or 500 μ g dihydrotestosterone for two consecutive days have no effect on memory in adult rats on the spatial Morris water maze task (Frick et al., 2004), while chronic exposure to an AAS cocktail with equal combination of three steroids (testosterone cypionate, nandrolone decanoate, methandrostenolone; 7.5 mg/kg) for 4 weeks in female adolescent mice did not affect learned fear (Costine et al., 2010). Correspondingly, our study showed no differences on the IAT in AAS-treated periadolescent females. Since the exact timing of adolescence is a matter of dispute in laboratory rats, and given the consensus of females reaching puberty before males (for review see Spear, 2000), it is possible that sex-specific effects on IAT might be due to differences in the surge of gonadal hormones during development. Although we do not have specific measures of puberty in our rats, puberty is defined as the attainment of sexual maturation and the time of gonadarche (Graber, 1998). In male rats, the onset of puberty has been established at approximately PN-40, the time of preputial separation (Feinberg et al., 1997; Korenbrot et al., 1977; Shin et al., 2009). In female rats, puberty is marked by the vaginal opening, which occurs around PN-33-36 (Engelbregt et al., 2000; Gabriel et al., 1992; Ojeda et al., 1976). This raises the possibility that the majority of the females in our study may have been in a post-pubertal stage, while males might be pre-pubertal. Nevertheless, other studies have demonstrated that androgen-treated females enhance their cognitive performance on the IAT and Y maze (Frye and Lacey, 2001). Differences in type of androgens (natural versus synthetic), their metabolites, developmental stages (adults versus periadolescents) and/or the hormonal manipulation (ovariectomized or intact) might account for differences in Frye's studies and ours, respectively. Even though we used intact females, no differences were detected between females in diestrus or proestrus, ruling out the possibility of endogenous hormonal status having a role on avoidance learning. This result contrasts from previous findings indicating that learning in adult female rodents differ through the estrus cycle (Gupta et al., 2001; Milad et al., 2009). In fact, differences in learning and greater variability among female rats, as opposed to male rats, are usually associated with changes in hormonal status across the cycle. For instance, contextual fear conditioning in female rats fluctuates across the estrous cycle, with less freezing during proestrus compared to estrus (Markus and Zecevic, 1997; Milad et al., 2009). In a study to test if ovarian steroids play a role in fear conditioning, sham-operated (intact) female rats extinguished fear faster than males and also than ovariectomized (OVX) females (Gupta et al., 2001).

As 17 β -meT holds the potential to modulate memory circuitries for emotional memory formation, many studies have focused on the neural mechanisms that mediate learning and memory, in which the amygdala and hippocampus have been identified as key structures for these processes (for review see Phelps, 2004). The amygdala and hippocampus are parts of the limbic system, which subserve tasks related to memory and emotional regulation (Okada et al., 2011; Salado-Castillo et al., 2011; Sindreu and Storm, 2011). These regions are abundant in androgen sensitive substrates, making them responsive and regulated by steroid hormones (Kerr et al., 1995; Roselli et al., 1989; Wood and Newman, 1999). The increased androgen response in these brain regions during puberty (Ahmed et al., 2008) could explain why an acute exposure to a single steroid is enough to induce cognitive impairments.

The amygdala is involved in mediating influences of emotional arousal and stress on learning and memory. Specifically, the basolateral nucleus of the amygdala (BLA) is greatly implicated in the modulation of memories of meaningful events (for review see LeDoux, 2000; McGaugh et al., 2002; Rodrigues et al., 2004). Evidence indicates that memory formation on the IAT may be mediated by the BLA (Liang, 1999; Tomaz et al., 1992). In fact, intra-BLA injections of different drugs and neurotransmitter agents modulate memory in this task (McGaugh et al., 1996; 2002). In this line of evidence, and as discussed before, intra-amygdalar and intra-hippocampal testosterone injections impaired spatial memory and interfered with avoidance learning (Naghdi et al., 2001, 2003; Harooni et al., 2008).

However, it is established that the BLA does not work alone in the modulation of memory, and performs its role via potent interactions with other cortical and limbic structures such as the hippocampus (McIntyre et al., 2003). A previous study showed a cooperative interaction between the BLA and the ventral tegmental area (VTA) on IAL (Nazari-Serenjeh and Rezayof, 2012). It has also been found that outburst projections from the BLA lead to IAL neuroplastic responses in hippocampal pyramidal neurons (Farmer and Thompson, 2012). Thus, the reciprocal relationship between the BLA and the VTA and hippocampus in memory consolidation of inhibitory avoidance learning may be dependent on cooperative interactions between the glutamatergic and GABAergic systems via NMDA and GABA-A receptors.

Although androgens are known to act primarily through the AR, non-genomic pathways are rapid and transient alternative responses in acute exposure. Non-genomic steroid activity typically involves the rapid induction of conventional second messenger signal transduction cascades. This might include increases in free intracellular calcium, and activation of protein kinase A (PKA), protein kinase C (PKC), and mitogen-activated (MAP) kinase. The existence of a novel membrane-bound AR has been postulated by a number of authors (Konoplya and Popoff, 1992; Leung et al., 2001; Sato et al., 2010).

For instance, membrane ARs may serve as non-genomic transducers of androgen action in the hippocampal formation (Tabori et al., 2005), affecting dendritic spine synapse density in the CA1 hippocampal region of the male rat (Kovacs et al., 2003; Leranthe et al., 2003). In fact, second messenger activation by androgens has been documented to be mediated through G-protein coupled receptors (Leung et al., 2001; Lieberherr and Grosse, 1994). These effects occur within seconds to minutes, being too fast to involve changes in transcription and protein synthesis (for review see Heinlein and Chang, 2002; Michels and Hoppe, 2008). The inhibitory GABAergic system, which plays an important role in learning and memory (Erhlich et al., 2009) has shown to be modulated by 17 β -meT (Jones et al., 2006; Penatti et al., 2011; Yang et al., 2002, 2005). In this regard, it has been found that extinction memory (for review see Barad et al., 2006) and inhibitory avoidance learning (Khajepour et al., 2011) are modulated through GABA-A receptors in the BLA. Particularly, intra-BLA microinjection of muscimol, a GABA-A receptor agonist, impaired

dexamethasone-induced memory; while bicuculline, a competitive GABA-A receptor antagonist, increased memory retrieval using the IAT (Khajehpour et al., 2011).

Since we suggest a sex-specific effect of the hormonal milieu on avoidance learning in periadolescent rats, it is plausible to suggest a sex-specific GABAergic transmission in which males have higher GABA-A receptor Bmax (maximal number of binding sites) than females (Juptner and Hiemke, 1990). However, it is important to note that this study reported that not all brain regions portray the Bmax sex difference. In addition, other studies have shown opposite results, with Stefanova (1998) concluding that females have more GABA-expressing neurons in the BLA.

Other point of regulation to attain sex-specific effects on behavior is at the GABA receptor subunit level. This was evidenced by the increase in GABA-A receptor $\alpha 2$ subunit mRNA after 17 β -meT (7.5 mg/kg) treatment in the hypothalamus of adolescent females, but not male mice. This differential expression of the receptor subunit was accompanied by a decrease in spontaneous inhibitory synaptic current frequency in this brain region (Penatti et al., 2005). Still, limbic areas such as the amygdala and hippocampus have greater GABAergic response to neurosteroids than the hypothalamus (Wilson and Biscardi, 1997). As a matter of fact, in males, neuroactive steroids produced greater GABA-activated chloride influx in the amygdala as compared to the hypothalamus; a trend not seen in females (Wilson and Biscardi, 1997). We suggest this region-specific modulation of GABAergic currents can also be attributed to AAS, but this must be taken cautiously, as Yang (2002, 2005) has shown that anabolic steroids have actions on the GABA-A receptor that are distinct from those associated with neurosteroid binding. These differences were established only in the medial preoptic area and hypothalamus, therefore actions on memory-related regions such as the amygdala and hippocampus remain to be determined.

Taken together, it is suggested that pharmacological doses of steroids has the potential to allosterically modulate GABA-A receptors that might result in alteration of GABA-induced currents and the subsequent modulation of learning and memory through androgen-sensitive brain regions. Future electrophysiological and molecular studies will warrant the understanding of the mechanism of action of 17 β -meT through the GABAergic system in avoidance learning.

5. Conclusion

Our data suggests that cognitive impairment in male animal models is not restricted to adults, but also that at earlier developmental stages, subjects are vulnerable to be affected by hormonal disturbances. This study highlights the sex-specific response to a single AAS dose in periadolescent rats when tested in paradigms that measure cognitive processes and memory storage. Further studies will be needed to determine if this effect could also be observed in humans. The data presented in this study should be taken into account by parents, educators and the sports community.

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References

- Ahmed EI, Zehr JL, Schulz KM, Lorenz BH, DonCarlos LL, Sisk CL. Pubertal hormones modulate the addition of new cells to sexually dimorphic brain regions. *Nat Neurosci*. 2008; 11(9):995–997. [PubMed: 19160494]
- Alexander GM, Packard MG, Hines M. Testosterone has rewarding affective properties in male rats: implications for the biological basis of sexual motivation. *Behav Neurosci*. 1994; 108(2):424–428. [PubMed: 8037886]
- Andreano JM, Cahill L. Sex influences on the neurobiology of learning and memory. *Learn Mem*. 2009; 16(4):248–266. [PubMed: 19318467]
- Barad M, Gean PW, Lutz B. The role of the amygdala in the extinction of conditioned fear. *Biol Psychiatry*. 2006; 60(4):322–328. [PubMed: 16919522]
- Barreto-Estrada JL, Barreto J, Fortis-Santiago Y, Rivera-Ramos I, Fortis-Santiago A, Jorge JC. Modulation of affect after chronic exposure to the anabolic steroid 17alpha-methyltestosterone in adult mice. *Behav Neurosci*. 2004; 118(5):1071–1079. [PubMed: 15506889]
- Bing O, Heilig M, Kakoulidis P, Sundblad C, Wiklund L, Eriksson E. High doses of testosterone increase anticonflict behaviour in rat. *Eur Neuropsychopharmacol*. 1998; 8(4):321–323. [PubMed: 9928924]
- Blakemore SJ, Burnett S, Dahl RE. The role of puberty in the developing adolescent brain. *Hum Brain Mapp*. 2010; 31(6):926–933. [PubMed: 20496383]
- Blasberg MR, Langan CJ, Clark AS. The effects of 17 -Methyltestosterone, Methandrostenolone, and Nandrolone Decanoate on the Rat Estrous Cycle. *Physiol & Behavior*. 1997; 61:265–272.
- Castillo EM, Comstock RD. Prevalence of use of performance-enhancing substances among United States adolescents. *Pediatr Clin No Amer*. 2007; 54(4):663–675.
- Chaudhari P, Sharma P, Barhate N, Kulkarni P, Mistry C. Solubility enhancement of hydrophobic drugs using synergistically interacting cyclodextrins and cosolvent. *Current Science*. 2007; 92(11):1586–1591.
- Clark AS, Henderson LP. Behavioral and Physiological Responses to Steroids. *Neurosci Biobehav*. 2003; 27(5):413–436.
- Cooper, RL.; Goldman, JM.; Vandenbergh, JG. Monitoring the estrous cycle in the laboratory rodent by vaginal lavage. In: Tyson, CA.; Witschi, H., editors. *Methods in Toxicology Chapter 4 Vol 18*. Vol. 3B. Academic Press Incorporated; Orlando, FL: 1993. p. 45-54.
- Costine BA, Oberlander JG, Davis MC, Penatti CA, Porter DM, Leaton RN, Henderson LP. Chronic anabolic androgenic steroid exposure alters corticotropin releasing factor expression and anxiety-like behaviors in the female mouse. *Psychoneuroendocrinol*. 2010; 35(10):1473–1485.
- Edinger KL, Lee B, Frye CA. Mnemonic effects of testosterone and its 5alpha-reduced metabolites in the conditioned fear and inhibitory avoidance tasks. *Pharmacol Biochem Behav*. 2004; 78(3):559–568. [PubMed: 15251265]
- Ehrlich I, Humeau Y, Grenier F, Ciochi S, Herry C, Luthi A. Amygdala inhibitory circuits and the control of fear memory. *Neuron*. 2009; 62(6):757–771. [PubMed: 19555645]
- Engelbregt MJ, Houdijk ME, Popp-Snijders C, Delemarre-van de Waal HA. The effects of intra-uterine growth retardation and postnatal undernutrition on onset of puberty in male and female rats. *Pediatr Res*. 2000; 48(6):803–807. [PubMed: 11102550]
- Farmer GE, Thompson L. Learning-dependent plasticity of hippocampal CA1 pyramidal neuron postburst after hyperpolarizations and increased excitability after inhibitory avoidance learning depend upon basolateral amygdala inputs. *Hippocampus*. 2012; 22(8):1703–1719. [PubMed: 22367983]
- Feinberg MJ, Lumia AR, McGinnis MY. The effect of anabolic-androgenic steroids on sexual behavior and reproductive tissues in male rats. *Physiology and Behavior*. 1997; 62:23–30. [PubMed: 9226338]
- Frankola KA, Flora AL, Torres AK, Grissom EM, Overstreet S, Dohanich GP. Effects of early rearing conditions on cognitive performance in prepubescent male and female rats. *Neurobiol Learn Mem*. 2010; 94(1):91–99. [PubMed: 20403447]

- Frick KM, Fernandez SM, Bennett JC, Prange-Kiel J, MacLusky NJ, Leranath C. Behavioral training interferes with the ability of gonadal hormones to increase CA1 spine synapse density in ovariectomized female rats. *Eur J Neurosci.* 2004; 19(11):3026–3032. [PubMed: 15182310]
- Frye CA, Edinger KL, Lephart ED, Walf AA. 3alpha-androstenediol, but not testosterone, attenuates age-related decrements in cognitive, anxiety, and depressive behavior of male rats. *Front Aging Neurosci.* 2010; 2(15):1–21. [PubMed: 20552041]
- Frye CA, Lacey EH. Posttraining androgens' enhancement of cognitive performance is temporally distinct from androgens' increases in affective behavior. *Cogn Affect Behav Neurosci.* 2001; 1(2): 172–182. [PubMed: 12467112]
- Frye CA, Petralia SM, Rhodes ME. Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3alpha,5alpha-THP. *Pharmacol Biochem Behav.* 2000; 67(3):587–596. [PubMed: 11164090]
- Frye CA, Seliga AM. Testosterone increases analgesia, anxiolysis, and cognitive performance of male rats. *Cogn Affect Behav Neurosci.* 2001; 1(4):371–381. [PubMed: 12467088]
- Gabriel SM, Roncancio JR, Ruiz NS. Growth hormone pulsatility and the endocrine milieu during sexual maturation in male and female rats. *Neuroendocrinology.* 1992; 56:619–628. [PubMed: 1488093]
- Graber, JA. Brooks-Gunn J. Puberty. In: Blechman, EA.; Brownell, KD., editors. *Behavioral medicine and women: a comprehensive handbook.* Guilford Press; New York, NY: 1998. p. 51-58.
- Graham MR, Evans P, Davies B, Baker JS. AAS, growth hormone, and insulin abuse: psychological and neuroendocrine effects. *Ther Clin Risk Manag.* 2008; 4(3):587–597. [PubMed: 18827854]
- Grissom EM, Hawley WR, Bromley-Dulfano SS, Marino SE, Stathopoulos NG, Dohanich GP. Learning strategy is influenced by trait anxiety and early rearing conditions in prepubertal male, but not prepubertal female rats. *Neurobiol Learn Mem.* 2012; 98(2):174–181. [PubMed: 22705447]
- Gupta RR, Sen S, Diepenhorst LL, Rudick CN, Maren S. Estrogen modulates sexually dimorphic contextual fear conditioning and hippocampal long-term potentiation (LTP) in rats. *Brain Res.* 2001; 888(2):356–365. [PubMed: 11150498]
- Harooni HE, Naghdi N, Sepehri H, Rohani AH. Intra hippocampal injection of testosterone impaired acquisition, consolidation and retrieval of inhibitory avoidance learning and memory in adult male rats. *Behav Brain Res.* 2008; 188:71–77. [PubMed: 18054400]
- Hartgens F, Kuipers H. Effects of androgenic anabolic steroids in athletes. *Sports Med.* 2004; 34:513–554. [PubMed: 15248788]
- Heinlein CA, Chang C. The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions. *Mol Endocrinol.* 2002; 16(10):2181–2187. [PubMed: 12351684]
- Heinsbroek RP, van Haaren F, van de Poll NE. Sex differences in passive avoidance behavior of rats: sex-dependent susceptibility to shock-induced behavioral depression. *Physiol Behav.* 1988; 43(2): 201–206. [PubMed: 3212056]
- Jarvik ME, Kopp R. An improved one trial passive avoidance learning situation. *Psychol Rep.* 1967; 1:221–224. [PubMed: 6078370]
- Jones BL, Whiting PJ, Henderson LP. Mechanisms of anabolic androgenic steroid inhibition of mammalian α -subunit-containing GABAA receptors. *J Physiol.* 2006; 573:571–593. [PubMed: 16543268]
- Jüptner M, Hiemke C. Sex differences in GABAA receptor binding in rat brain measured by an improved in vitro binding assay. *Exp Brain Res.* 1990; 81(2):297–302. [PubMed: 2168842]
- Kerr JE, Allore RJ, Beck SG, Handa RJ. Distribution and hormonal regulation of androgen receptor (AR) and AR messenger ribonucleic acid in the rat hippocampus. *Endocrinol.* 1995; 136:3213–3221.
- Khajehpour L, Alizadeh-Makvandi A, Kesmati M, Eshagh-Harooni H. Involvement of basolateral amygdala GABAA receptors in the effect of dexamethasone on memory in rats. *J Zhejiang Univ Sci B.* 2011; 12(11):900–908. [PubMed: 22042654]
- Konoplya EF, Popoff EH. Identification of the classical androgen receptor in male rat liver and prostate cell plasma membranes. *Int. J. Biochem.* 1992; 24:1979–1983. [PubMed: 1473610]

- Korenbrod CC, Huhtaniemi IT, Weiner RI. Preputial separation as an external sign of pubertal development in the male rat. *Biology of Reproduction*. 1977; 17:298–303. [PubMed: 889997]
- Kouvelas D, Pourzitaki C, Papazisis G, Dagklis T, Dimou K, Kraus MM. Nandrolone abuse decreases anxiety and impairs memory in rats via central androgenic receptors. *Int J Neuropsychopharmacol*. 2008; 11(7):925–934. [PubMed: 18405416]
- Kovacs EG, MacLusky NJ, Leranath C. Effects of testosterone on hippocampal CA1 spine synaptic density in the male rat are inhibited by fimbria/fornix transection. *Neuroscience*. 2003; 122:807–810. [PubMed: 14622923]
- LeDoux JE. Emotion circuits in the brain. *Annu Rev Neurosci*. 2000; 23:155–184. [PubMed: 10845062]
- Leranath C, Petnehazy O, MacLusky NJ. Gonadal hormones affect spine synaptic density in the CA1 hippocampal subfield of male rats. *J Neurosci*. 2003; 23:1588–1592. [PubMed: 12629162]
- Leung GPH, Cheng-Chew SB, Wong PYD. Nongenomic effect of testosterone on chloride secretion in cultured rat efferent duct epithelia. *Am. J. Physiol. Cell Physiol*. 2001; 280:C1160–C1167. [PubMed: 11287329]
- Liang KC. Pre- or post-training injection of buspirone impaired retention in the inhibitory avoidance task: involvement of amygdala 5-HT_{1A} receptors. *Eur J Neurosci*. 1999; 11(5):1491–5000. [PubMed: 10215901]
- Lieberherr M, Grosse B. Androgens increase intracellular calcium concentration and inositol 1,4,5-triphosphate and diacylglycerol formation via a pertussis toxin-sensitive G protein. *J. Biol. Chem*. 1994; 269:7217–7223. [PubMed: 8125934]
- Lofstsson T, Masson M, Brewster ME. Self-association of cyclodextrins and cyclodextrin complexes. *J. Pharm. Sci*. 2004; 93:1091–1099. [PubMed: 15067686]
- Magnusson K, Hånell A, Bazov I, Clausen F, Zhou Q, Nyberg F. Nandrolone decanoate administration elevates hippocampal prodynorphin mRNA expression and impairs Morris water maze performance in male rats. *Neurosci Lett*. 2009; 467(3):189–193. [PubMed: 19782718]
- Maravelias C, Dona A, Stefanidou M, Spiliopoulou C. Adverse effects of anabolic steroids in athletes. A constant threat. *Toxicol Lett*. 2005; 158(3):167–175. [PubMed: 16005168]
- Markus EJ, Zecevic M. Sex differences and estrous cycle changes in hippocampus-dependent fear conditioning. *Psychobiol*. 1997; 25(3):246–252.
- McDermott CM, Liu D, Schrader LA. Role of gonadal hormones in anxiety and fear memory formation and inhibition in male mice. *Physiol Behav*. 2012; 105(5):1168–1174. [PubMed: 22226989]
- McGaugh JL, Cahill L, Roozendaal B. Involvement of the amygdala in memory storage: interaction with other brain systems. *Proc Natl Acad Sci USA*. 1996; 93(24):13508–13514. [PubMed: 8942964]
- McGaugh JL, McIntyre CK, Power AE. Amygdala modulation of memory consolidation: interaction with other brain systems. *Neurobiol Learn Mem*. 2002; 78(3):539–552. [PubMed: 12559833]
- McIntyre CK, Marriott LK, Gold PE. Cooperation between memory systems: acetylcholine release in the amygdala correlates positively with performance on a hippocampus-dependent task. *Behav Neurosci*. 2003; 117(2):320–326. [PubMed: 12708528]
- Michels G, Hoppe UC. Rapid actions of androgens. *Front Neuroendocrinol*. 2008; 29(2):182–198. [PubMed: 17983646]
- Milad MR, Igwe SA, Lebron-Milad K, Novales JE. Estrous cycle phase and gonadal hormones influence conditioned fear extinction. *Neuroscience*. 2009; 164(3):887–895. [PubMed: 19761818]
- Millan MJ, Brocco M. The Vogel conflict test: procedural aspects, gamma-aminobutyric acid, glutamate and monoamines. *Eur J Pharmacol*. 2003; 463(1-3):67–96. [PubMed: 12600703]
- Mulcahey MK, Schiller JR, Hulstyn MJ. Anabolic steroid use in adolescents: identification of those at risk and strategies for prevention. *Phys Sportsmed*. 2010; 38(3):105–113. [PubMed: 20959703]
- Muller M, Aleman A, Grobbee DE, de Haan EH, van der Schouw YT. Endogenous sex hormone levels and cognitive function in aging men: is there an optimal level? *Neurol*. 2005; 5:866–871.
- Naghdi N, Nafisy N, Majlessi N. The effects of intrahippocampal testosterone and flutamide on spatial localization in the Morris water maze. *Brain Res*. 2001; 897(1-2):44–51. [PubMed: 11282357]

- Naghdi N, Oryan S, Etemadi R. The study of spatial memory in adult male rats with injection of testosterone enanthate and flutamide into the basolateral nucleus of the amygdala in Morris water maze. *Brain Res.* 2003; 972:1–8. [PubMed: 12711072]
- Nazari-Serenjeh F, Rezayof A. Cooperative interaction between the basolateral amygdala and ventral tegmental area modulates the consolidation of inhibitory avoidance memory. *Prog Neuropsychopharmacol Biol Psychiatr.* 2012; 40:54–61.
- Oberlander JG, Henderson LP. Corticotropin-releasing factor modulation of forebrain GABAergic transmission has a pivotal role in the expression of anabolic steroid-induced anxiety in the female mouse. *Neuropsychopharmacol.* 2012; 37(6):1483–1499.
- Ojeda SR, Wheaton JE, Jameson HE, McCann SM. The onset of puberty in the female rat: changes in plasma prolactin, gonadotropins, luteinizing hormone-releasing hormone (LHRH), and hypothalamic LHRH content. *Endocrinology.* 1976; 98(3):630–638. [PubMed: 770154]
- Okada G, Okamoto Y, Kunisato Y, Aoyama S, Nishiyama Y, Yoshimura S, Onoda K, Toki S, Yamashita H, Yamawaki S. The effect of negative and positive emotionality on associative memory: an fMRI study. *PLoS One.* 2011; 6(9):e24862. [PubMed: 21935483]
- Packard MG, Cornell AH, Alexander GM. Rewarding affective properties of intra-nucleus accumbens injections of testosterone. *Behav Neurosci.* 1997; 111(1):219–224. [PubMed: 9109641]
- Pawlowski S, Sauer A, Shears JA, Tyler CR, Braunbeck T. Androgenic and estrogenic effects of the synthetic androgen 17alpha-methyltestosterone on sexual development and reproductive performance in the fathead minnow (*Pimephales promelas*) determined using the gonadal recrudescence assay. *Aquat Toxicol.* 2004; 68(3):277–291. [PubMed: 15159053]
- Penatti CA, Oberlander JG, Davis MC, Porter DM, Henderson LP. Chronic exposure to anabolic androgenic steroids alters activity and synaptic function in neuroendocrine control regions of the female mouse. *Neuropharmacol.* 2011; 61(4):653–664.
- Penatti CA, Porter DM, Henderson LP. Chronic exposure to anabolic androgenic steroids alters neuronal function in the mammalian forebrain via androgen receptor- and estrogen receptor-mediated mechanisms. *J. Neurosci.* 2009; 29:12484–12496. [PubMed: 19812324]
- Penatti CA, Porter DM, Jones BL, Henderson LP. Sex-specific effects of chronic anabolic androgenic steroid treatment on GABA(A) receptor expression and function in adolescent mice. *Neurosci.* 2005; 135(2):533–543.
- Pérez-Acevedo NL, Lathroum L, Jorge J. The neurosteroid 3-alpha Diol modulates place preference when infused in the basolateral amigdala according to sex. *Behav Neurosci.* 2006; 120:632–640. [PubMed: 16768615]
- Phelps EA. Human emotion and memory: interactions of the amygdala and hippocampal complex. *Curr Opin Neurobiol.* 2004; 14(2):198–202. [PubMed: 15082325]
- Rivas-Arancibia S, Vázquez-Pereyra F. Hormonal modulation of extinction responses induced by sexual steroid hormones in rats. *Life Sci.* 1994; 54:363–367.
- Rodrigues SM, Schafe GE, LeDoux JE. Molecular mechanisms underlying emotional learning and memory in the lateral amygdala. *Neuron.* 2004; 44(1):75–91. [PubMed: 15450161]
- Roselli CE, Handa RJ, Resko JA. Quantitative distribution of nuclear androgen receptors in microdissected areas of the rat brain. *Neuroendocrinol.* 1989; 49(5):449–453.
- Salado-Castillo R, Sánchez-Alavéz M, Quirarte GL, Martínez García MI, Prado-Alcalá RA. Enhanced training protects memory against amnesia produced by concurrent inactivation of amygdala and striatum, amygdala and substantia nigra, or striatum and substantia nigra. *Front Behav Neurosci.* 2011; 5(83):1–7. [PubMed: 21267359]
- Sato SM, Johansen JA, Jordan CL, Wood RI. Membrane androgen receptors may mediate androgen reinforcement. *Psychoneuroendocrinology.* 2010; 35(7):1063–1073. [PubMed: 20137860]
- Schneider-Rivas S, Paredes-Carbajal C, Mascher D, Angoa-Pérez M, Jaramillo-González E, Borgonio-Pérez G, Rivas-Arancibia S. Effects of testosterone and growth hormone on long-term retention and extinction of a passive avoidance response in young and aged rats. *Int J Neurosci.* 2007; 117(10):1443–1456. [PubMed: 17729155]
- Shin JH, Kim TS, Kang IH, Kang TS, Moon HJ, Han SY. Effects of postnatal administration of diethylstilbestrol on puberty and thyroid function in male rats. *J Reprod Dev.* 2009; 55(5):461–466. [PubMed: 19444002]

- Shughrue PJ, Lane MV, Merchenthaler I. Regulation of progesterone receptor messenger ribonucleic acid in the rat medial preoptic nucleus by estrogenic and antiestrogenic compounds: an in situ hybridization study. *Endocrinology*. 1997; 138(12):5476–5484. [PubMed: 9389534]
- Sindreu C, Storm DR. Modulation of neuronal signal transduction and memory formation by synaptic zinc. *Front Behav Neurosci*. 2011; 5(68):1–12. [PubMed: 21267359]
- Sisk CL, Foster DL. The neural basis of puberty and adolescence. *Nat Neurosci*. 2004; 10:1040–1047. [PubMed: 15452575]
- Spear L. Modeling adolescent development and alcohol use in animals. *Alcohol Res Health*. 2000; 24(2):115–123. [PubMed: 11199278]
- Stefanova N. Gamma-aminobutyric acid-immunoreactive neurons in the amygdala of the rat - sex differences and effect of early postnatal castration. *Neurosci Lett*. 1998; 255(3):175–177. [PubMed: 9832201]
- Tabori NE, Stewart LS, Znamensky V, Romeo RD, Alves SE, McEwen BS, Milner TA. Ultrastructural evidence that androgen receptors are located at extranuclear sites in the rat hippocampal formation. *Neuroscience*. 2005; 30(1):151–163. [PubMed: 15561432]
- Tomaz C, Dickinson-Anson H, McGaugh JL. Basolateral amygdala lesions block diazepam-induced anterograde amnesia in an inhibitory avoidance task. *Proc Natl Acad Sci USA*. 1992; 89(8):3615–3619. [PubMed: 1565657]
- Tsutsui KT, Wood RI, Craft RM. Anabolic-androgenic steroid effects on nociception and morphine antinociception in male rats. *Pharmacol Biochem Behav*. 2011; 99(3):500–508. [PubMed: 21586302]
- Vázquez-Pereyra F, Rivas-Arancibia S, Loaeza-Del Castillo A, Schneider-Rivas S. Modulation of short term and long term memory by steroid sexual hormones. *Life Sci*. 1995; 56:255–260.
- Wilson MA, Biscardi R. Influence of gender and brain region on neurosteroid modulation of GABA responses in rats. *Life Sci*. 1997; 60(19):1679–1691. [PubMed: 9129123]
- Wood RI, Newman SW. Androgen receptor immunoreactivity in the male and female Syrian hamster brain. *J Neurobiol*. 1999; 39(3):359–370. [PubMed: 10363909]
- Yang P, Jones BL, Henderson LP. Mechanisms of anabolic androgenic steroid modulation of alpha(1)beta(3)gamma(2L) GABA(A) receptors. *Neuropharmacol*. 2002; 43(4):619–633.
- Yang P, Jones BL, Henderson LP. Role of the alpha subunit in the modulation of GABAA receptors by anabolic androgenic steroids. *Neuropharmacol*. 2005; 49(3):300–316.

Highlights

- During periadolescence, a single acute dose of 17 β -methyltestosterone impairs inhibitory avoidance learning.
- The effect of a single acute dose of 17 β -methyltestosterone, on inhibitory avoidance learning, is sex-specific.
- A single acute dose of 17 β -methyltestosterone did not affect anxiety-related behaviors.
- Cognitive impairment after a single AAS exposure highlights the need of education for the prevention of steroid abuse.

Figure 1. Effect of AAS on the IAT in adolescent rats

(A) Control male rats showed a significant increase in the crossover latency (sec) when compared Day 1 to Day 3. In contrast, during retention (Day 3) AAS-treated male rats showed a significant decreased in this latency. (B) Although a non-significant increase was observed in the crossover latency (sec) in control female rats when compared Day 1 to Day 3, they were not affected by AAS during retention (Day 3). **Two-Way ANOVA RM; $p < 0.005$. Males: Control $n=9$, AAS $n=10$; Females: Control $n=30$, AAS $n=30$. Error bars represent SEM.

Fig. 2. Effect of AAS on the EPM in adolescent rats

Exposure to AAS did not affect the time spent in the open arms (A, B), the open arms entries (C, D), or the total number of entries (E, F) on the EPM in either male (A, C, E) or female (B, D, F) rats. Control n=10, AAS n=10 for each male or female. Error bars represent SEM.

Table 1

Effect of AAS on risk assessment behaviors (RAB) on the EPM in adolescent rats

RAB	Males		Females	
	Control	AAS	Control	AAS
HD	8.8 ± 0.77	10.1 ± 0.50	4.4 ± 0.24 ^{**}	6.6 ± 1.25
FBA	0.8 ± 0.29	1.0 ± 0.33	1.0 ± 0.45	0.6 ± 0.40
SAP	1.8 ± 0.35	2.3 ± 0.30	0.4 ± 0.24 [*]	0.4 ± 0.24

Values are presented as mean ± SEM. Flat back approach (FBA), stretched attend posture (SAP), head dipping (HD). Control n=10, AAS n=10. Two-way ANOVA,

^{*}
p<0.05,

^{**}
p<0.005; control females different from control males.