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Testosterone attenuates and the selective estrogen receptor modulator, raloxifene, potentiates amphetamine-induced locomotion in male rats

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ABSTRACT

Although sex steroids are known to modulate brain dopamine, it is still unclear how testosterone modifies locomotor behaviour controlled, at least in part, by striatal dopamine in adolescent males. Our previous work suggests that increasing testosterone during adolescence may bias midbrain neurons to synthesise more dopamine. We hypothesised that baseline and amphetamine-induced locomotion would differ in adult males depending on testosterone exposure during adolescence. We hypothesised that concomitant stimulation of estrogen receptor signaling, through a selective estrogen receptor modulator (SERM), raloxifene, can counter testosterone effects on locomotion.

Male Sprague-Dawley rats at postnatal day 45 were gonadectomised (G) or sham-operated (S) prior to the typical adolescent testosterone increase. Gonadectomised rats were either given testosterone replacement (T) or blank implants (B) for six weeks and sham-operated (i.e. intact or endogenous testosterone group) were given blank implants. Subgroups of sham-operated, gonadectomised and gonadectomised/testosterone-replaced rats were treated with raloxifene (R, 5 mg/kg) or vehicle (V), daily for the final four weeks. There were six groups (SBV, GBV, GTV, SBR, GBR, GTR). Saline and amphetamine-induced (1.25 mg/kg) locomotion in the open field was measured at PND85.

Gonadectomy increased amphetamine-induced locomotion compared to rats with endogenous or with exogenous testosterone. Raloxifene increased amphetamine-induced locomotion in rats with either endogenous or exogenous testosterone. Amphetamine-induced locomotion was negatively correlated with testosterone and this relationship was abolished by raloxifene.

Lack of testosterone during adolescence potentiates and testosterone exposure during adolescence attenuates amphetamine-induced locomotion. Treatment with raloxifene appears to potentiate amphetamine-induced locomotion and to have an opposite effect to that of testosterone in male rats.

Introduction

Circulating androgens and estrogens, produced mainly by the gonads, cross the blood brain barrier and directly modulate brain function in regions with cells expressing androgen (AR) and estrogen (ER) receptors. While many neuronal types express sex steroid receptors, we are particularly interested in sex-steroid responsiveness of midbrain dopamine neurons, which are known to express AR and ERs (Creutz and Kritzer, 2004; Kritzer and Creutz, 2008; Perez et al., 2003; Purves-Tyson et al., 2012; Ravizza et al., 2002; Shughrue et al., 1997). Dopamine-related brain functions are varied and include modalities such as movement, reward, pleasure, motivation, compulsion and perseveration, attention and working memory and influence decision making, addiction and psychosis (Arnsten, 2013; Brisch et al., 2014; Leyton and Vezina, 2014). Male adolescence is a period of increasing exposure to circulating testosterone, as well as a period of higher incidence of risk taking behavior and onset of schizophrenia, both of which are characterized by changes in dopamine (Christie et al., 1988; Leyton and Vezina, 2014; Markham, 2012). Previously, we found that male castration prior to adolescence can lead to altered social

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interaction, a drop in social rank and changed sensorimotor gating in primates (Morris et al., 2010; Richards et al., 2009). It is, however, unclear if and how developmental changes in testosterone may contribute to the regulation of other more commonly studied dopamine-dependent behaviors in rodents. Therefore, a focus of this study was to examine the effect of testosterone during male adolescence on a pharmacologically-induced behavior related to dopamine neurotransmission, i.e amphetamine-induced locomotion, in adulthood.

We recently under took a molecular examination as to how testosterone over adolescence may modulate dopamine parameters in the nigrostriatal pathway in male rats. We reported that increasing testosterone over the male adolescent period (postnatal day [PND] 45-60) resulted in an increase in dopamine synthesis potential (tyrosine hydroxylase [TH] protein expression) in the young adult substantia nigra (Purves-Tyson et al., 2012) but not in the striatum (Purves-Tyson et al., 2014), but also found in late adolescent rhesus macaques that circulating testosterone levels correlated positively with TH levels in the putamen (Morris et al., 2010). We also reported, in young adult rats, that increasing testosterone over the adolescent period increased dopamine receptor D2 (DRD2) gene expression in both the substantia nigra and dorsal striatum (Purves-Tyson et al., 2014). Further, gene expression of enzymes involved in dopamine transport and metabolism were also increased by adolescent testosterone in the young adult nigrostriatal pathway (Purves-Tyson et al., 2012, 2014). However, young adult striatal dopamine levels were not changed by adolescent testosterone; rather, striatal dopamine turnover (a measure of dopaminergic activity) was increased upon testosterone removal, and this stimulatory effect was attenuated by testosterone replacement (Purves-Tyson et al., 2014). From these molecular and biochemical observations, it is unclear whether the apparent increase in dopamine synthesis capacity in the substantia nigra and the modulation of other dopamine pathway molecules in the substantia nigra and striatum as a result of changed testosterone during adolescence contributes to an increase, or a decrease, of overall dopamine tone or in dopamine-related behaviors in adulthood.

In rodents, a well-described behavioral response to amphetamine, is an increase in locomotion. This hyperlocomotive response may be due to changes in multiple neurotransmitter systems as amphetamine increases monoamine release and inhibits reuptake by reversing the direction of the transporters (Jones et al., 1998; Sulzer et al., 1995, 2005). In particular, the increase in dopamine concentration in the synapse induced by amphetamine, has been linked to the locomotor-enhancing effects of amphetamine as they are attenuated by dopamine D2 receptor blockade (Lapin and Rogawski, 1995; O'Neill and Shaw, 1999). Although other neurotransmitter systems (e.g. other catecholamines, serotonin, glutamate) can also contribute, either directly or indirectly, to the amphetamine-induced locomotor response (Sulzer et al., 2005), in this study we have emphasized the contribution of dopamine due to our molecular measures relating to the modulation of dopamine function by testosterone, although we have not measured dopamine directly (Purves-Tyson et al., 2012, 2014).

Although sex steroid effects on different aspects of dopamine activity have received much attention [reviewed in (Sanchez et al., 2010)], the role of androgens in modulating dopamine pathways in males, particularly during adolescence, is not fully understood. As sex hormones exert unique organizational effects on brain and evolving behavior during adolescence, it is important to study this developmental period. Preclinical studies in mostly adult male rats report that testosterone can both increase (de Souza Silva et al., 2009; Thiblin et al., 1999) and decrease (Beatty et al., 1982; Dluzen and Ramirez, 1989; Forgie and Stewart, 1994; Menniti and Baum, 1981) striatal dopamine activity. Testosterone treatment increased dopamine turnover but not dopamine synthesis in the striatum of adult male rats (Thiblin et al., 1999) and acute intranasal testosterone treatment of adult male rats increased dopamine in the striatum (de Souza Silva et al., 2009). Other studies have shown that testosterone removal by castration of adult male rats increased spontaneous locomotion (Dluzen and Ramirez, 1989) and increased the locomotor response to amphetamine (Beatty et al., 1982; Forgie and Stewart, 1994) and that both effects were reversed by exogenous testosterone replacement. A further study in male rats, investigating sex steroid modulation during adolescence, reported that gonadectomy and testosterone replacement immediately prior to adolescence reduced amphetamine-induced locomotion compared to non-testosterone replaced gonadectomised in adulthood (Menniti and Baum, 1981). In this study, we also have investigated amphetamine-induced locomotion in adulthood after circulating testosterone was altered during adolescence.

There is evidence that estrogen treatment of adult male rats modulates aspects of the dopamine pathway in the striatum that may contribute to reduced dopamine neurotransmission, e.g. estrogen decreased DRD2 mRNA levels in the striatum (Lammers et al., 1999). The selective estrogen receptor modulator, raloxifene, can act as an ER agonist in the brain, as seen in the hippocampus where both raloxifene and estrogen administration restore choline acetyltransferase activity in ovariectomised female rats (Wu et al., 1999). The many studies investigating estrogen effects on dopamine pathways [reviewed in (Sanchez et al., 2010)] indicate that estrogen receptor-mediated effects can both decrease and increase different measures of dopamine neurotransmission, and since molecular alterations in dopamine-related pathways in male rats appeared to be mediated by AR signaling (Purves-Tyson et al., 2014), we tested if concomitant stimulation of ER signaling through raloxifene may be able to modify testosterone effects on locomotion. In this study, we therefore also tested the extent to which raloxifene treatment in young adulthood (after testosterone modulation during adolescence) impacts amphetamine-induced locomotive behavior both in the absence of testosterone (i.e. gonadectomised) and in the presence of endogenous (sham-operated) or exogenous testosterone (i.e. gonadectomy with testosterone replacement by subdermal silastic implant) in adult male rats.

Materials and methods

Animals

All animal experiments were approved by the Animal Care and Ethics Committee of the University of New South Wales (Ethics number ACEC 13/5A) in accordance with the National Health and Medical Research Council of Australia's Code of Practice for the Care and Use of Animals for Experimental Purposes. Male Sprague-Dawley rats were used for all experiments (Animal Resource Centre, Perth, WA, Australia). Rats were received at PND31 or 32 and group housed (3–4/cage) in 12/12 h light/dark phases with constant humidity and temperature and free access to water and standard rat chow.

Gonadectomy, testosterone replacement and raloxifene treatment

Surgical procedure

For surgery and hormone replacement, rats were anaesthetised with an isofluorane vaporisor set at 5% to induce anaesthesia and then set at 3% for maintenance of anaesthesia. Rats were gonadectomised via a midline abdominal incision and bilateral removal of the testes after ligating the vas deferens and associated blood vessels. Sham-operated animals underwent abdominal surgery but gonads were left in place. Silastic implants were placed subdermally between the shoulder blades at time of gonadectomy with a small incision closed with 1–2 silk sutures. Implants were 1 cm long, internal diameter 1.47 mm, outer diameter 1.95 mm and filled with crystalline steroid (ends sealed with silastic adhesive). At experiment end the presence of the silastic implants was confirmed. The testosterone implants have been characterized in previous studies and achieve physiological or supra-physiological, steady-state serum testosterone levels and maintain seminal vesicle weights comparable to that

in sham-operated animals (Purves-Tyson et al., 2007, 2012; Singh et al., 1995). Body weight measurements were taken weekly throughout the experiment.

Treatments groups

Male Sprague-Dawley rats experience a gradual increase in circulating testosterone between 45 and 60 days of age (Saksena and Lau, 1979; Walker et al., 2012) and at ~60 days of age are considered young adults and by ~90 days of age are considered adults. Male rats were gonadectomised (G) or sham operated (S) at 45 days of age (prior to the adolescent testosterone increase) and given continuous replacement testosterone (T) in the form of testosterone filled or empty silastic implants (Blanks, B) for six weeks. Two weeks following gonadectomy or sham operations daily subcutaneous injections of raloxifene (R, 5 mg/kg) or vehicle (V, 5% dimethylsulphoxide, 47.5% monopropyleneglycol and 47.5% normal saline [0.9% NaCl]) were started and continued daily for four weeks with testosterone or blank implants remaining in place. This dose of raloxifene (5 mg/kg) was selected as it fully restores hippocampal choline acetyltransferase activity in ovariectomised rats in a manner similar to estrogen (Wu et al., 1999). All together, there were six groups of rats (n = 6-11 rats/group): 1. sham-operated with empty (blank) implant and vehicle injections (SBV); 2. gonadectomy with empty implant and vehicle injections (GBV); 3. gonadectomy with testosterone implant and vehicle injections (GTV); 4. sham operated with empty implant and raloxifene injections (SBR); 5. gonadectomy with empty implant and raloxifene injections (GBR); and 6. gonadectomy with testosterone implant and raloxifene injections (GTR).

Amphetamine-induced locomotor activity

Locomotor activity tests (PND85 i.e. 39 days post surgery) were conducted in four open field apparatus (65×65×65 cm, Tru Scan Activity System, Coulbourn Instruments, USA). Movement in each arena was measured by infrared beams. Horizontal activity was the main measure of locomotion.

Locomotion tests were always commenced between 9:30 and 10:00 am to ensure sham-operated rats were in a similar phase in their testosterone circadian rhythm. On day of locomotion testing, rats were placed in the test arenas and locomotor activity measured during a 30 minute habituation period. Animals were then briefly removed from the apparatus and received a vehicle injection (isotonic 0.9% saline solution) and returned to the same apparatus and locomotion measured for a further 30 minutes of testing (considered as "saline baseline" activity). The animals were then removed from the apparatus a second time and received an amphetamine injection (1.25 mg/kg, intraperitoneally, National Measurement Institute, Sydney, Australia). They were immediately returned to the same arena and locomotion response to amphetamine (amphetamine-induced locomotion) was monitored for a further 2 hours. This low dose of amphetamine was selected as it is within the standard dose range (0.2–2.0 mg/kg) used in adult male rats to induce increased locomotion but not stereotypy, which occurs at higher doses (5–10 mg/kg) (Randrup and Munkvad, 1974; Sharp et al., 1987) and, in our hands, produced locomotor effects comparable to previously published data using a similar saline/amphetamine injection paradigm in adult male Sprague-Dawley rats (Byrne et al., 2013). Locomotion data was analyzed over the entire 180 minute testing period.

Seminal vesicle measurements, serum collection and sex steroid measurements

At experiment end (PND88, three days after behavioural assessment) animals were sacrificed by isofluorane aneasthesia and decapitation. Trunk blood was collected in serum tubes, left at room temperature for approximately 1 h and centrifuged at 12000 rpm at 4 °C for 18 min. Serum was stored at −80 °C until required for sex steroid measurements. Seminal vesicles were removed and weight of each seminal vesicle recorded. The average weight of the two seminal vesicles for each rat was then determined and this value used to determine group means.

Sex steroids were quantified in serum using stable isotope dilution liquid chromatography-tandem mass spectroscopy as described (Harwood and Handelsman, 2009) and adapted for rodents (McNamara et al., 2010). The limit of detection for testosterone is 0.01 ng/ml. In thirteen out of fourteen gonadectomised rats without testosterone replacement (GBV and GBR groups), testosterone was not detectable and to allow for statistical comparisons a value of 0.01 ng/ml was assigned to those samples.

Statistical analysis

Statistical analyses were conducted using SPSS software (IBM SPSS Statistics, version 10) or GraphPad Prism (Graphpad Software, version 6) and $p < \text{or} = 0.05$ was considered statistically significant. Data are shown as mean ± standard error of the mean (SEM). Seminal vesicle weight (g) and testosterone levels (ng/ml) were compared using one-way ANOVA with 'treatment' as the independent variable with Fisher's least significant differences (LSDs) *post hoc* analysis. Body weight over the duration of the experiment was analysed with two-way repeated measures ANOVA with a Greenhouse-Geisser correction with 'treatment' as the between subjects factor and 'time' as the within subjects factor. Significant main effect of treatment was followed up with Tukey's *post hoc* analysis and a significant interaction between time and treatment was followed up with Sidak's pairwise comparisons.

Locomotion data is presented as distance moved (cm) in 5 min time bins over the full 180 min period (as per (Byrne et al., 2013)). A single rat in each of the GTR, SBV and SBR groups was removed from the study as their activity did not increase in response to amphetamine relative to baseline activity and were therefore defined as non-responders. Outliers (n = 3) were removed by analysing total distance moved (i.e. area under the curve) after amphetamine injection by Grubbs test for outliers (GraphPad Software, online calculators). Two outliers were removed from the SBV group and one outlier from the GBR group.

The distance moved in the open field arena in 5 min time bins over the 180 min period was analysed with two-way repeated measures ANOVA with a Greenhouse-Geisser correction with 'treatment' as the between subjects factor and 'time' as the within subjects factor. Significant main effect of treatment was followed up with Tukey's *post hoc* analysis and a significant interaction between time and treatment was followed up with Sidak's pairwise comparisons. All six test groups were analysed together and all 6 line graphs are shown in Fig. 3A. However, for ease of viewing the data, the comparisons between groups are presented on different graphs with some groups represented on more than one graph (Figs. 4, 5, 6). In all cases, data are presented as mean ± standard error of the mean (SEM) and $p < 0.05$ was considered statistically significant. The total distance moved after amphetamine injection was analyzed with one-way ANOVA with treatment as the independent variable followed by Fisher's LSDs (Fig. 3D).

To test for significant differences in distance travelled in both the habituation period and the saline baseline period, the total distance moved during each of these 30 min periods was compared using one-way ANOVA with treatment as the independent variable followed by Fisher's LSDs.

Eta squared (η^2) is reported when analysis was by ANOVA and Cohen's *d* is reported for pair-wise comparisons (0.2 is a small effect size, 0.5 is a modest effect size and >0.8 is a large effect size). Eta squared was calculated manually (Field, 2005) and Cohen's *d* was calculated using Ellis, P.D. (2009), "Effect size calculators," website. www.polyu.edu.kh/mm/effectsizefaqs/calculators/calculators.html.

To determine the relationship between testosterone and amphetamine-induced locomotion, the 2 hour period following amphetamine was also divided into four 30 min time periods and Pearson's correlations were performed in groups with testosterone (SBV, GTV) and total distance moved during all six 30 min time periods. To determine how this relationship was changed by raloxifene treatment, Pearson's correlations were then performed in groups with both testosterone and raloxifene treatment (SBR with endogenous testosterone, GTR with replaced testosterone) and total distance moved during each 30 minute time period.

Results

Confirmation of sex steroid replacement by serum measurements and organ weight

As expected, there was a significant effect of treatment group when examining seminal vesicle weights [$F(5,45) = 78.4$, $p < 0.0001$, $\eta^2 = 0.89$] (Fig. 1A). Gonadectomy reduced seminal vesicle weight when compared to sham operated animals (SBV) in both the GBV (SBV vs. GBV, $p < 0.0001$, $d = 8.6$) and the GBR (SBV vs. GBR, $p < 0.0001$, $d = 8.6$) groups and there were no differences between the gonadectomised groups as a result of raloxifene treatment (GBV vs. GBR, $p = 0.97$). Seminal vesicles were partly restored to 76% and 88% of the sham group (SBV) by testosterone replacement in the vehicle and raloxifene (SBV vs. GTV $p < 0.002$, $d = 1.4$ and SBV vs. GTR $p = 0.08$, $d = 0.7$) groups, respectively. There were no differences in seminal vesicle weight between the groups with gonadectomy and testosterone replacement with and without raloxifene (GTV vs. GTR, $p > 0.05$). Raloxifene treatment of intact rats significantly reduced seminal vesicle weight (SBV vs. SBR, $p < 0.0001$, $d = 1.8$).

Similarly, there was a significant effect of treatment group when examining circulating testosterone levels measured six weeks after replacement [$F(5,33) = 16.56$, $p < 0.0001$, $\eta^2 = 0.7$] (Fig. 1B). In intact animals, circulating testosterone was 2.15 ± 0.32 ng/ml and this was reduced to 0.01 ± 0.0 ng/ml by gonadectomy (SBV vs. GBV, $p < 0.0001$, $d = 3.4$). Testosterone implants restored circulating testosterone levels in the GTV (0.98 ± 0.23 ng/ml) and the GTR (0.77 ± 0.19 ng/ml) groups (GBV vs. GTV and GBV vs. GTR, both $p < 0.05$, $d = 2.7$ and 2.3, respectively). Raloxifene treatment significantly reduced endogenous testosterone levels by 41% from 2.15 ± 0.32 ng/ml in the SBV group to 1.27 ± 0.25 ng/ml in the SBR group (SBV vs. SBR, $p = 0.005$, $d = 1.2$). This effect of raloxifene on circulating testosterone was maintained in a larger cohort of rats (10-14 per group, data not shown).

Over the six week experiment, there was a trend for a main effect of treatment on body weight [$F(5,25) = 2.3$, $p < 0.072$, $\eta^2 = 0.32$], a significant main effect of time on body weight [$F(6,150) = 1116.4$, $p < 0.0001$, $\eta^2 = 0.94$] and a significant interaction between time and treatment on body weight [$F(30, 150) = 8.9$, $p < 0.0001$] (Fig. 2). At days 35 and 42 post gonadectomy there was a reduction in body weight of gonadectomised rats compared to sham-operated rats at both time points (SBV vs. GBV, $p < 0.05$, $d = 1.5$ for both) and this was prevented by testosterone replacement (SBV vs. GTV, $p > 0.05$) (Fig. 2). There was a significant reduction in body weight due to raloxifene treatment of sham-operated rats at 35 and 42 days post gonadectomy (SBV vs. SBR, $p < 0.001$, $d = 2.3$ and 2.8, respectively) (Fig. 2). Testosterone replacement prevented the trend for a lack of weight gain experienced by gonadectomised rats treated with raloxifene [GBV vs. GBR, $p = 0.079$, $d = 1.6$ at 42 days post gonadectomy, GBR vs GTR, $p < 0.05$ at 35 ($d = 1.1$) and 42 ($d = 1.3$) days post gonadectomy] (Fig. 2).

Modulation of baseline and amphetamine-induced locomotion by testosterone and raloxifene

Locomotion (distance travelled) was assessed over a 180 minute testing period, which included 30 minutes of habituation in the open field chamber and 30 minutes in the chamber following a saline injection (saline baseline) prior to a further 120 minutes following an amphetamine injection. When distance travelled was analyzed by two-way repeated measures ANOVA over the 180 minute test period there was a significant main effect of treatment [$F(5,37) = 7.7$, $p < 0.0001$, $\eta^2 = 0.51$] as well as a significant main effect of time [$F(35,1295) = 80.3$, $p < 0.0001$, $\eta^2 = 0.62$] and a significant interaction between time and treatment [$F(175, 1295) = 2.3$, $p < 0.0001$] (Fig. 3A). Refer to online version for colour figures.

Locomotion differed between groups during the habituation and the saline baseline periods

There were significant differences in locomotor activity between groups during the habituation and saline injection periods. To explore this further, the total distance moved for each animal in each treatment group in the habituation and the saline baseline 30 minute periods was assessed separately by one-way ANOVA. There was a significant effect of treatment during the habituation period [$F(5,40) = 2.6$, $p = 0.038$, $\eta^2 = 0.34$] (Fig. 3B). During the habituation period, there was a trend for gonadectomised rats to be more active in the novel environment than the intact rats (GBV vs. SBV, $p = 0.056$, $d = 1.1$). Similarly, the intact rats treated with raloxifene (SBR) and the gonadectomised rats treated with raloxifene only (GBR) moved more than gonadectomised rats with both testosterone replacement and raloxifene (SBR vs. GTR, $p = 0.023$, $d = 1.0$; GBR vs. GTR, $p = 0.012$, $d = 1.5$) (Fig. 3B).

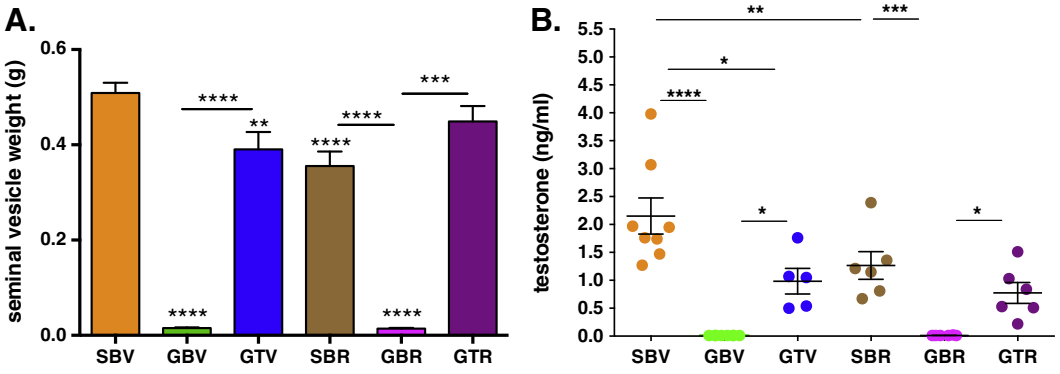
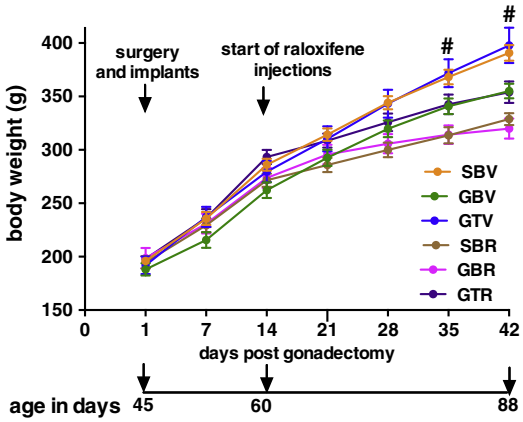


Fig. 1. Effect of gonadectomy, testosterone replacement and raloxifene treatment on seminal vesicle weight and circulating testosterone levels in male rats. 1A. Seminal vesicles were smaller following gonadectomy compared with intact rats and this was largely restored by testosterone replacement irrespective of raloxifene or vehicle treatment. However, seminal vesicle weight was reduced in intact animals when treated with raloxifene (SBV vs. SBR). Unless indicated with a line, asterix denote comparisons with intact group (SBV). 1B. Circulating testosterone was reduced by gonadectomy and increased by testosterone replacement, although to significantly lower than endogenous levels (SBV vs. GTV). Endogenous circulating testosterone was significantly reduced by raloxifene treatment (SBV vs, SBR) in the behavioural cohort. **** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.



# Fisher's LSDs *post hoc* comparisons	35 days post gonadectomy significance, Cohen's *d*	42 days post gonadectomy significance, Cohen's *d*
SBV vs. GBV	*, 1.5	*, 1.5
GBV vs. GTV	*, 1.2	*, 1.2
SBV vs. GTV	ns	ns
SBV vs. SBR	***, 2.3	***, 2.8
GBV vs. GBR	ns	p=0.08, 1.6
GTV vs. GTR	ns	p=0.06, 1.1
GBR vs. GTR	*, 1.1	*, 1.3
GBR vs. SBR	ns	ns

Fig. 2. Effect of gonadectomy, testosterone replacement and raloxifene treatment on body weight in male rats. At days 1 (i.e day following surgery), 7, 14, and 28 days post gonadectomy there were no significant differences in body weight between groups. At days 35 and 42 (experiment end) post gonadectomy there were significant effects of treatment (indicated by # on the graph, and presented in the table). The statistical differences between treatment groups at 35 and 42 days post gonadectomy are shown in the table. Error bars show SEM. *** $p < 0.001$; * $p < 0.05$.

There was also a significant effect of treatment on locomotor activity during the saline baseline period [$F(5,38) = 6.1$, $p = 0.0003$, $\eta^2 = 0.45$] (Fig. 3C). In the 30 minutes following saline injection (i.e. saline baseline period), testosterone removal by gonadectomy (GBV) increased the distance travelled compared to the intact rats (GBV vs. SBV, $p = 0.048$, $d = 0.9$) and this was attenuated by testosterone replacement (GBV vs. GTV, $p = 0.009$, $d = 1.5$) (Fig. 3C). Locomotion of intact rats was not altered by raloxifene treatment during the saline baseline period (SBV vs. SBR, $p > 0.05$). However, in gonadectomised rats there was a trend for raloxifene treatment to increase locomotion at saline baseline (GBV vs. GBR, $p = 0.068$, $d = 0.8$) and this effect was attenuated by testosterone (GBR vs. GTR, $p < 0.0001$, $d = 2.5$) (Fig. 3C).

Testosterone attenuated amphetamine-induced hyperlocomotion

The total distance moved (in 120 minutes) for each animal in each treatment group following amphetamine injection was also assessed by one-way ANOVA. There was a significant effect of treatment following amphetamine injection [$F(5,33) = 5.1$, $p = 0.0014$, $\eta^2 = 0.44$] (Fig. 3D). There was a significant increase in distance traveled when testosterone was removed (SBV vs. GBV, $p < 0.05$, Cohen's $d = 1.2$) and this was prevented by testosterone replacement (SBV vs. GTV, $p > 0.05$; GBV vs. GTV, $p < 0.05$, Cohen's $d = 1.1$). Intact rats treated with raloxifene travelled further than intact rats without raloxifene (SBV vs. SBR, $p < 0.05$, Cohen's $d = 1.3$) as did gonadectomised rats treated with raloxifene compared to gonadectomised rats without raloxifene (GBV vs. GBR, $p < 0.05$, Cohen's $d = 1.1$). There was a trend for gonadectomised rats treated with raloxifene to travel further than both the intact rats with raloxifene and the gonadectomised rats with replacement testosterone (SBR vs. GBR, $p < 0.1$, Cohen's $d = 0.9$; GBR vs. GTR, $p < 0.05$, Cohen's $d = 1.1$).

The locomotion data was also analysed with two-way repeated measures ANOVA (as reported above, see Fig. 3A). Removal of circulating testosterone during adolescence by gonadectomy potentiated the hyperlocomotion response induced by amphetamine (Fig. 4). Within 30 minutes of amphetamine injection, gonadectomised rats (GBV) without testosterone showed a greater distance travelled above the other two groups. The 5 minute pairwise comparisons with p values and Cohen's d are shown in Fig. 4. *Post hoc* comparisons between the SBV and GBV groups are presented below the SBV group, and the *post hoc* comparisons between the GBV and GTV groups are shown above the GBV group in Fig. 4. There were no statistically significant differences in distance travelled in 5 minute time bins between sham-operated rats with endogenous testosterone or gonadectomised rats with testosterone replacement (SBV vs. GTV) (Fig. 4).

Raloxifene potentiated amphetamine-induced hyperlocomotion in the presence of endogenous testosterone

Four weeks of raloxifene treatment of sham-operated male rats (i.e. with endogenous testosterone, SBV) potentiated amphetamine-induced locomotion (SBV vs. SBR) by maintaining increased locomotion for a longer time (Fig. 5). The distance travelled by intact rats (SBV) and intact rats treated with raloxifene (SBR) increased at the same rate following amphetamine injection (Fig. 5, SBV vs. SBR; $p > 0.05$ for 5 minute time bins between 60 minutes and 115 minute). The distance travelled by the SBV group began to decline 45 minutes after amphetamine injection (i.e. at 105 minutes) whereas the distance travelled by the intact group with raloxifene (SBR) continued to increase until 60 minutes after amphetamine injection (i.e. 115 minutes) when distance travelled also started to decline towards baseline, but remained significantly higher than the intact rats without raloxifene (Fig. 5, SBV vs. SBR between 135 minutes and 165 minutes p was between <0.05 and <0.001 for many 5 minute time bins). The 5 minute pairwise comparisons with p values and Cohen's d are shown in Fig. 5

Raloxifene potentiated amphetamine-induced hyperlocomotion in rats without testosterone

The group with the greatest distance travelled in total in response to amphetamine injection was the gonadectomised rats without testosterone replacement but treated with raloxifene (GBR) (Figs. 3A and 6). When gonadectomised rats with testosterone replacement were treated with raloxifene (GTR) they appeared to start returning toward lower baseline levels of locomotion slightly before the group without testosterone replacement (GBR). When comparing distance travelled in 5 minute time bins there was a trend for the distance travelled by the GTR group to be less than the GBR group at 170 minutes ($p < 0.1$, Cohen's $d = 1.6$) and the distance travelled by the GTR group was significantly less at 175 minutes ($p < 0.01$, Cohen's $d = 1.6$) compared to the GBR group.

However, raloxifene treatment significantly increased the dopamine-potentiating effect of amphetamine on locomotion in gonadectomised rats with testosterone replacement (GTR) as compared to gonadectomised rats with testosterone without raloxifene (GTV) ($p = 0.02$, Cohen's $d = 1.6$) and when comparing distance travelled in 5 minute time bins, this was evident from 15 minutes following the amphetamine injection (i.e. 95 minutes) when the distance travelled by the GTV group peaked and started to decline toward baseline and the GTR group continued to increase the distance travelled until 110 minutes. The 5 minute pairwise

comparisons with *p* values and Cohen's *d* are shown in Fig. 6 and in figure legend.

Testosterone was negatively correlated with the amount of amphetamine-induced locomotion

Pearson's correlations revealed an inverse relationship between locomotion after amphetamine injection and circulating testosterone level at time of sacrifice (i.e. 3 days after behavioral testing), when analysing the groups with either endogenous or exogenous testosterone but no raloxifene treatment (SBV, GTV) (Fig. 7). The distance moved after amphetamine injection was divided into 30 minute time blocks and the relationship between the total distance moved in each of the two 30 min time blocks prior to amphetamine injection and each of the four 30 minute time blocks after amphetamine injection and terminal circulating testosterone levels was determined with Pearson's correlations. There were no significant correlations between distance moved and testosterone levels during the habituation period (0-30 minutes, r = -0.35, $p = 0.27$) or the saline baseline period (30-60 minutes, r = 0.02, $p = 0.95$). However, after amphetamine injection there was a negative correlation between serum testosterone and distance travelled in each of the last two 30 minute time bins (SBV + GTV; 120-150 minutes, r = -0.70, $p = 0.012$ and 150-180 minutes, r = -0.76, $p = 0.004$) (Fig. 7). This indicates that the higher the terminal serum testosterone concentration, the less amphetamine-induced locomotion. These negative correlations were not found when levels of testosterone and levels of activity were examined in rats treated with raloxifene (SBR + GTR; 120-150 minutes, r = 0.37, $p = 0.30$ and 150-180 minutes, r = 0.34, $p = 0.33$) (Fig. 7).

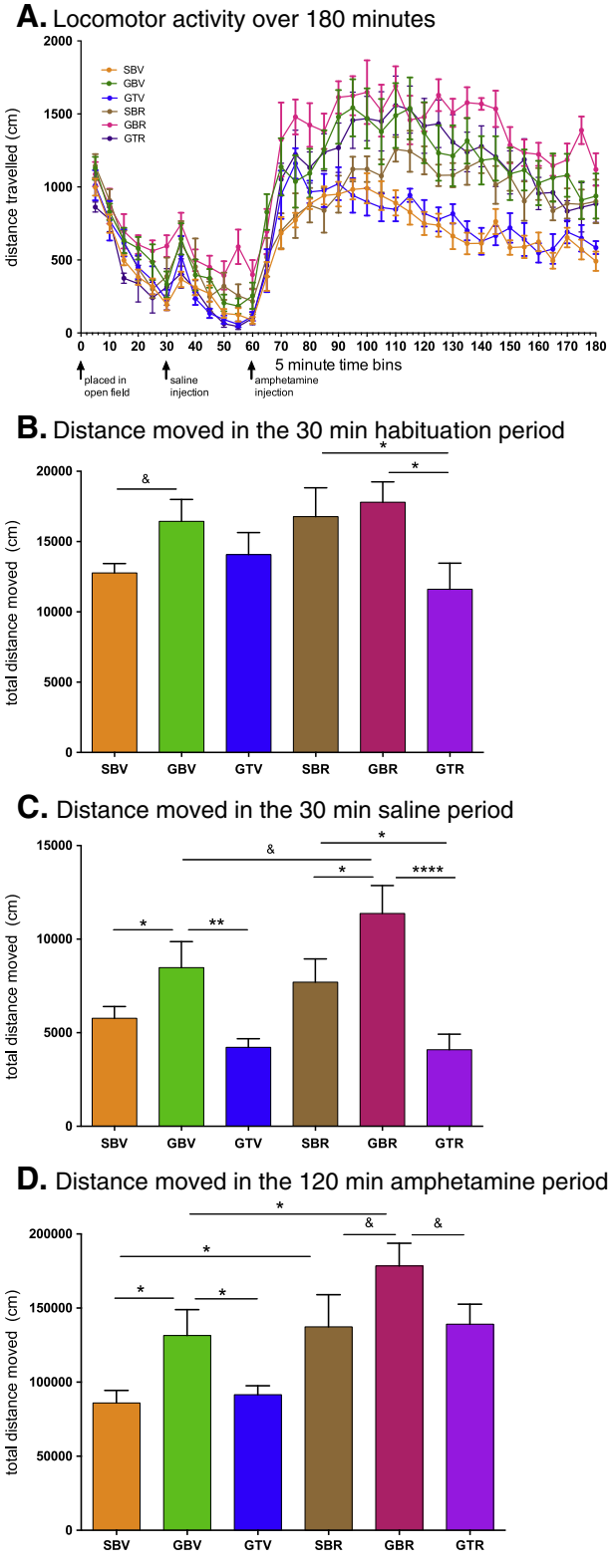


Fig. 3. The effect of gonadectomy and testosterone replacement on amphetamine-induced locomotion during the habituation and saline baseline periods. A. Distance traveled over the 3 h observation period in 5 minute time bins showing the 30 min habituation period starting at time 0 min when animals were placed in the chamber and time 30 min when saline injection was administered. Amphetamine was injected at 60 min and rats were observed for a further 120 min. When distance travelled was analysed there was a significant effect of treatment [between subjects, $F(5,37) = 7.7$, $p < 0.0001$, $\eta^2 = 0.51$] as well as a significant effect of time [within subjects, $F(35, 1295) = 80.3$, $p < 0.0001$, $\eta^2 = 0.62$] and a significant interaction between time and treatment [$F(175,1295) = 2.3$, $p < 0.0001$]. B. There was a significant effect of treatment during the habituation period [$F(5, 40) = 2.6$, $p = 0.038$, $\eta^2 = 0.34$]. C. There was a significant effect of treatment in the saline baseline period [$F(5,38) = 6.1$, $p = 0.0003$, $\eta^2 = 0.45$). D. There was a significant effect of treatment following the amphetamine injection [$F(5,33) = 5.1$, $p = 0.0014$, $\eta^2 = 0.44$]. & < 0.1, ** $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$,**** $p < 0.0001$.

Discussion

Summary of findings

We report that testosterone removal prior to adolescence increases the adult locomotive response, while both endogenous and exogenous testosterone exposure during adolescence decrease the adult locomotive response, following administration of amphetamine. We report that raloxifene treatment increases the locomotion response to amphetamine in intact rats with circulating testosterone. We also show that in gonadectomised rats without circulating testosterone, raloxifene increases the locomotor response to amphetamine and in gonadectomised rats with exogenous testosterone, raloxifene largely overrides the attenuating effect of testosterone on amphetamine-induced locomotion. Further, we show a negative correlation between circulating testosterone levels and degree of amphetamine-induced hyperlocomotion, and we find that this relationship is lost when rats are treated with raloxifene. This demonstrates that not only can raloxifene potentiate amphetamine-induced locomotion alone, but at the single concentration used in this study, it appears to largely override any locomotor blunting changes related to testosterone levels.

Baseline locomotion differs between treatment groups

The increased locomotion in response to a saline injection in gonadectomised rats compared to gonadectomised rats with testosterone replacement or sham-operated rats could be due to many different effects. In our previous study, we reported an increase in baseline dopamine turnover, a measure of dopamine activity, in the dorsal striatum following two weeks of gonadectomy compared to intact rats and this

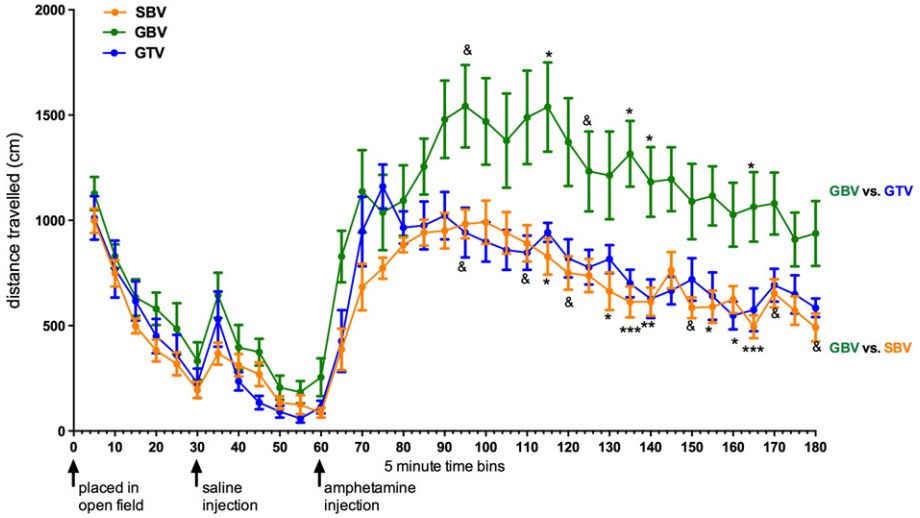


Fig. 4. The effect of gonadectomy and testosterone replacement on amphetamine-induced locomotion. The distance traveled following amphetamine injection was greater in the gonadectomised group compared to either group with circulating testosterone (GBV vs. SBV and GBV vs. GTV). There was no difference in distance travelled between the intact animals and gonadectomised animals with testosterone replacement. & $p < 0.1$ Cohen's $d = 1.1$–1.3, * $p < 0.05$ Cohen's $d = 1.2$–1.4, ** $p < 0.01$ Cohen's $d = 1.5$, *** $p < 0.001$,**** $p < 0.0001$. Cohen's $d = 1.5$–1.8.

was prevented by testosterone replacement (Purves-Tyson et al., 2014). This provides some evidence that testosterone modulation has the potential to directly change dopamine neurotransmission and that changes in dopamine may mediate sex hormone effects on locomotion, although dopamine-related behaviors were not measured in our previous study and dopamine was not measured directly in the current study. In addition, testosterone increased dopamine transporter mRNA and protein (Purves-Tyson et al., 2014) and in DAT knockout mice the absence of DAT translates into a marked increase in locomotion in a novel environment due to the lack of dopamine reuptake (Gainetdinov and Caron, 2003), and this may contribute to why rats without circulating testosterone in the study reported herein (and therefore we presume less DAT) show an increase in locomotion during habituation and after saline injection. Raloxifene treatment of gonadectomised rats also led to a marked increase in locomotion during the saline baseline period and this was also prevented if the rats had circulating testosterone. As such, this decrease in dopamine activity in response to a novel environment or saline (i.e. not injection of a dopamine-potentiating drug) in animals with testosterone present may be due to a truncated time course of dopamine action and this observation would be consistent with a more rapid clearance of dopamine by DAT via increased levels of DAT.

Although these differences in locomotion were identified at baseline, amphetamine-induced locomotion data was not normalized to saline baseline data. Since we find evidence that both the numerator (amphetamine-induced locomotion) and the denominator (baseline locomotion) may be changed by sex steroid modulation and raloxifene treatment, the normalization procedure may result in skewed data rather than "controlling" for individual differences in activity for each rat. As such, that data has not been normalized and is this a potential weakness of our study. However, the changes in baseline locomotion may be an important aspect of sex steroid modulation of behavior and requires consideration when future studies are designed.

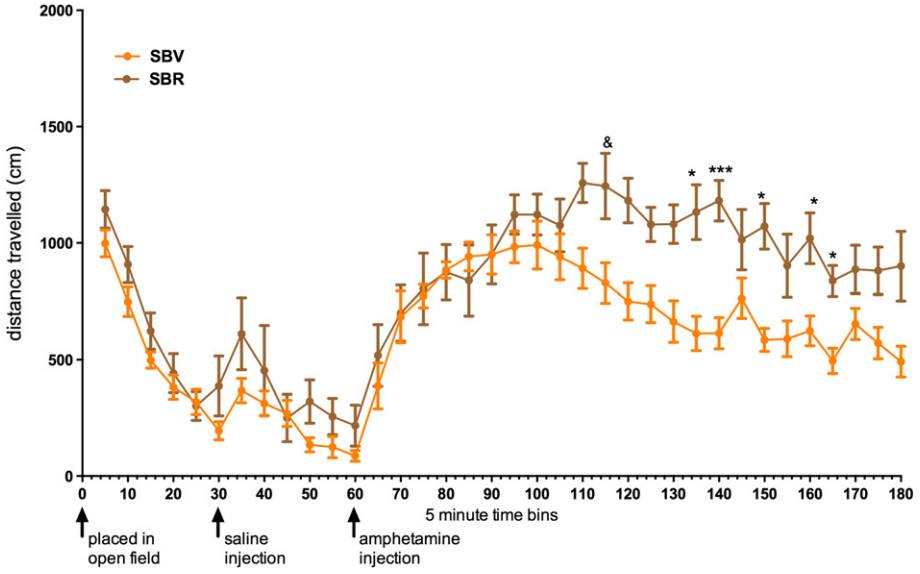


Fig. 5. The effect of raloxifene on amphetamine-induced locomotion in rats with *endogenous* circulating testosterone. The distance travelled by the SBV and SBR groups following amphetamine injection was similar until 45 minutes after injection at which point the SBR group continued to increase the distance travelled whereas the intact group began to decrease the distance travelled and the difference in distance travelled reached a trend at 115 minutes and statistical significance at 135 minutes after injection. & $p < 0.1$ Cohen's $d = 1.2$, * $p < 0.05$ Cohen's $d = 1.5$ - 1.9, *** $p < 0.001$ Cohen's $d = 2.2$.

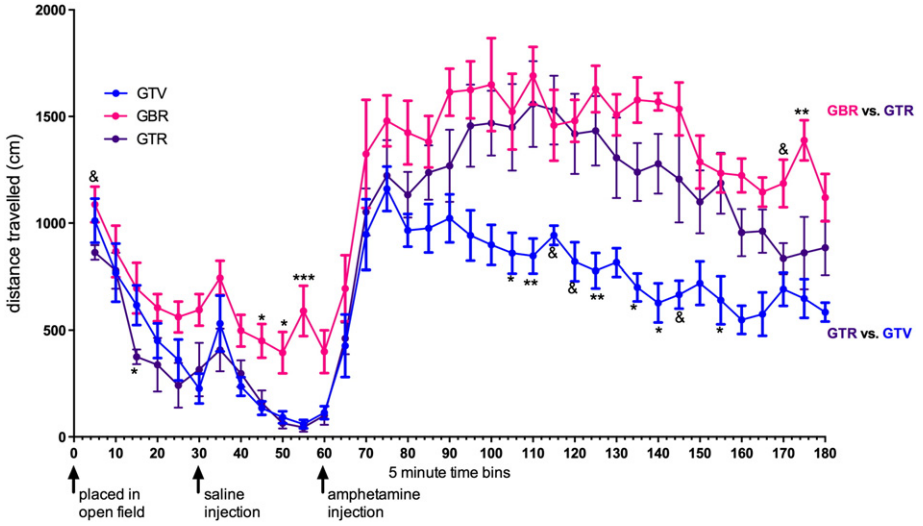


Fig. 6. The effect of raloxifene on amphetamine-induced locomotion in rats with or without *exogenous* circulating testosterone. Raloxifene treatment significantly increased the dopamine-potentiating effect of amphetamine on locomotion in gonadectomised rats with testosterone replacement (GTR) as compared to gonadectomised rats with testosterone without raloxifene (GTV). When comparing GTR vs. GTV, Cohen's *d* was 2 at 115 min and 1.5 at both 120 and 145 min. When $p < 0.05$ between GTR vs.GTV, Cohen's *d* was between 1.7 and 2.2 and at 125 min Cohen's *d* was 2.1. When comparing GBR vs. GTR Cohen's *d* was 1.6 at both 170 ($p < 0.1$) and 175 min ($p < 0.01$). & $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Testosterone decreases amphetamine-induced locomotion

Testosterone has been implicated in the modulation of dopamine neurotransmission, although with conflicting evidence on the direction of change. Some studies, all in adult male rats, indicate that testosterone increases striatal dopamine and enhances the effect of dopamine-potentiating drugs (de Souza Silva et al., 2009; Thiblin et al., 1999), whilst others indicate that testosterone removal increases striatal dopamine (Beatty et al., 1982; Dluzen and Ramirez, 1989; Forgie and Stewart, 1994). Our data reported herein, although we have not directly measured dopamine release in the behaving rats, concur with the studies that indicate that testosterone removal increases striatal dopamine activity in response to the dopamine-potentiating drug, amphetamine, and that testosterone replacement of gondectomised male rats reverses this effect. Our study differs from these previous studies as our sex steroid manipulation was prior to the adolescent increase in testosterone but our behavioural testing was in adulthood, whereas most of these previous studies began in adulthood. Importantly our study concurs with that of (Menniti and Baum, 1981) in which sex steroid manipulation was also prior to the adolescent increase in circulating steroids and behavioral testing in adulthood and as such both studies encompass the adolescent developmental period that is well known to undergo remodeling of the dopamine system e.g. (Teicher et al., 1993). It is known that peri-adolescent rodents are less sensitive to amphetamine treatment than adult rodents and amphetamine produces smaller behavioral activation in early adolescent rats compared to adult rats (Bolanos et al., 1998; Laviola et al., 1999). Thus, our data may reflect an impact of testosterone on this remodeling of the dopaminergic system.

Neurotransmitter systems other than dopamine may also contribute to the changes in amphetamine-induced locomotion reported herein. Amphetamine has complex acute drug effects that may also involve norepinephrine, epinephrine, serotonin and glutamate or combinations of these neurotransmitters. For example agonists of metabotropic glutamate receptors 2/3 attenuate amphetamine-induced locomotion (Pehrson and Moghaddam, 2010) and estrogen has been shown to activate metabotropic receptor 2 signaling (Boulware et al., 2005). Agonism of the serotonin 1A (5-HT_{1A}) receptor and antagonism of the 5-HT_{2A} receptor attenuates amphetamine-induced dopamine release, thus changes in serotonin pathways can also impact amphetamine-induced locomotion (Di Giovanni et al., 2010) and as such sex steroid modulation and raloxifene treatment may modulate other neurotransmitter systems that may contribute to changes in amphetamine-induced locomotion and this requires investigation in future studies.

In addition, testosterone may be influencing the locomotor response to amphetamine via direct modulation of dopamine neurotransmission. We have previously reported changes in gene expression of a number of dopamine pathway-related molecules in the nigrostriatal system in response to removal and replacement of testosterone over adolescence (Purves-Tyson et al., 2012, 2014) that may shed light on the underlying molecular changes induced by testosterone to contribute to this effect on amphetamine-induced locomotion. Our published molecular data (Purves-Tyson et al., 2012, 2014) suggests that while testosterone may prime the midbrain neurons to make and release more dopamine (through increases in TH and VMAT2), it may concomitantly also increase the ability to control the intensity and duration of dopamine action by increasing re-uptake (DAT) and breakdown enzymes that serve to terminate dopamine action (Napolitano et al., 1995; Nirenberg et al., 1996). However, in response to amphetamine, which reverses the direction of the dopamine transporter (i.e. results in efflux of dopamine into the synaptic cleft) (Heikkila et al., 1975; Seiden et al., 1993), it is harder to reconcile increased DAT gene expression in testosterone replaced rats with the overall decreased amphetamine-induced locomotion that we report here. Indeed, DAT overexpressing mice show increased locomotor responses to amphetamine compared to wild type mice (Salahpour et al., 2008). However, the actions of dopamine autoreceptors (DRD2 short) may also contribute to the decrease in amphetamine-induced locomotion in testosterone-replaced rats.

Indeed, we have previously reported an increase in DRD2 pan and DRD2 short (DRD2S) mRNA at the dopamine cell bodies (substantia nigra) as well as in the dorsal striatum (i.e. DR mRNA in the cell bodies of striatal target neurons) in male rats (Purves-Tyson et al., 2014). Presynaptic DRD2S autoreceptors inhibit dopamine release and postsynaptic DRD2S receptors inhibit DRD1 responses (Rouge-Pont et al., 2002; Usiello et al., 2000). Although we also reported an increase in postsynaptic DRD2 mRNA levels, which could contribute to increased locomotion (Lapin and Rogawski, 1995; O'Neill and Shaw, 1999), the locomotion response reported herein indicates that the balance of these dopamine receptor changes as a result of increased testosterone levels, may contribute to a system with overall less dopamine activity as measured by amphetamine-induced locomotion. In addition, the molecular changes in the dopamine pathway may be offset or further modulated by additional changes in other

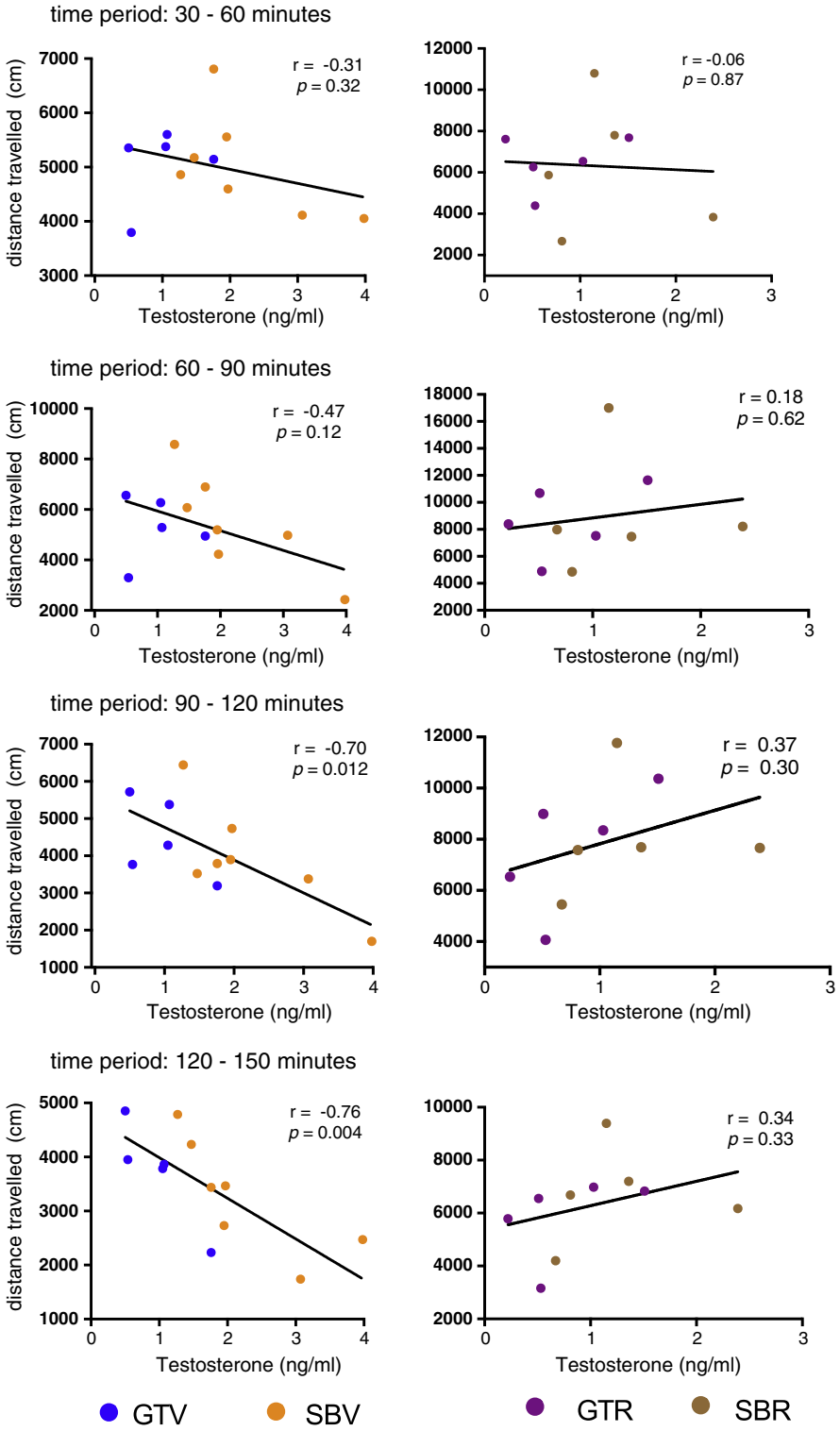


Fig. 7. The negative relationship between amphetamine-induced locomotion and circulating testosterone level is abolished by raloxifene treatment. The total distance moved was divided into 30 minute time bins and Pearson's correlations with circulating testosterone levels revealed a negative relationship between circulating testosterone level and the distance travelled and this was lost when rats were treated daily with 5 mg/kg raloxifene.** $p < 0.01$, *** $p < 0.001$.

neurotransmitter systems controlling amphetamine-induced locomotion. We propose that the cumulative effect of less DAT, less presynaptic DRD2S (i.e less presynaptic autoreception) and slower dopamine metabolism may contribute to a more gradual return toward baseline locomotion levels (after amphetamine injection) in gonadectomised rats without testosterone compared to both sham and testosterone-replaced rats with the converse occurring in rats with circulating testosterone. Therefore, we propose that the presence or absence of testosterone over the adolescent period and into adulthood may modulate the response of the dopamine system to amphetamine in adulthood and this would be consistent with the testosterone-induced changes in dopamine pathway-related molecules we and others have reported [reviewed in (Sanchez et al., 2010)]. Studies using specific dopamine receptor antagonists and

dopamine reuptake blockers would help to clarify the contribution of the components of the dopamine pathway to these changes. It is important to note that it is a limitation of this study that we have not directly measured dopamine levels in the behaving rats and this would be necessary to confirm the direct modulation of dopamine neurotransmission by testosterone and/or raloxifene.

It must also be considered that testosterone in the male can be metabolized to 17β-estradiol and have effects via ERs. Testosterone can be by metabolized to dihydrotestosterone (DHT) which acts via AR but is further metabolized by 3α-hydroxysteroidodehyrogenase to 3β-androstanediol which acts via ERβ (Celotti et al., 1992). 3β-androstanediol and action of upstream steroidogenic enzymes (5α-reductase) have been linked to sensorimotor gating deficits following dopamine activation (Devoto et al., 2012; Frau et al., 2014). This suggests the possibility that raloxifene is having the opposite effect to the estrogen-driven effects from testosterone metabolites and may therefore be acting as an estrogen receptor antagonist. The effects reported here do not elucidate whether testosterone actions are direct and/or indirect via it's metabolites. However, in the study by Menniti and Baum, 1981, comparing gonadectomised (at 40 days of age) and hormone replaced (with silastic implants for 2–3 weeks) male rats indicated that estradiol replacement increased amphetamine (1.5 mg/kg)-induced locomotion compared to gonadectomised rats and DHT or testosterone replacement reduced amphetamine-induced locomotion (Menniti and Baum, 1981). When the combination of DHT and estradiol was used for replacement, DHT partially attenuated the estradiol-induced increase in locomotion. This data indicates that testosterone effects on amphetamine-induced locomotion are therefore most likely not via ER agonism. Additional experiments using specific blockers of testosterone metabolism (e.g. inhibition of 5α-reductase) would be needed to further clarify how testosterone is exerting its effects. Also, further experiments using ERα and ERβ specific agonists may help to determine which ER(s) are involved in mediating the raloxifene effects. However, we cannot rule out that there may be complex agonist/antagonist actions at ERs when both testosterone (and it metabolites 17β-estradiol and 3β-androstanediol) and raloxifene are present in vivo.

Raloxifene treatment of intact rats increases amphetamine-induced locomotion

An important aspect of this study was to determine how raloxifene modulates amphetamine-induced locomotion in the presence and absence of testosterone. Evidence suggests that estrogen can modulate dopamine neurotransmission and amphetamine-induced locomotion (Menniti and Baum, 1981; Sanchez et al., 2010). We sought to determine whether concomitant stimulation of ER was able to modulate testosterone effects on amphetamine-induced locomotion. Raloxifene, in our study, appears to be acting in a similar manner to estrogen in the study by Menniti and Baum (1981) and therefore, most likely as an ER agonist. We found that ER stimulation by the SERM raloxifene appeared to have the opposite effect on locomotor behaviour as compared to testosterone in adult male rats. Raloxifene treatment of rats with endogenous testosterone potentiated amphetamine-induced hyperlocomotion both by increasing locomotion to a higher initial level after injection of amphetamine and by slowing the return to baseline locomotion levels. While we found potentiation of amphetamine-induced locomotion by raloxifene with low to no circulating testosterone levels (gondectomised and saline-replaced animals), we also found an overall effect of raloxifene on circulating testosterone levels in intact animals. This is in contrast to what has been reported in humans, that raloxifene increases circulating testosterone in males (Duschek et al., 2004; Uebelhart et al., 2004), as in our experiments, raloxifene treatment reduced circulating testosterone levels in intact male rats (~40%). This difference in the effect of raloxifene on testosterone levels between male humans and male rats may be the result of a species difference or it may be a result of an age difference. The studies in humans were in elderly or middle-aged men who had low endogenous testosterone (Duschek et al., 2004; Uebelhart et al., 2004) and our animals are young adults, the time of peak testosterone levels, at the time of raloxifene administration. Thus, in sham-operated animals the reduction in circulating testosterone by raloxifene may contribute to the hyperlocomotion effects of raloxifene in this group (SBR). However, the activity potentiating effects of raloxifene are also found in the testosterone-replaced animals and in gonadectomised rats with undetectable levels of testosterone, where in these animals raloxifene cannot influence gonadal synthesis of circulating testosterone levels. Thus, our results suggest that raloxifene acting independently of testosterone or acting in conjunction with decreasing testosterone levels, can increase locomotor activity in adult male rats treated with amphetamine. Therefore, the ultimate effect of raloxifene treatment of intact male rats would be a result of the net effect of indirect action via testosterone modulation and AR action and direct action via ER. The loss of the negative correlation between testosterone levels and amphetamine-induced locomotion when raloxifene is present further supports raloxifene treatment is capable of inducing opposing effects on amphetamine-induce locomotion to testosterone.

Raloxifene has been proposed for use in men with schizophrenia as it has been shown to ameliorate psychotic symptoms in women with schizophrenia (Kulkarni et al., 2002; Usall et al., 2011) and improves cognitive function in both males and females with schizophrenia (Weickert et al., in Press). Our data raises important questions as to its predicted clinical effect in men or whether it may have differential affects on psychosis depending on the sex steroid background of the patient. In the clinic, treatment of males with raloxifene would be in the context of endogenous circulating testosterone and thus, sex steroid/SERM interactions need to be understood in the context of males and their endogenous levels of testosterone. This study highlights the importance of understanding the effect of raloxifene in the context of different genders or in people/animals with altered sex steroid receptors (Weickert et al., 2008).

Limitations of the study

There are a number of technical aspects that require consideration when interpreting our data. It is important to note the differences between endogenous circulating testosterone and replaced testosterone that may impact the data. Endogenous testosterone levels increase gradually over adolescence (between 45 days of age and 60 days of age) and also exhibit daily circadian rhythms, whereas implants supply an immediate high steady state level. Although we did not detect any differences between the GTV and SBV groups in this study, it should be considered that these differences in testosterone dynamics could contribute to changes in aspects such as dominance status that may ultimately impact on neurotransmitter systems and associated behaviors.

A further limitation of this study is that only one dose of raloxifene (5 mg kg^{-1} day^{-1}), the effects of which are typically dose-dependent, was selected (Wu et al., 1999). Now that a raloxifene-induced effect has been identified in males, it will be important for future studies to examine a raloxifene dose response curve. In addition there is some evidence in the literature that sex steroids can modulate the liver parahydroxylation of amphetamine (Forgie and Stewart, 1994) and thereby, modulate amphetamine-induced behaviors. This seems unlikely to be the main or only contributor to the effects found in our study, as we can detect direct molecular changes in brain in dopamine signaling molecules in rats treated with similar sex steroid manipulation strategies (Purves-Tyson et al., 2014).

Conclusions

This study contributes to our knowledge of how testosterone over adolescence may contribute to changes in the response to drugs such

as amphetamine in adulthood and how this may be modulated by raloxifene. It raises important considerations regarding sex steroid based interventions for psychotic disorders involving dopaminergic dysregulation and thus warrants further investigation. We conclude that testosterone during adolescence attenuates locomotor responses to amphetamine in adulthood and estrogen receptor modulation by raloxifene treatment in young adulthood (after adolescent testosterone modulation), potentiates locomotor responses to amphetamine in adulthood.

Acknowledgments

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