



Effects of testosterone administration and gonadectomy on incentive downshift and open field activity in rats

Nadia Justel^a, Eliana Ruetti^a, Mariana Bentosela^a, Alba E. Mustaca^a, Mauricio R. Papini^{b,*}

^a Instituto de Investigaciones Médicas, CONICET-UBA, Argentina

^b Texas Christian University, United States

ARTICLE INFO

Article history:

Received 2 May 2012

Accepted 4 May 2012

Keywords:

Testosterone

Gonadectomy

Incentive contrast

Consummatory extinction

Open field activity

Frustration

Anxiety

Rats

ABSTRACT

Previous research showed that the effects of incentive downshift in male rats are attenuated by a pretrial opportunity to ejaculate. Because ejaculation raises testosterone (T) levels and has anxiolytic-like effects in male rats, the present experiments were designed to assess the role of T and gonadectomy (GDX) on two situations involving incentive downshift. In consummatory successive negative contrast, a downshift from 32% to 4% sucrose leads to consummatory suppression. T alleviates such suppression (Experiment 1), but GDX does not affect it (Experiment 3). In consummatory extinction, animals are downshifted from 32% sucrose to an empty sipper tube. T enhances consummatory extinction (Experiment 2), but GDX does not affect it (Experiment 4). In agreement with published results, T increases (Experiment 2) and GDX reduces (Experiment 4) activity in the central area of an open field, thus behaviorally validating these manipulations. The results are discussed in terms of the anxiolytic-like properties of androgen hormones.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

The present research is concerned with the emotional consequences of incentive downshifts. Previous research indicates that sexual behavior produces an anxiolytic-like effect reducing the impact of various types of stressors [1]. For example, male rats allowed ejaculations 20 h and immediately before being exposed to an unexpected downshift from 32% to 4% sucrose exhibit attenuated disruption of consummatory behavior, compared to both controls that did not ejaculate and controls with the same ejaculatory experience, but exposed only to 4% sucrose (i.e., unshifted controls) [2]. Because various types of anxiolytic drugs have a similar attenuating effect on this so-called consummatory successive negative contrast (cSNC) effect [3–5], the effect of ejaculatory behavior on cSNC was interpreted as an anxiolytic-like effect. Moreover, ejaculatory behavior shared an important property with benzodiazepine anxiolytics like chlordiazepoxide, namely, they were both effective in reducing cSNC in the second downshift trial, but not in the first downshift trial [2,3].

Two pieces of evidence suggest that the effect of copulation on cSNC could be mediated by testosterone (T) levels. First, male mating behavior increases circulating levels of T in rats [6,7] and other mammals [8,9].

Second, androgens have been reported to have anxiolytic effects in other situations involving emotional stress. For example, male rats administered T are less disrupted during punished drinking testing in the Vogel paradigm [10] and exhibit decreased signs of anxiety in the elevated plus maze [11,12], open field test [13], defensive burying test [14], and defensive freezing [13,15] relative to vehicle-treated rats. Moreover, increasing endogenous androgen release by sexual stimuli also increases exploratory behavior in the open arms of the elevated plus maze, in male mice [11]. Removing the testes (gonadectomy, GDX), the primary source of T, results in higher levels of behavior indicative of anxiety in a variety of tasks, in male rats. For example, GDX male rats exhibit decreased activity in the central area of the open field and in the open arms of the elevated plus maze, reduced drinking in Vogel's punished drinking test, and increased freezing behavior compared to intact controls [13–19]. T administration can reverse some of the effects of GDX [13–19].

The present experiments were designed to test two hypotheses. First, exogenous T treatment has an anxiolytic-like effect on cSNC, increasing the consummatory behavior of downshifted animals without affecting the behavior of unshifted controls, relative to vehicle-treated controls. Second, GDX has an anxiogenic-like effect on cSNC, suppressing consummatory behavior of downshifted animals without affecting the behavior of unshifted controls, relative to sham-operated controls. In addition, the effects of T treatment and GDX were tested in two other tasks: consummatory extinction (cE) and the open field test (OF). In cE animals are given access to 32% sucrose and then downshifted to an empty sipper

* Corresponding author at: Department of Psychology, Texas Christian University, Box 298920, Fort Worth, TX 76129, United States. Tel.: +1 817 257 6084.

E-mail address: m.papini@tcu.edu (M.R. Papini).

tube. In the OF test animals are placed in an open arena and their locomotor activity is measured.

There is evidence that cE is faster (i.e., reduced consummatory behavior) after treatments that reduce the disrupting effects of frustration on appetitive behavior, including partial reinforcement and small-magnitude incentives [20], and ethanol administration [21]. Partial reinforcement and ethanol diminish cSNC by increasing consummatory responding—the opposite behavioral outcome (see discussion in [20,21]). Both situations induce avoidance of the sipper tube given the incentive downshift. However, whereas in cSNC there is still an incentive capable of inducing approach behavior, in cE there is no support for approach to the sipper tube. Thus, any factor presumed to reduce anxiety (i.e., reducing avoidance), should increase consummatory behavior in the cSNC situation (i.e., reduce cSNC), but it should reduce consummatory behavior in the cE situation (i.e., enhance cE). Such factors may be said to promote acceptance of the new incentive conditions. On this basis, it was predicted that exogenous T treatment would facilitate cE whereas GDX would retard cE, relative to their respective controls.

In the OF test, animals treated with anxiolytics show an enhanced tendency to explore the central location of the field [22]. Thus, it was expected that exogenous T treatment would increase activity in the central area of the OF. Conversely, GDX should increase cSNC, retard cE, and reduce activity in the central area of the OF.

2. Experiment 1

In the cSNC situation used in the present Experiments 1 and 3, one group of rats is given access to 32% sucrose for 10 daily trials (preshift) followed by access to 4% sucrose for an additional 5 daily trials (postshift). During postshift trials, downshifted rats exhibit less consummatory behavior than an unshifted control group always given access to 4% sucrose. Previous experiments suggest that the effects of T administration on consummatory behavior may depend on the timing or length of its administration relative to training experience. For example, unpublished experiments showed that acute T treatment during the 5 postshift trials had no effect on cSNC (see also General Discussion). However, when the same postshift treatment was combined with additional T administration before the start of training (pretraining administration), T facilitated recovery from cSNC [23]. Pretraining T administration also had an unexpected facilitatory effect on 4% sucrose intake (not so on 32% intake). Thus, the preshift terminal performance of T-treated groups was different from that of vehicle controls. As done when a treatment yields different terminal preshift performance (e.g., when cSNC is tested in nondeprived animals; see [24]), the facilitatory effect of T was detected by computing the proportion of each posttrial value, for each animal, relative to the performance on Trial 10.

The differences between postshift treatment having no effect on cSNC (unpublished experiments) and pretraining plus postshift treatment facilitating recovery from cSNC [23] suggest that the timing and/or length of the T treatment may determine the extent to which such treatment modulates the cSNC effect. Therefore, a new T administration protocol was implemented in the present experiment, differing from the unpublished studies mentioned above (T only on postshift Trials 11–15; 5 days of T treatment) and from published research (T administered 6 days before Trial 1 and also before Trials 11–15; 11 days of T treatment) [23], in that T was administered between Trials 5–15, starting 6 trials before the first downshift event (which occurred on Trial 11) and continuing during all postshift trials (11 days of T treatment). Thus, unlike in previous studies, T was here administered before 6 preshift trials (Trials 5–10), and like previous experiments, T was also administered before each postshift trial (Trials 11–15). The length of T treatment was the same as in pretraining experiments [23], but the timing was different.

2.1. Method

2.1.1. Subjects

The subjects were 36 male Wistar rats, housed individually, with free access to water. Animals were 105 days old at the start of the experiment and experimentally naïve. Animals were weighed daily. The average ad libitum weight was 315 g (range: 250–361 g). The amount of food was gradually reduced over days until each animal reached 85% of its ad libitum weight. This level of deprivation was maintained throughout the experiment by administering the appropriate amount of food at least 20 min after the end of the daily trial. Animals were kept in a daily light–dark cycle of 12 h (lights on at 07:00 h). The housing and testing rooms were maintained at a constant temperature (around 22 °C) and humidity (around 60–70%).

2.1.2. Apparatus

Rats were trained in 5 conditioning boxes (MED Associates, Fairfax, VT). Each box measured 24.1 cm in length, 29.2 cm in width, and 21 cm in height. The floor was made of aluminum bars (0.4 cm in diameter, 1.1 cm apart from center to center). In the center of a lateral wall, there was a 5-cm hole, 3.5 cm deep, 1 cm above the floor level, through which a sipper tube could be manually introduced from the outside. When fully inserted, the sipper tube protruded 2 cm into the box. A photocell was located just in front of the tip of the sipper tube, inside this hole. Goal-tracking time (measured in 0.01-s units) was automatically recorded by a computer that measured the cumulative amount of time that the photocell was activated during the trial. Goal-tracking time correlates with fluid intake for the two sucrose concentrations used in this experiment [20] and it has been used concurrently with fluid intake yielding the same results [25,26]. Most dependent measures used to assess the cSNC effect, including goal-tracking time, lick frequency, fluid intake, rearing, and ambulation, are variable across experiments yielding somewhat different results. For example, lick frequency usually leads to higher terminal levels of licking in the 32% sucrose conditions than in the 4% sucrose condition, but in some cases the opposite has been reported (see p. 56 in [27]). Goal-tracking time appears to be sensitive to satiation effects induced by consumption of 32% sucrose and thus has a tendency to decrease toward the end of the trial [28], thus yielding in some cases lower terminal performance in the group exposed to the higher sucrose concentration. In a study involving seven sucrose concentrations, from 0 to 64%, under similar conditions to those used in the present experiments, the peak goal-tracking time was at the 16% value, with 32% and 64% yielding lower values [29]. Still, during the critical postshift trials, both groups receive the same 4% sucrose solution and, therefore, differential satiety during the trial cannot account for the cSNC effect. Each box was enclosed in a sound and light-attenuating cubicle equipped with a source of white noise and diffused house light. The sucrose solutions (w/v) were prepared by mixing 320 g or 40 g of commercial sugar in 1 L of tap water to obtain the 32% and 4% sucrose solutions used in the experiment.

2.1.3. Drug preparation

T propionate (purchased from Droguería Saporiti, Buenos Aires, Argentina) in a dose of 25 mg/kg (in a volume of 1.42 ml/kg, dissolved in olive oil) was administered (sc) 30 min before the start of training, on Trials 5 to 15. Control subjects received an equivalent dose of olive oil, the vehicle. The current parameters worked best in pilot studies that tested doses of 15, 20, and 25 mg/kg, administered 30 or 60 min before a test in an elevated plus maze.

2.1.4. Training procedure

Training started when animals were at the target weight. Animals were randomly assigned to four groups ($n=9$). Groups 32/T and 32/V had access to 32% sucrose during Trials 1–10 and then were downshifted to 4% sucrose during Trials 11–15. Groups 4/T and 4/V had access to 4% sucrose on Trials 1–15. A day before the first trial, each animal was

exposed to the assigned sucrose concentration in its cage. The water bottle was filled with 20 ml of the corresponding sucrose solution and made available for 40 min. This procedure was intended to attenuate taste neophobia. Animals in Groups 32/T and 4/T received a dose of T 30 min before each one of Trials 5 to 15. Animals in Groups 32/V and 4/V received an equal-volume vehicle injection 30 min before Trials 5 to 15. Animals were tested in squads of five. The order of the squads was randomized over the days, but each animal was always trained in the same box. A trial started with placing the animal in the conditioning box; the sipper tube was already inserted and available. The trial lasted 5 min from the first time the photocell was activated. When the trial ended, the animal was placed in its cage, taken to the housing room, and each conditioning box was swept with a damp towel. Goal-tracking times were subjected to analysis of variance with an alpha value set at the 0.05 level for all tests.

2.2. Results

The results are plotted in Fig. 1 and were analyzed with a Contrast (32%, 4% sucrose) \times Hormone (T, V) \times Trial (1–10) mixed model, with trial as a repeated-measure factor. Hormone was included as a factor in the analysis to determine whether there was an assignment bias before the downshift event. This analysis indicated significant effects for the contrast by trial interaction, $F(9, 288) = 2.15, p < 0.03$, significantly higher performance in the groups given access to 32% sucrose than to 4% sucrose, $F(1, 32) = 8.58, p < 0.01$, and also a significant increase of goal-tracking times across trials, $F(9, 288) = 64.47, p < 0.001$. All other effects were not significant, $F_s < 1.06, p_s > 0.40$. A second analysis based on the same factors, but restricted to Trials 5–10, when animals received the T treatment, provided similar results. There was a contrast by trial significant interaction, $F(5, 160) = 2.97, p < 0.02$, and a significant increase across trials, $F(5, 160) = 13.98, p < 0.001$. None of the other factors reached significance, $F_s < 2.99, p_s > 0.06$. Thus, there was no evidence in preshift trials that the assignment to the T vs. vehicle treatments was biased.

Fig. 1 also shows the results of the postshift trials. The T treatment attenuated cSNC, increasing the goal-tracking scores of downshifted rats without affecting the scores of unshifted controls. A Contrast \times Hormone \times Trial (11–15) analysis indicated a significant contrast by hormone interaction, $F(1, 32) = 4.17, p < 0.05$, and a significant difference between the T vs. vehicle treatments, $F(1, 32) = 13.58, p < 0.002$. Also significant were the contrast by trial interaction, $F(4, 128) = 28.22, p < 0.001$, the difference between downshifted and unshifted groups, $F(1, 32) = 26.51, p < 0.001$, and the change across trials, $F(4, 128) = 46.68, p < 0.001$. Other effects were not significant, $F_s < 1$.

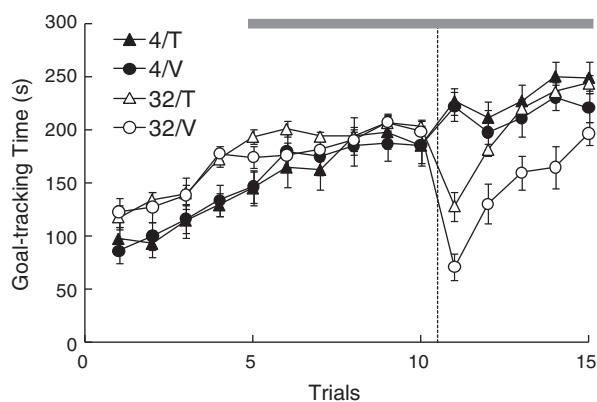


Fig. 1. Goal-tracking times of groups exposed to an incentive downshift (32) or unshifted controls (4), and treated with testosterone (T) or vehicle (V) in Experiment 1. The bar on the top marks the trials in which animals received T or V administration. The dashed vertical line marks the transition from preshift to postshift trials.

Inspection of Fig. 1 suggests that the triple interaction was not significant because recovery from cSNC progressed in parallel in 32/T and 32/V. However, 32/T obviously reaches the level of its contrast control, 4/T, much faster than 32/V reaches its own contrast control 4/V. To determine whether these trends were significant, further analyses were computed for each contrast comparison, on each postshift trial, with the following results. A comparison of Groups 32/V and 4/V indicated that the cSNC effect was significant on Trials 11–14, $F_s(1, 16) > 5.94, p_s < 0.03$, but not on Trial 15, $F(1, 16) = 2.55, p > 0.13$. However, Group 32/T was significantly below Group 4/T only on Trial 11, $F(1, 16) = 39.32, p < 0.001$; these groups were not significant, but marginally so, on Trial 12, $F(1, 16) = 4.30, p = 0.055$, and not significant on Trials 13–15, $F_s < 1$. Thus, statistically, T reduced the cSNC effect from lasting 4 trials (Trials 11–14 in vehicle groups) to lasting a single trial (Trial 11 in T groups). Furthermore, pair wise comparisons between the two downshifted groups, 32/T vs. 32/V, indicated significant differences in all 5 postshift trials, $F_s(1, 16) > 8.58, p_s < 0.02$, whereas comparisons between the unshifted controls, 4/T vs. 4/V, yielded only nonsignificant effects, $F_s(1, 16) < 2.65, p_s > 0.12$. Therefore, T reduced the cSNC effect whether measured in relation to an unshifted control also treated with T or in relation to a downshifted group treated with the vehicle.

3. Experiment 2

In the cSNC situation, animals are downshifted from a large to a nonzero incentive magnitude (e.g., 32% to 4% sucrose, as in Experiment 1). If, however, animals are downshifted to an empty tube and consummatory behavior directed at the sipper tube is the main measure, then the procedure would be equivalent to a typical extinction procedure in which the reinforcer is withheld. If the incentive in the present situation is sucrose, then the absence of sucrose in a situation equal to that of training can be technically described as consummatory extinction (cE). cE is an interesting situation because it tends to produce opposite outcomes relative to instrumental extinction (iE), that is, the situation in which the dependent variable is anticipatory behavior [30]. In rats, iE tends to be faster after training with continuous, rather than partial reinforcement and after training with a large, rather than small incentive [31], whereas cE follows the opposite trend [20]. Similarly, cE also tends to produce opposite outcomes in terms of consummatory behavior. For example, ethanol reduces cSNC by increasing goal-tracking times [32], but it enhances cE by reducing goal-tracking times [21]. Conversely, naloxone increases cSNC by reducing goal-tracking times, but it retards cE by increasing goal-tracking times [33,34]. If this opposite pattern generalizes to the effects of T, then one would predict that T-treated rats would exhibit lower goal-tracking times than vehicle-treated rats in the cE situation.

Experiment 2 also introduced an open field test as a way of validating the T treatment implemented in these experiments. If this treatment has anxiolytic-like properties, then it should replicate a known effect. For example, GDX reduces entries into the central area of an open field, whereas T replacement reverses this result [13]. Thus, the present T treatment was predicted to increase entries in the central area of an open field, without necessarily affecting general activity across the entire arena. Ambulation in the central area of the open field is usually considered an indicator of anxiety, whereas ambulation in the periphery of the arena is a measure of general activity [13,22,35].

3.1. Method

3.1.1. Subjects

The subjects were 13 male Wistar rats, about 120 days old, with previous experience with access to 4% sucrose (but no downshift experience). The average ad libitum weight was 443 g (range: 381–478 g). Other features were as described in Experiment 1.

3.1.2. Apparatus

The 5 conditioning boxes described in Experiment 1 were also used in the cE situation. An open field (120×120×30 cm, LxWxH), made of wood, and divided into 16 equal squares was used to assess activity. A light bulb (100 W) was suspended on top of the open field to provide illumination. A single trial was administered and it was recorded with a video camera (Sony DCR 308, 25× optical zoom). An entry into one of the 16 squares required the animal to have all four feet inside the square. Two dependent measures were recorded: an entry into any of the squares (total entries) and an entry into any of the 4 central squares (central entries).

3.1.3. Training procedure

Animals were randomly assigned to Groups T ($n = 7$) or V ($n = 6$). The same procedure used in Experiment 1 was implemented for the cE part. The plan was to administer 10 acquisition trials followed by 3 extinction trials. However, by mistake, T was not administered on Trial 5 as planned. To keep the number of T administrations before the downshift the same as in the previous experiment, animals received 11 acquisition trials. Thus, the first extinction trial (Trial 12) was preceded by 6 T injections, on Trials 6–11. Extinction trials were the same as acquisition trials, except that the sipper tube was empty. T was also administered before each extinction trial. The OF test was administered a day after the last extinction trial (Trial 14) and also after T administration (thus, there were a total of 10 T or vehicle injections). The OF test lasted 5 min. Each animal was tested once in the OF. All these tests were videotaped for later scoring. The observer who scored the videos was blind to the treatment of the subjects. A second observer scored 100% of the trials. Interobserver reliability, assessed in terms of Pearson's coefficient of correlation, yielded a significant positive correlation, $r(11) = 0.99, p < 0.01$.

3.2. Results

Fig. 2 shows the results of the cE test. A Hormone × Trial (1–11) analysis indicated only significant changes across trials, $F(10, 110) = 15.63, p < 0.001$. The hormone and hormone by trial interaction effects were not significant, $F_s < 1$. A similar analysis restricted to Trials 6–11, when animals received the hormonal treatment, yielded nonsignificant results for all three factors, $F_s < 1.28, p_s > 0.28$. Thus, consistent with the results of the previous experiment, there was no evidence that T had an effect on acquisition performance. Fig. 2 also shows the extinction performance. A Hormone × Trial (12–14) analysis revealed that goal-tracking times were significantly lower for Group T than for Group V, $F(1, 11) = 6.71, p < 0.03$. There was also a significant extinction effect, $F(2, 22) = 5.24, p < 0.02$, but the interaction effect was not significant,

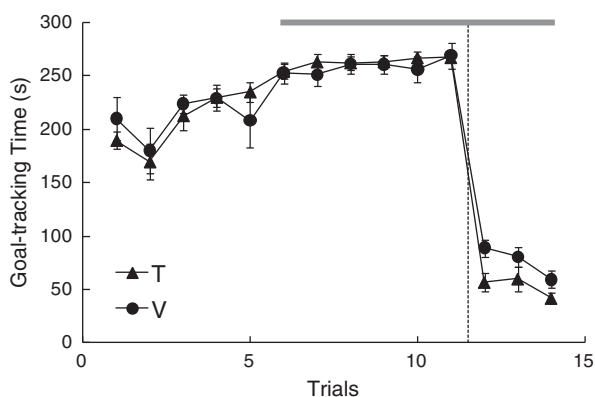


Fig. 2. Goal-tracking times of groups exposed to 32% sucrose on Trials 1–11 and then shifted to extinction on Trials 12–14 in Experiment 2. T: testosterone treatment. V: vehicle treatment. The horizontal line on the top marks the trials in which animals received T or V administration. The dashed vertical line marks the transition from acquisition to extinction trials.

$F < 1$. Consistent with the previous findings cited above, T had the opposite effect on cE relative to cSNC: T suppressed consummatory behavior in the former, but it enhanced it in the latter.

Fig. 3 displays the results of the open field test. Whereas T had no effect on overall activity, $F < 1$, it significantly increased activity in the central panels of the open field, $F(1, 11) = 5.22, p < 0.05$. This result demonstrates that the T treatment implemented in these experiments has similar effects to those reported in analogous experiments in other labs [13].

4. Experiment 3

Behaviors sensitive to T treatment are usually also sensitive to the removal of T by GDX, but in the opposite direction. The effects of GDX are also usually reversed by exogenous T administration. Central entries in the open field test are an example of this pattern of results [13]. It was expected that the cSNC effect would be enhanced by GDX. The two experiments reported here differ only in terms of the length of time from the removal of the testis and the first incentive downshift event on Trial 11. In Experiment 3a, there was a 28-day interval, whereas in Experiment 3b there was a 49-day interval. Given the absence of GDX effects obtained in Experiment 3a, the length of this interval was extended in Experiment 3b to ensure the depletion of circulating T at the time of the first incentive downshift episode. Circulating T can no longer be detected 4 weeks after GDX [36,37].

4.1. Method

4.1.1. Subjects and apparatus

The subjects were 39, 90-day old (Experiment 3a) and 32, 105-day old (Experiment 3b) male, experimentally naive Wistar rats. Average weights were 361 g (range: 293–417 g) in Experiment 3a, and 361 g (range: 300–433 g) in Experiment 3b. Maintenance conditions and conditioning boxes were as described in Experiment 1.

4.1.2. Surgical procedure

Rats were castrated 28 days (Experiment 3a) or 49 days (Experiment 3b) before the scheduled day for the first incentive downshift event (on Trial 11). Rats were anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg). For both GDX and Sham operations, the sac of the scrotum and the underlying tunica were incised. For the GDX operation, the vasa deferentia were ligated and the testes removed. The incision was sutured and the animals were observed daily for signs of infection. Antibiotics were administered as needed.

4.1.3. Training procedure

Approximately 7 days before the start of the experiments, animals were deprived to an 85% of their ad libitum weight, as done in previous

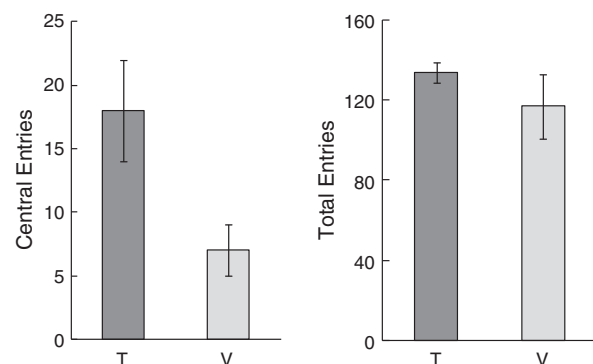


Fig. 3. Entries in the central area (left panel) or overall area (right panel) of the open field for animals treated with either testosterone (T) or vehicle (V). Results from Experiment 2.

experiments. Training in the cSNC situation was exactly as described in Experiment 1.

4.2. Results

Fig. 4 shows the results from Experiments 3a (top) and 3b (bottom). GDx had no appreciable effects on cSNC or on preshift performance. Contrast \times Surgery \times Trial analyses for each experiment produced similar results. For Trials 1–10, 32% sucrose groups performed significantly above 4% groups, $F_s > 9.55$, $p_s < 0.005$, and there was a significant increased of goal-tracking time scores across trials, $F_s > 19.67$, $p_s < 0.001$. All other effects were not significant, $F_s < 1.86$, $p_s > 0.05$. For Trials 11–15, there were significant trial by contrast interactions, $F_s > 8.99$, $p_s < 0.001$, contrast, $F_s > 9.44$, $p_s < 0.005$, and trial effects, $F_s > 30.19$, $p_s < 0.001$. The remaining factors, all of which involved the GDx manipulation, were not significant, $F_s < 1.39$, $p_s > 0.24$. As a whole, the evidence for the cSNC effect suggests that the effects of exogenous T administration and GDx are not symmetrical.

5. Experiment 4

This experiment had two goals. First, to determine the effects of GDx on cE, as a way of extending the results to another situation involving incentive downshift. Second, to determine whether GDx reduces central entries in the open field as described in experiments from other labs [13].

5.1. Method

The subjects were 15 male Wistar rats, about 6 months old, with previous experience in a similar cSNC experiment. Of these 15 animals, 10 were previously assigned to a 4% unshifted control condition

and 5 were previously assigned to a 32-to-4% downshift condition. All 15 rats had received 3 injections of T (same dose and route as in Experiment 1). Approximately 2 months intervened between the two experiments. During that period, animals were fed ad libitum. Animals were assigned to the surgery condition matching their previous experience as much as possible. Thus, Group GDx included 5 rats that previously experienced 4% sucrose and 3 rats previously assigned to 32% sucrose; similarly, Group sham had 5 and 2 rats previously assigned to 4% and 32% conditions, respectively. GDx and sham surgeries were performed as described in Experiment 3 and 38 days before the first extinction trial. After recovery from surgery, animals were deprived to 85% of the new ad libitum weights. The procedures for testing cE and open field activity were as described for Experiment 2, except that in this case there were 10 acquisition trials in cE testing.

5.2. Results

Fig. 5 shows the results of cE testing. A Surgery \times Trial (1–10) analysis indicated a significant trial effect, $F(9, 117) = 19.59$, $p < 0.001$, but nonsignificant surgery or interaction effects, $F_s < 1$. In extinction, there was a nonsignificant tendency for GDx rats to perform above sham rats, $F(1, 13) = 3.91$, $p = 0.069$. There was a significant extinction effect, $F(2, 26) = 4.50$, $p < 0.03$, but a nonsignificant interaction, $F < 1$. Because of the marginal surgery effect, group comparisons for each extinction trial were calculated; GDx animals were not significantly different from sham animals on any of the three extinction trials, $F_s(1, 13) < 1.98$, $p_s > 0.18$. Thus, the most conservative conclusion is that there was no evidence of a GDx effect on cE.

Fig. 6 shows the results of the open field test. As expected, GDx reduced entries in the central area of the open field, $F(1, 13) = 10.60$, $p < 0.007$, but did not affect overall activity in the open field, $F < 1$. This provides a behavioral validation of the GDx surgeries. However, the difference between the sham group of the present experiment and the vehicle group of Experiment 2, which were treated similarly, is somewhat disconcerting. There are two discrepancies between these experiments that might account for this difference. One is previous experience of the animals and the other are the results of the cE testing that preceded OF testing. Therefore, these results must be taken with caution.

6. General discussion

The administration of T 6 days before an incentive downshift and also before the downshift trials reduced the cSNC effect (Experiment 1) and enhanced cE (Experiment 2). These effects are consistent with previous results using pretraining and postshift T administration [23],

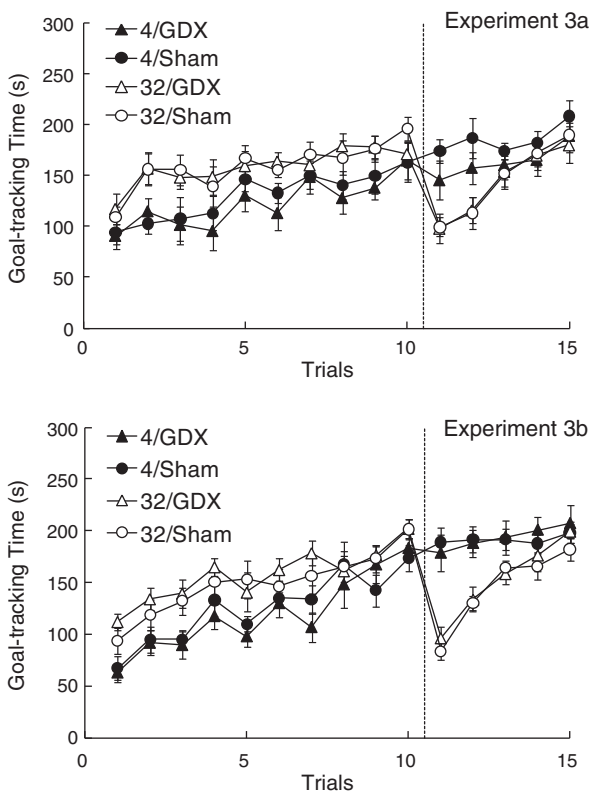


Fig. 4. Goal-tracking times of groups exposed to an incentive downshift (32) or unshifted controls (4), and gonadectomized (GDx) or control surgery (Sham) in Experiments 3a (top panel) and 3b (bottom panel). The dashed vertical line marks the transition from preshift to postshift trials.

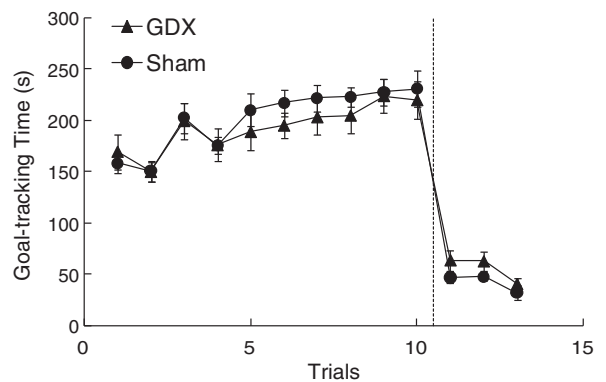


Fig. 5. Goal-tracking times of groups exposed to 32% sucrose on Trials 1–10 and then shifted to extinction on Trials 11–13 in Experiment 4. GDx: gonadectomy surgery. Sham: control surgery. The dashed vertical line marks the transition from acquisition to extinction trials.

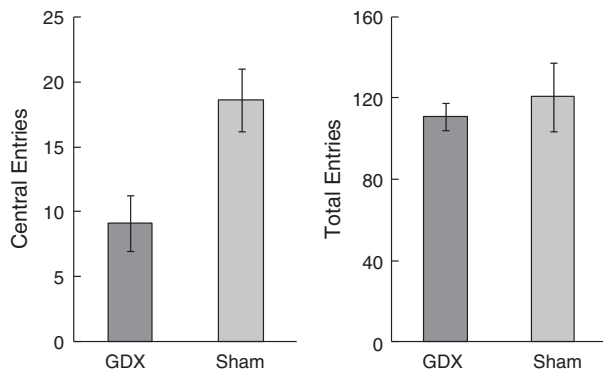


Fig. 6. Entries in the central area (left panel) or overall area (right panel) of the open field for animals that had received gonadectomy (GDX) or control surgery (Sham). Results from Experiment 4.

although the effects were stronger with the present treatment protocol. The effect of T on cSNC is consistent with an anxiolytic interpretation analogous to that used to account for the reduction of the cSNC effect after ejaculation [2], after ethanol administration [5,32], and after treatment with benzodiazepine anxiolytics [4,38]. However, anxiolytics and T have different effects when administered prior to the first downshift trial. Whereas anxiolytics are generally ineffective [27], T was clearly effective in reducing cSNC on the first downshift trial (see Fig. 1). A major procedural difference is, of course, that whereas anxiolytics are administered acutely on Trial 11 [3], T was administered daily during the previous 6 days before Trial 11, as well as prior to Trial 11. When T was administered before the start of training, as well as prior to each postshift trial, it also reduced the cSNC [23]. However, as mentioned in the introduction to Experiment 1, unpublished experiments showed that acute T administration prior to postshift trials (i.e., without earlier T exposure) has no measurable effects on cSNC.

The effect of T on cE is consistent with effects of magnitude and schedule of reinforcement [20] and the effects of ethanol [21]. The effect of T on both cSNC and cE cannot be attributed to drug-induced generalization decrement because administration began 6 days before the downshift. Similarly, these effects cannot be attributed to T's sensory or motor effects because the treatment did not have effects before the downshifts and, in the case of cSNC, there were no observable effects on the unshifted control group. Moreover, T- and vehicle-treated groups did not differ in overall activity in the open field. However, interpreting these effects as involving a reduction in anxiety is consistent with an increase in activity in the central area of the open field after T treatment (Experiment 2). Interestingly, anxiolytics such as chlordiazepoxide are notable by their failure to reduce cSNC during the first downshift trial, except when the animal has had prior downshift experience [30,38]. Unlike chlordiazepoxide, the contrast-reducing effects of the present T treatment were detected already during the first downshift trial. This effect suggest that in addition to downshift experience, it might be possible to open a window of opportunity allowing benzodiazepine anxiolytics to reduce the cSNC effect during the first downshift trial using the current treatment protocol with T.

Unpublished data (mentioned in the introduction to Experiment 1) from experiments involving T administration only in days when animals were experiencing the incentive downshift (acute administration), in both the cSNC and cE situations, produced no detectable effects. Moreover, acute T administration did not affect activity in the central area of the open field. One consequence of the chronic protocol used in the present experiments (see also [23]) is that animals had six opportunities to experience the physiological aftereffects of T administration while drinking sucrose solutions before the downshift event. Similar repeated exposures were used in other studies that reported an anxiolytic-like effect of T or of treatments that are known to induce T release, such as

ejaculation [7]. For example, in one experiment only males that ejaculated in 5 socio-sexual encounters with females were selected before testing them in the cSNC situation [2]. In another experiment mice were preexposed to eight 3-min sessions with an opposite-sex partner before testing them for anxiety in the elevated plus maze [11]. Therefore, it seems that T activation in advance of the anxiogenic event is needed before anxiolytic-like effects become evident.

These conclusions concerning the anxiolytic-like effects of testosterone in incentive downshift situations are tempered by the negative results obtained with GDX in Experiments 3–4. These negative results appear especially compelling in the case of cSNC, where there is no hint of a GDX effect on consummatory behavior. A floor effect seems unlikely given that goal-tracking times are actually increasing during postshift trials (see Figs. 4 and 5). These negative results must also be viewed in light of GDX's effects in the open field test. As expected, GDX reduced activity in central area activity in the open field, an outcome consistent with the interpretation that GDX increases anxiety levels, thus having the opposite effect to T administration. Thus, the addition and depletion of circulating T do not have symmetrical effects in incentive downshift situations. The failure of GDX to enhance cSNC suggests that other regulatory pathways are available that reduce the impact of the incentive downshift event. For example, blockage of opioid receptors via naloxone administration enhances the response to incentive downshifts [34,39,40]. Thus, the natural release of endogenous opioids during the downshift event may override the lower levels of circulating T induced by GDX.

Acknowledgments

The research reported here was supported by Grants PICT 25335/04, 38020/05 from Agencia de Promoción y Desarrollo, Secretaría de Ciencia y Técnica. All the experimental procedures reported in this article were approved by the Institutional Animal Ethics Committee, University of Buenos Aires. Address correspondence concerning this article to Nadia Justel, Laboratorio de Psicología Experimental y Aplicada, Instituto de Investigaciones Médicas (IDIM-CONICET), Combatientes de Malvinas 3150, 1427-Buenos Aires, Argentina (nadiajustel@gmail.com).

References

- [1] Fernández-Guasti A, Roldán-Roldán G, Saldívar A. Reduction in anxiety after ejaculation in the rat. *Behavioral Brain Research* 1989;32:23–9.
- [2] Freidin E, Kamenetzky G, Mustaca AE. Anxiolytic-like effect of ejaculation upon frustration. *Learn Behav* 2005;33:277–86.
- [3] Flaherty CF, Grigson PS, Lind S. Chlordiazepoxide and the moderation of the initial response to reward reduction. *Q J Exp Psychol* 1990;42B:87–105.
- [4] Mustaca AE, Bentosela M, Papini MR. Consummatory successive negative contrast in mice. *Learn Motiv* 2000;31:272–82.
- [5] Kamenetzky G, Mustaca AE, Papini MR. An analysis of the anxiolytic effects of ethanol on consummatory successive negative contrast. *Av Psicol Latinoam* 2008;26:135–44.
- [6] Edinger K, Frye C. Sexual experience of male rats influences anxiety-like behavior and androgen levels. *Physiol Behav* 2007;92:443–53.
- [7] Kamel F, Mock EJ, Wright WW, Frankel AI. Alterations in plasma concentrations of T, LH, and prolactin associated with mating in the male rat. *Horm Behav* 1975;6:277–88.
- [8] Harding CF, Feder HH. Relation between individual differences in sexual behavior and plasma T levels in the guinea pig. *Endocrinology* 1976;98:1198–205.
- [9] Herndon JG, Turner JJ, Collins DC. Ejaculation is important for mating-induced T increases in male rhesus monkeys. *Physiol Behav* 1981;27:873–7.
- [10] Bing O, Heilig M, Kakoulidis P, Sundblad C, Wiklund L, Eriksson E. High doses of testosterone increase anticonflict behavior in rat. *Eur Neuropsychopharm* 1998;8:321–3.
- [11] Aikey J, Nyby J, Anmuth D, James P. Testosterone rapidly reduces anxiety in male house mice (*Mus musculus*). *Horm Behav* 2002;42:448–60.
- [12] Bitran D, Kellog C, Hilvers R. Treatment with an anabolic-androgenic steroid affects anxiety-related behavior and alters the sensitivity of cortical GABA_A receptors in the rat. *Horm Behav* 1993;27:568–83.
- [13] Edinger K, Frye C. Testosterone's analgesic, anxiolytic, and cognitive-enhancing effects may be due in part to actions of its 5 α -reduced metabolites in the hippocampus. *Behav Neurosci* 2004;118:1352–64.
- [14] Fernández-Guasti A, Martínez-Mota L. Orchidectomy sensitizes male rats to the action of diazepam on burying behavior latency: role of testosterone. *Pharmacol Biochem Behav* 2003;75:473–9.

- [15] Edinger K, Frye C. Testosterone's anti-anxiety and analgesic effects may be due in part to actions of its 5- α -reduced metabolites in the hippocampus. *Psychoneuroendocrinology* 2005;30:418–30.
- [16] Edinger K, Frye C. Intrahippocampal administration of an androgen receptor antagonist, flutamide, can increase anxiety-like behavior in intact and DHT-replaced male rats. *Horm Behav* 2006;50:216–22.
- [17] Fernández-Guasti A, Martínez-Mota L. Anxiolytic-like actions of testosterone in the burying behavior test: Role of androgen and GABA-benzodiazepine receptors. *Psychoneuroendocrinology* 2005;30:762–70.
- [18] Frye C, Edinger K. Testosterone's metabolism in the hippocampus may mediate its anti-anxiety effects in male rats. *Pharmacol Biochem Behav* 2004;78:473–81.
- [19] Svensson A, Soderpalm B, Engel J. Gonadectomy enhances shock-induced behavioral inhibition in adult male rats: implications for impulsive behavior. *Pharmacol Biochem Behav* 2000;65:731–6.
- [20] Mustaca AE, Freidin E, Papini MR. Extinction of consummatory behavior in rats. *Int J Comp Psychol* 2002;1:1–10.
- [21] Kamenetzky G, Mustaca AE, Pedrón V, Cuenya L, Papini MR. Ethanol facilitates consummatory extinction. *Behav Processes* 2009;82:352–4.
- [22] Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur J Pharmacol* 2003;463:3–33.
- [23] Justel N, Ruetti E, Mustaca AE, Papini MR. Effects of pretraining treatment with testosterone on successive and anticipatory negative contrast. *Physiol Behav* 2012;105:933–7.
- [24] Flaherty CF, Coppotelli C, Potaki J. Effect of chlordiazepoxide on the response to repeated reductions in sucrose concentration in free-fed rats. *Physiol Behav* 1996;60:1291–8.
- [25] Papini MR, Mustaca AE, Bitterman ME. Successive negative contrast in the consummatory responding of didelphid marsupials. *Anim Learn Behav* 1988;16:53–7.
- [26] Riley EA, Dunlap WP. Successive negative contrast as a function of deprivation condition following shifts in sucrose concentration. *Am J Psychol* 1979;92:59–70.
- [27] Flaherty CF. *Incentive relativity*. Cambridge, UK: Cambridge University Press; 1996.
- [28] Pellegrini S, Muzio RN, Mustaca AE, Papini MR. Successive negative contrast after partial reinforcement in the consummatory behavior of rats. *Learn Motiv* 2004;35:303–21.
- [29] Papini MR, Pellegrini S. Scaling relative incentive value in consummatory behavior. *Learn Motiv* 2006;37:357–78.
- [30] Mustaca AE, Bentosela M, Ruetti E, Kamenetzky G, Cuenya L, Justel N, et al. Similitudes y discrepancias en dos modelos animales de frustración. In: Richaud de Minzi MC, Moreno E, editors. *Recientes desarrollos iberoamericanos en investigación en ciencias del comportamiento*, Vol. 2. Buenos Aires, Argentina: CIIPME-CONICET; 2009. p. 921–40.
- [31] Wagner A. Effects of amount and percentage of reinforcement and number of acquisition trials on conditioning and extinction. *J Exp Psychol* 1961;62:234–42.
- [32] Becker HC, Flaherty CF. Influence of ethanol on contrast in consummatory behaviour. *Psychopharmacology* 1982;77:253–8.
- [33] Norris JN, Daniel AM, Papini MR. Spontaneous recovery of consummatory behavior, but not of consummatory successive negative contrast. *Learn Motiv* 2008;39:296–312.
- [34] Pellegrini S, Wood MD, Daniel AM, Papini MR. Opioid receptors modulate recovery from consummatory successive negative contrast. *Behav Brain Res* 2005;164:239–49.
- [35] Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods* 1985;14:149–67.
- [36] Edinger K, Lee B, Frye C. Mnemonic effects of testosterone and its 5 α -reduced metabolites in the conditioned fear and inhibitory avoidance tasks. *Pharmacol Biochem Behav* 2004;78:559–68.
- [37] Frye C, Edinger K, Seliga A, Wawrzycki J. 5 α -reduced androgens may have actions in the hippocampus to enhance cognitive performance of male rats. *Psychoneuroendocrinology* 2004;29:1019–27.
- [38] Flaherty CF, Grigson PS, Rowan GA. Chlordiazepoxide and the determinants of contrast. *Anim Learn Behav* 1986;14:315–21.
- [39] Daniel AM, Ortega LA, Papini MR. Role of the opioid system in incentive downshift situations. *Neurobiol Learn Mem* 2009;92:439–50.
- [40] Norris JN, Perez-Acosta AM, Ortega LA, Papini MR. Naloxone facilitates appetitive extinction and eliminates escape from frustration. *Pharmacol Biochem Behav* 2009;94:81–7.