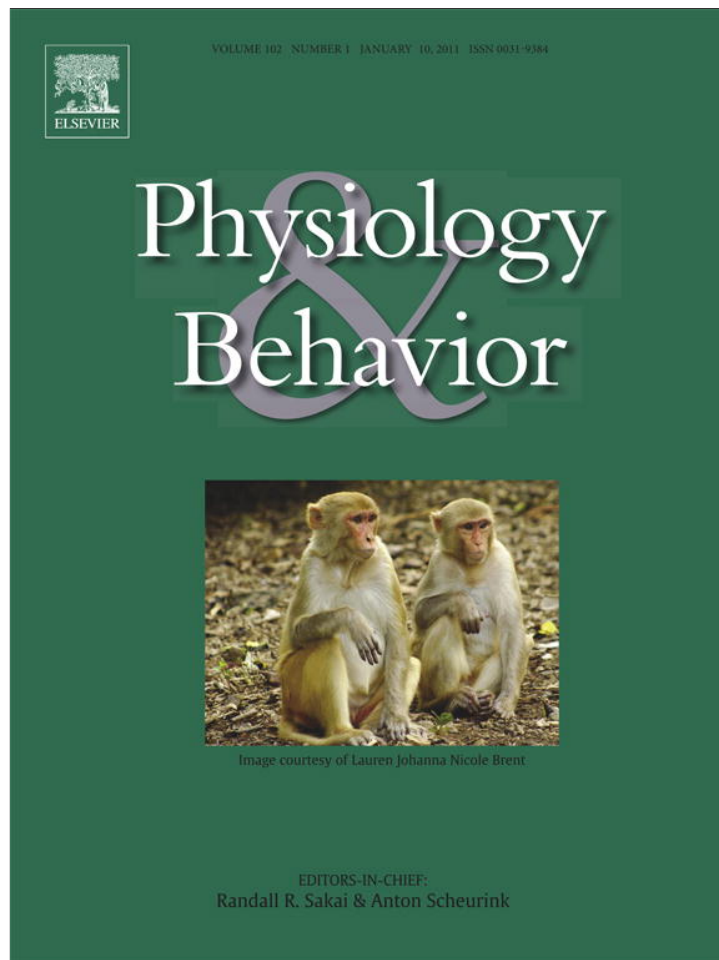


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Effects of pretraining treatment with testosterone on successive and anticipatory negative contrast

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ABSTRACT

Previous research indicated that the suppression of consummatory behavior that follows incentive downshift in male rats is attenuated by testosterone (T) administration during training. The present experiments were designed to assess the role of pretraining T administration on two incentive contrast situations in consummatory behavior: successive negative contrast (cSNC) and anticipatory negative contrast (cANC). In cSNC (Experiment 1), a downshift from 32% to 4% sucrose leads to behavioral suppression relative to an unshifted, 4% sucrose condition (the cSNC effect). Pretraining T administration enhanced consummatory behavior directed at 4% sucrose, without affecting behavior directed at 32% sucrose. This effect obscured a reduction in the cSNC effect by the T treatment that was only detected when a proportional measure of behavior was used. In cANC (Experiment 2), groups received access to two bottles per day separated by a short midtrial interval. Consumption of 4% sucrose is suppressed when the second bottle offers 32% sucrose, relative to 4% sucrose (the cANC effect). Pretraining T did not affect the cANC effect, known to be insensitive to treatment with anxiolytics. These results suggest an anxiolytic-like effect of testosterone in adjustment to incentive downshifts.

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1. Introduction

Rats exposed to a downshift in sucrose concentration display less consummatory behavior than rats exposed only to the lower sucrose concentration. This phenomenon, called consummatory successive negative contrast (cSNC), is modulated by anxiolytic, opioid, and cannabinoid drugs [1–6]. cSNC is also influenced by testosterone (T) treatment. In one experiment [7], rats were assigned to either 32% or 4% sucrose on Trials 1–10 and then all rats received 4% sucrose for Trials 11–15 (half downshifted from 32% to 4% sucrose and the rest unshifted controls). T (or vehicle) treatment started before Trial 5 and continued every day to Trial 15. T attenuated cSNC and led to a faster recovery to unshifted levels of consummatory behavior; however, T affected performance neither before the downshift nor in unshifted controls, implying that the effect was specific to the downshift event. The same treatment also resulted in an increase in activity in the central area of an open field [7]. These effects on cSNC and open field tests were consistent with the reduction in cSNC reported after ejaculation [8], which is known to result in a surge in endogenous T release [9]. All together, these effects can be interpreted as arising from the anxiolytic-like effects of T on behavior.

The anxiolytic effects of T treatment interact with the mode of administration of such treatment. For example, unpublished data from

experiments involving T administration only in days when animals were experiencing the incentive downshift or open-field testing (acute administration) produced no detectable effects in either case. One consequence of the chronic protocol [7] is that animals had six opportunities to experience the physiological aftereffects of T administration while drinking sucrose solutions before they were exposed to the incentive 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 [9]. For example, Freidin et al. [8] selected only male rats that ejaculated in 5 socio-sexual encounters with females before testing them in the cSNC situation. In an experiment with mice, [10] subjects were preexposed to eight 3-min sessions with an opposite-sex partner before testing them for anxiety in the elevated plus maze. Therefore, it seems that repeated T activation, whether directly by administering T or indirectly by exposing animals to situations that induce T release [8–10], in advance of the anxiogenic event is needed before anxiolytic-like effects can be detected behaviorally.

The aim of the present experiments is to understand the role played by the steroid hormone T in situations involving negative emotions induced by incentive devaluations. Previous research suggests that the timing of hormonal treatment relative to behavioral testing can determine the experimental outcome. Therefore, the present experiments explore the effects of pretraining T treatment on two situations involving incentive comparisons: cSNC (Experiment 1) and consummatory anticipatory negative contrast (cANC; Experiment 2). As reviewed above, cSNC involves emotional activation. However,

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the cANC situation is known to be less vulnerable to anxiolytic treatments [11], although it involves the same incentives used in the cSNC situation.

2. Experiment 1

2.1. Method

2.1.1. Subjects

The subjects were 31 male Wistar rats, experimentally naïve, and housed individually with free access to water throughout the experiment. Animals were 100 days old at the start of the experiment. Animals were weighed daily. The average ad libitum weight was 314 g (range: 270–367 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 [12]. 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) before the start of training during 6 consecutive days immediately before Trial 1. Moreover, the drug was also administered before Trials 11–15, 30 min before the start of the trial. Control subjects received an equivalent dose of olive oil, the vehicle. The dose and administration procedure were tested in preliminary unpublished experiments. The present dose was chosen because pilot research showed it to be effective in modulating consummatory behavior.

2.1.4. Training procedure

Once animals reached the target deprivation weight, they received 6 daily injections (T or vehicle). Training started a day after the sixth injection. Animals were randomly assigned to four groups ($n=8$, except for 4/V $n=7$). 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 for 6 consecutive days before Trial 1 and also 30 min before each postshift trial (Trials 11–15). Animals in Groups 32/V and 4/V received an equal-volume vehicle injection according to the same schedule. 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. As far as possible, each conditioning box was used to train animals assigned to each of the conditions of the experiment. 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. Preshift data 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. The analysis indicated significant main effects of Hormone, $F(1, 27) = 5.11, p < 0.04$, Contrast, $F(1, 27) = 12.71, p < 0.002$, and Trials $F(9, 243) = 32.86, p < 0.001$. Also significant were three second-order interactions: Contrast by Hormone, $F(1, 27) = 5.53, p < 0.03$, Contrast by Trial, $F(9, 243) = 3.11, p < 0.002$, and Hormone by Trial, $F(9, 243) = 2.6, p < 0.008$. There was also a significant Contrast by Hormone by Trial three-way interaction, $F(9, 243) = 3.18, p < 0.001$.

To elucidate the triple interaction, LSD pair wise analyses with the error term from the main analysis were computed with the following results. First, a comparison of unshifted groups differing in hormonal treatment (Groups 4/T vs. 4/V), indicated significantly different goal-tracking times on Trials 4–10, $F_s(1, 27) > 4.77, p_s < 0.04$, but not on Trials 1–3, $F(1, 27) < 3.76, p_s > 0.06$. Downshifted groups with different hormonal treatments (Groups 32/T vs. 32/V) were not different in any of the trials, $F_s < 1, p_s > 0.46$. Second, vehicle groups receiving different contrast treatment (Groups 32/V vs. 4/V) differed on Trials 2–4 and 6–9, $F_s(1, 27) > 8.75, p_s < 0.006$; these groups were not significantly different on the remaining trials, $F_s(1, 27) < 3.86, p_s > 0.06$. T-treated groups receiving different contrast treatments (Groups 32/T vs. 4/T) were different only on Trial 1, $F(1, 27) = 15.71, p < 0.001$; for other trials, $F_s(1, 27) < 2.98, p_s > 0.09$. Therefore, pretraining T treatment increased goal-tracking times for the animals given access to 4% sucrose, but had no effect on animals drinking 32% sucrose.

The effects of T on incentive downshift are also presented in Fig. 1 (see Trials 11–15). The T treatment seemed to have attenuated cSNC,

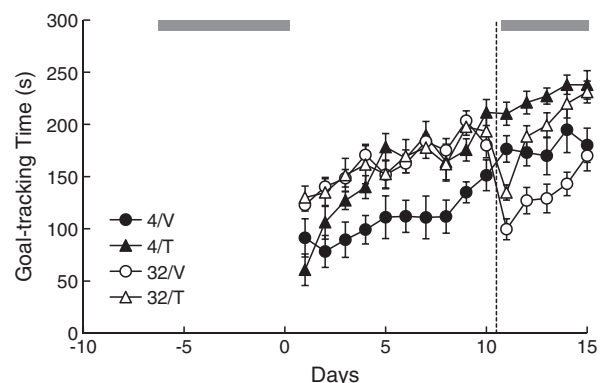


Fig. 1. Goal-tracking times (s) of groups exposed to an incentive downshift (32) or unshifted controls (4), and treated with testosterone (T) or vehicle (V) in Experiment 1. T or V were administered for 6 consecutive days before the start of training and also during postshift trials (marked by a gray line on top of the graph). T was administered at a dose of 25 mg/kg, in a volume of 1.42 ml/kg, dissolved in olive oil, administered subcutaneously. The dashed vertical line marks the transition from preshift to postshift trials.

increasing goal-tracking times in downshifted rats in comparison with downshifted vehicle animals. However, T also increased consumption of 4% sucrose in unshifted animals, as seen above in the analysis of preshift Trials 1–10. A Contrast \times Hormone \times Trial (11–15) analysis indicated significant main effects of Contrast, $F(1, 27) = 16.93, p < 0.001$, Hormone, $F(1, 27) = 33.71, p < 0.001$, and Trial, $F(4, 108) = 20.31, p < 0.001$. There was also a significant Contrast by Trial interaction, $F(4, 108) = 7.82, p < 0.001$. Other effects were not significant, $F_s < 1$, including the three-way interaction. The implication is, therefore, that T increased the performance of rats with access to 4% sucrose independently of the incentive downshift event and without affecting the recovery from the cSNC effect.

Fig. 1 suggests that not only pretraining T elevated goal-tracking times relative to vehicle controls, but there was also an indication that T accelerated recovery from cSNC. To minimize the potential confusion introduced by different terminal levels of preshift performance (i.e., Trial 10), the postshift goal-tracking time for each animal and each trial (x_{st}) was transformed according to the formula: Trial 10 / (Trial 10 + x_{st}). This transformation was used when nondeprived rats produced different terminal levels of preshift performance [13], as in the present experiment. The results are shown in Fig. 2. A Contrast \times Hormone \times Trial analysis revealed significant main effects for Contrast, $F(1, 27) = 25.28, p < 0.001$, and Trial, $F(4, 108) = 15.59, p < 0.001$. The effect for T was marginal, $F(1, 27) = 4.06, p < 0.06$. There were also significant second-order interactions: T by Contrast, $F(1, 27) = 14.46, p < 0.002$, and Contrast by Trial, $F(4, 108) = 8.15, p < 0.001$. Other interactions, including the three-way interaction, were not significant, $F < 1$. LSD pair wise test with the error term from the main analysis indicated that whereas vehicle treated groups exhibited significant contrast, $F(1, 27) = 17.53, p < 0.001$, downshifted and unshifted T-treated groups failed to differ, $F(1, 27) = 1.54, p > 0.22$. Therefore, eliminating group differences in preshift performance allowed the detection of an anxiolytic-like effect by the pretraining T treatment.

3. Experiment 2

If the present T treatment affects consummatory behavior directed at 4% sucrose independently of incentive downshift, then it should elevate such performance in other incentive contrast situations. cANC was selected because extensive pharmacological research indicates a different profile to that of cSNC [11]. For example, the benzodiazepine anxiolytic chlordiazepoxide (6, 12, and 20 mg/kg, ip), which reduces

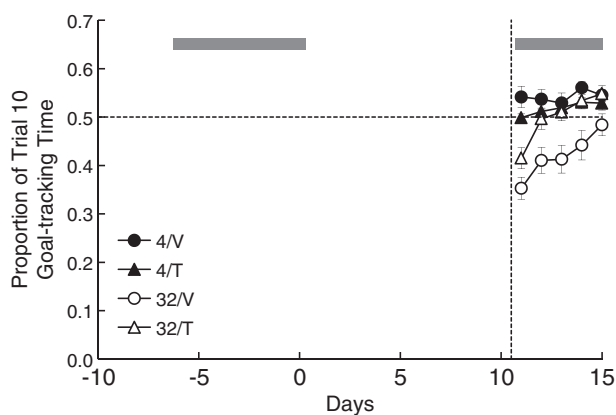


Fig. 2. Proportion of goal-tracking time on each postshift trial (Trials 11–15) relative to the scores of each animal on the last preshift trial (Trial 10), according to the formula $T10/(T10 + x)$, where x is the score of each animal on each postshift trial. The dashed horizontal line indicates the indifference point, 0.5, in which the score in postshift trials would equal that on Trial 10. The dashed vertical line and gray lines are as in Fig. 1. T: testosterone. V: vehicle.

cSNC, has no effect on cANC [14]. On the basis of results like these, Flaherty [11] argued that cANC, unlike cSNC, “has nothing in common with animal models of anxiety.”

In cANC, each day the rat is exposed to two trials separated by a short midtrial interval. In the experimental group (4–32), animals have access to 4% sucrose followed by access to 32% sucrose each day. In the control group (4–4), animals have access to 4% sucrose followed again by access to 4% sucrose each day. cANC is observed when there is lower consummatory responding in the first bottle in Group 4–32 than in Group 4–4, across days. Thus, anticipation of 32% sucrose suppresses performance directed at 4% sucrose. cANC is usually interpreted as a special case of Pavlovian conditioning in which the initial trial acts as a conditioned stimulus that signals the presentation of the second trial, which could be viewed as the unconditioned stimulus [11]. Therefore, suppression of consummatory behavior relates to the anticipation of a preferred sucrose solution, rather than to the anticipation of an aversive emotional state of frustration, as in cSNC.

The aim of this experiment was to determine whether rats receiving the same pretraining T treatment as in the previous experiment would show increased goal-tracking times directed at 4% sucrose relative to vehicle-treated rats.

3.1. Method

3.1.1. Subjects

The subjects were 32 male Wistar rats, housed individually, with free access to water. Animals were 104 days old at the start of the experiment and experimentally naïve. Animals were weighed daily. The average ad libitum weight was 318 g (range: 238–373 g). Other maintenance conditions, daily training times, and the conditioning boxes were as described for Experiment 1.

3.1.2. Training procedure

Once the animals reached the target weight, they received 6 daily injections (T or V). Training started a day after the sixth injection and consisted of seven daily trials in which rats had access to two solutions in a sequence. For all the rats, the first solution was 4% sucrose. This component (called first bottle) lasted 3 min, counting after the first interruption of the photocell. The second component (called second bottle) started after a midtrial interval of approximately 20 s. Animals were randomly assigned to one of four groups ($n = 8$). In the second bottle, two groups received access to 32% sucrose: 4–32/T, 4–32/V, whereas two groups received access to 4% sucrose: 4–4/T, and 4–4/V. The second bottle also lasted 3 min, starting with the first interruption of the photocell. The main dependent measure was the goal-tracking time (recorded as described in Experiment 1) during the first and second bottle. Six days before the start of the cANC training, animals received the administration of T or vehicle. Drugs and sucrose solutions were prepared as described in Experiment 1.

3.2. Results

One rat in Group 4–4/T failed to display consummatory behavior and was withdrawn from the experiment. Fig. 3 shows the results of this experiment. A cANC effect developed over trials in both groups, as seen in the top panel (first bottle). These results were subjected to a Contrast (4–32, 4–4) \times Hormone (T, V) \times Trial (1–7) analysis. First-bottle goal-tracking times for 4% sucrose were significantly lower in animals exposed to 32% sucrose in the second bottle than in animals exposed to 4% sucrose again, $F(1, 27) = 29.13, p < 0.001$. A significant main effect for Hormone was also found, $F(1, 27) = 11.93, p < 0.003$. There was also a main effect of Trials, $F(6, 162) = 17.61, p < 0.001$. Also significant were the Trial by Hormone interaction, $F(6, 162) = 2.29, p < 0.04$, and the Trial by Contrast interaction, $F(6, 162) = 8.72, p < 0.001$. However, the Contrast by Hormone interaction and the Contrast by Hormone by Trial three-way interaction

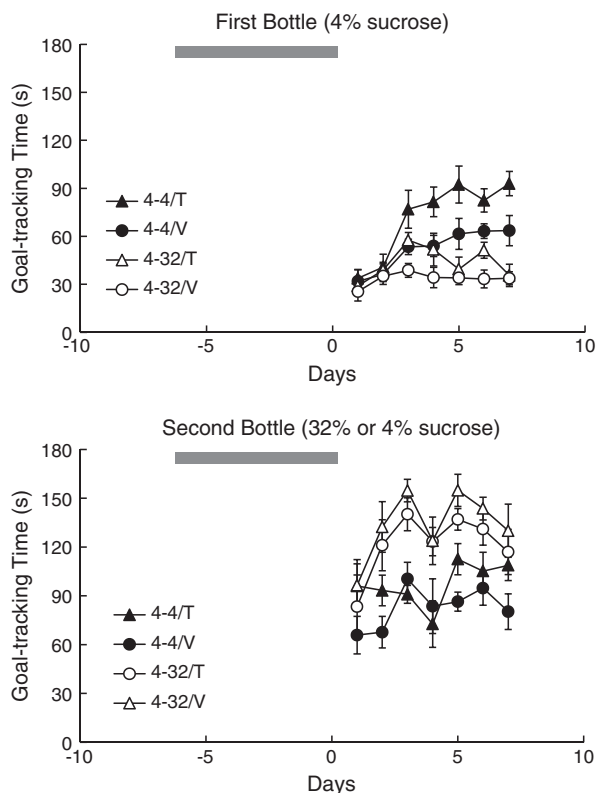


Fig. 3. Goal-tracking times of rats trained in an anticipatory negative contrast situation with two daily components in Experiment 2. In the first component (top panel), all rats had access to 4% sucrose. In the second component (bottom panel), rats in different groups had access either to the same 4% sucrose or to 32% sucrose in independent groups. Group labels refer to the sucrose concentration received in the second component, either 4% or 32% sucrose. Testosterone (T) or vehicle (V) was administered for 6 consecutive days before the start of training (marked by a gray line on top of each panel).

were both nonsignificant, $F_s < 1.54$, $p_s > 0.22$, thus providing no evidence that the effect of T treatment was specific to the cANC effect.

Fig. 3 (bottom panel) shows the results of the second bottle. Groups with access to 32% sucrose displayed higher goal-tracking times than groups with access to 4% sucrose. There was a hint of higher 4% sucrose scores in the group treated with T than in the vehicle group. A Contrast \times Hormone \times Trial analysis indicated the following results. There were significant main effects of Contrast, $F(1, 27) = 26.65$, $p < 0.001$, and Trial, $F(6, 162) = 7.07$, $p < 0.001$. The main effect of Hormone was not significant, $F < 1$. There was also a significant Contrast by Trial interaction, $F(6, 162) = 2.39$, $p < 0.04$. The hint mentioned above came closest to a significant value in terms of a Contrast by Hormone interaction, $F(1, 27) = 4.19$, $p = 0.051$. However, the Hormone by Trial interaction and the Contrast by Hormone by Trial interaction were not significant, $F_s < 1.05$, $p_s > 0.39$.

As shown in Fig. 3 (top panel), the initial levels of performance were similar across groups, so a transformation like that used in Experiment 1 was not warranted. However, T eventually affected behavior directed at the consumption of 4% sucrose. Therefore, we transformed the scores from each subject and trial in relation to the last training trial, Trial 7, using the same formula as before. A Contrast \times Hormone \times Trial analysis of first-bottle performance (Fig. 4, top panel) yielded significant main effects for Contrast, $F(1, 27) = 9.19$, $p < 0.006$, and Trial, $F(5, 135) = 14.83$, $p < 0.001$. There was also a significant second-order Contrast by Trial interaction, $F(5, 135) = 3.54$, $p < 0.006$. However, none of the effects involving T were significant, $F_s < 2.48$, $p_s > 0.12$. A similar analysis for second-bottle performance (Fig. 4, bottom panel) provided only a significant trial main effect

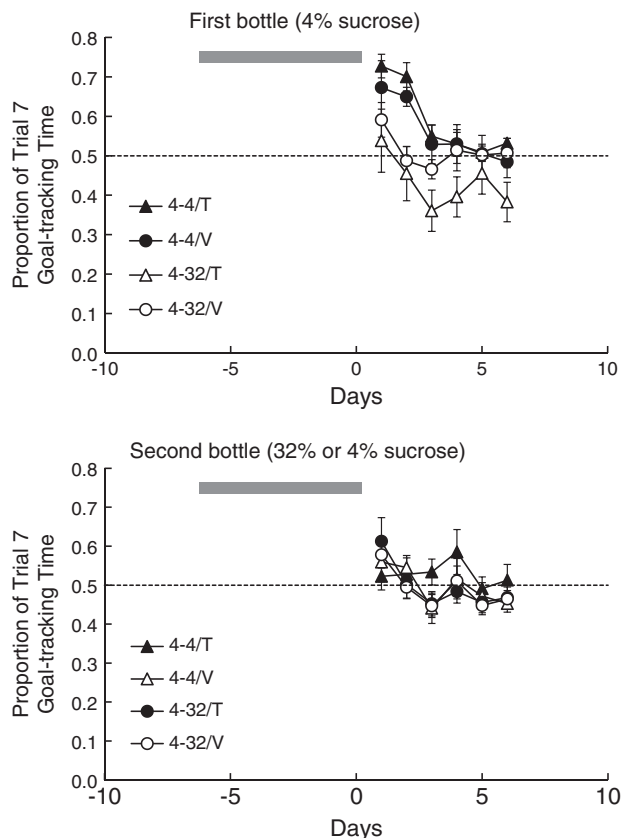


Fig. 4. Proportion of goal-tracking time on each trial (Trials 1–6) relative to the scores of each animal on the last trial (Trial 7), according to the formula $T7/(T7 + x)$, where x is the score of each animal on each trial. The top panel shows the results for the first bottle in each trial, whereas the bottom panel shows the results for the second bottle of each trial. The dashed horizontal line indicates the indifference point, 0.5, in which the score in each trial would equal that on Trial 7. The gray lines indicate the T (testosterone) or V (vehicle) pretraining treatments.

of Trial, $F(5, 135) = 9.72$, $p < 0.001$; other factors did not achieve significance, $F_s < 2.19$, $p_s > 0.05$. Therefore, the pretraining T treatment implemented in the present experiment had no detectable effect on cANC.

4. Discussion

Pretraining treatment with T facilitated consumption of 4% sucrose in two different situations involving consummatory negative contrast: successive and anticipatory. This facilitatory effect on 4% sucrose consumption was unrelated to the incentive contrast manipulation, unlike it was the case when T treatment was administered during the course of preshift-postshift trials in the cSNC situation [7]. In the latter case, T had an anxiolytic-like effect consistent with previous research showing that cSNC was reduced by prior ejaculatory activity [8]. This effect of T on consummatory behavior directed at the 4% sucrose solution obscured the anxiolytic-like effect of the treatment on contrast. Such effect was detected only transforming postshift data (Trials 11–15) to a proportion of the behavior recorded during the last preshift trial (Trial 10). This transformation has been previously used in experiments in which the manipulation produced different levels of preshift performance, as it is the case, for example, with food deprivation levels [11]. Under such conditions, it was clear that pretraining T attenuated the response to the incentive downshift manipulation relative to vehicle treatment, facilitating the recovery of goal-tracking times in downshifted rats.

Interestingly, such an effect of pretraining T was not observed in Experiment 2, in which a cANC design was used. Although cSNC

and cANC use the same sucrose concentrations, only cSNC seems to involve anxiety, a conclusion based on the selective effects of anxiolytic drugs, which do not influence behavior in anticipatory contrast situations [11]. It should be noted that animals in Experiment 1 received T administration both before training started and also during postshift trials. Thus, animals in Experiment 1 were exposed to a larger number of injections than animals in Experiment 2; T treatments were matched in terms of pretraining across experiments. Despite this limitation, the selectivity of the present T treatment reinforces the hypothesis that this hormone has an anxiolytic-like effect on cSNC [7].

This conclusion must be taken with caution because the effect was only detected in a proportional measure of behavior (see Fig. 2). There are at least two reasons why the anxiolytic-like effects of T were not as clearly detected with the present pretraining administration procedure. One possibility is that pretraining T enhances the palatability of sucrose thus leading to increased consumption. The lack of an effect with 32% sucrose may be explained either in terms of a response ceiling effect or by assuming that changes in palatability are less salient at relatively high sucrose concentrations (i.e., a palatability ceiling). However, there seems to be no available information on the effects of T treatment on sucrose palatability.

A second possibility is that pretraining T treatment enhances impulsivity, the tendency to invigorated responding in situations involving incentives. Available evidence indicates that T administration may increase impulsivity under some conditions [15–18]. For example, when male rats were evaluated in a punished-drinking situation the T-treated animals tolerated a higher number of electric shocks compared to vehicle-treated animals [16]. Similarly, T-treated rats show deficits in passive avoidance task [19], failing to inhibit behavior in a step-down situation. Again, the lack of an effect on animals consuming 32% sucrose may be explained in terms of a ceiling effect. The potential effects of pretraining T on taste palatability or impulsivity in the consummatory situations used in the present experiments deserve further attention.

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