CAN TESTOSTERONE INDUCE CONSUMMATORY SUCCESSIVE POSITIVE CONTRAST?

by

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Introduction

Basic emotions rely on the basic needs of organisms, such as feeding, drinking, thermoregulation, and reproduction. Emotions can be defined as states or psychological conditions that predispose the organism to act in a particular way. They likely evolved from basic mechanisms that confer animals the ability to avoid harm and seek resources (Paul, Hardind, & Mendl, 2005). Emotional responses can be expressed behaviorally when an animal avoids aversive consequences, looks for appetitive consequences, or when expectancies do not match actual outcomes (Crespi, 1942; Papini & Dudley, 1997; Papini, Wood, Daniel, & Norris, 2006). Emotional responses are especially strong (positive or negative) when predictive stimuli are accompanied by surprising outcomes, that is, when actual outcomes are significantly different from those expected on the basis of current signals (Papini & Dudley, 1997). Negative emotions, like frustration, anger, or rage, usually follow after environmental challenges. Positive emotions, like pleasure, euphoria, or relief, follow after environmental optimal conditions (Allport, 1921; Burgdorf & Panksepp, 2006). Some authors have proposed to emphasize emotional responses instead of emotions to avoid inferring internal events (Paul, Harding, & Mendl, 2005). Emotions have behavioral and physiological components. Cognitive factors, such as attention and memory, modulate the expression of emotional responses.

Physiology of Emotional Responses

Emotional responses have two kinds of elements: affective and cognitive. The affective element refers to the valence of the emotion, that is, whether this emotion is experienced as appetitive or aversive. This cognitive element refers to both the appraisal that can trigger the occurrence of particular emotions and the cognitive outputs that result from emotional states. Common behavioral reaction to stimuli that induce appetitive or aversive emotions are approach

and withdrawal/avoidance, respectively (Paul, Harding, & Mendl, 2005). Affective elements are regulated by lower brain areas, which in turn interact with other physiological systems (e.g., endocrine), to affect emotions (e.g., the increase of blood pressure after being exposed to a threatening situation). The midbrain, amygdala, hypothalamus, and brain stem are areas responsible for these processes (Panksepp, 2011). These structures are involved in reflexes which are relatively simple reactions to specific stimuli in the environment (Horn & Swanson, 2013). Each lower structure has a particular role to play.

The hypothalamus regulates the internal state of the organism, keeping the levels of some hormones below a certain physiological limit via negative feedback mechanisms. Such homeostasis is achieved through connections with the autonomic nervous system, the endocrine system, and some areas important for consummatory and reproductive behaviors (Brown, 1994; Horn & Swanson, 2013; Nelson, 1995). The amygdala plays a key role in detecting the affective components of the unconditioned and conditioned behavioral responses (LeDoux & Damasio, 2013). Reflexes build up more complex behaviors and are closely related to the affective portion of an emotion.

The cognitive elements of an emotion are regulated by more complex neural networks, which may differ depending on the emotion considered. In the case of fear conditioning, responses to fear-inducing stimuli are mediated by thalamo-cortical projections. Thalamic nuclei receive sensory inputs from the periphery and relay them to primary sensory areas in the neocortex and to the amygdala. The basolateral nucleus of the amygdala receives inputs from the thalamus and neocortex, and regulates emotional responses via output through its central nucleus by acting on caudal brain areas. The hippocampal formation participates in the storage of

acquired information related to the conditioning context and in situations involving time gaps (LeDoux & Damasio, 2013).

Neuroendocrinology of Emotion

The body has three different kinds of molecular messengers: neurotransmitters, hormones, and pheromones. (1) Neurotransmitters are released by neurons and travel short distances (in the nanometer order) to affect membrane receptors located in another neuron. (2) Hormones are molecular messengers released in the bloodstream to affect receptors located in other organs. Hormones are released by glands and by some brain areas (e.g., hypothalamus). They influence the probability of occurrence of several behaviors because they can affect membrane receptors inside the central nervous system. Hormones are usually controlled through negative feedback mechanisms. A high level of a hormone in the body releases inhibiting factors that reduce the production of that hormone. (3) Pheromones are molecular messengers released externally to affect other organisms (Nelson, 1995).

Hormones are regulated by the hypothalamus, which directly controls their release via nerve impulses to the posterior pituitary gland and indirectly via hormone releasing neurons located in the anterior part of this gland. The pituitary gland, in turn, affects the release of hormones in other glands (Horn & Swanson, 2013).

The neuronal control of hormone release depends on hypothalamic neurons. For example, magnocellular neurons of the paraventricular nucleus of the hypothalamus release oxytocin and vasopressin into the posterior pituitary gland. In mammals, these peptides regulate water balance and milk release. In the same nucleus, parvocellular neurons secrete peptides that travel through the blood stream to affect the release of a variety of hormones in the pituitary gland, including thyrotropin-releasing hormone, corticotropin-releasing hormone, gonadotropin-releasing

hormone, growth hormone-releasing hormone, prolactin releasing factor, and growth hormone release-inhibiting factor (Horn & Swanson, 2013).

The connection between hormones and emotional responses is highlighted by the following: (1) the hypothalamus interacts with other areas of the limbic system in order to regulate emotional reactions; and (2) hormones can act as neuromodulators and perhaps also as neurotransmitters in the brain (Holsboer & Ising, 2010; Nelson, 1995).

The hypothalamus is important because it integrates endocrine and behavioral responses. This area responds to the variations in neuromodulators in other areas of the limbic system, one of them being the amygdala. For instance, after selective lesions of the amygdala, autonomic responses that are controlled by the hypothalamus are also affected (Horn & Swanson, 2013).

Sexual Hormones and Emotion

Androgens and estrogens are the sex hormones of vertebrates. They are called sex hormones because they are dimorphic: while females tend to have higher concentrations of estrogens, males tend to have higher concentrations of androgens.

The biologically significant estrogens are estradiol, estrone, and estriol. Estrogens are produced in the ovaries and also in the adrenal gland. These hormones participate in the formation of corpus luteus in the genital duct, have functions in water and calcium metabolism; and they regulate sexual and parental behaviors in both sexes (Brown, 1994).

Androgens are principally produced in the interstitial cells of the testes (and in lesser amount in female ovaries) and in the adrenal gland; Sertoli cells, located in the seminiferous tubules in the testes, are the source of androgen-binding proteins that carry androgens through the blood. The most important androgen is testosterone (T); other androgens include androstenedione and dihydrotestosterone (DHT). Androgens have many functions, including

spermatogenesis, maintenance of the genital tract and the accessory sex organs (prostate, seminal vesicles, and bulbourethral glands), and supporting male secondary sex characters (e.g., pattern and density of body hair in humans, comb size in rooster). They are also important in the regulation of sexual, aggressive, and other social behaviors (Brown, 1994; Nelson, 1995).

Estrogens and androgens regulate the physiology and behavior of vertebrates via different mechanisms. First, these hormones act on brain structure early in life (prenatal and perinatal) shaping stable and, in some instances, dimorphic circuits and structures. These hormonal effects tend to be permanent and are usually called organizational effects. Second, these hormones can also act in a transient way in mature animals, regulating the physiology and behavior of the organism in a more dynamic way; these effects are usually called activational effects and they are conditional on the previous organizational effects (Nelson, 1995). For example, injections of androgens do not fully activate male sexual behavior rats if castration occurs when rats were less than 10 days old at the moment of the surgical procedure (Gerall, Hendricks, Johnsons & Bounds, 1967).

The organizational effects of T are established early in life; the activational effect usually implies intracellular receptors that are followed by gene expression in the brain. Some effects of T are produced by nongenomic mechanisms (Nyby, 2008). These physiological mechanisms affect the probability of occurrence of some behaviors by influencing the motivational and emotional states of the organism. In turn, emotional and motivational factors can modulate some of these hormonal actions (Nelson, 1995).

The physiological mechanisms by which T acts on the brain are diverse. These effects are important for proper sexual development, the production of behavioral traits, and morphological changes (Balthazart, Tlemçani, & Ball, 1996; Nelson, 1995). First, T can be reduced to some of

its metabolites such as DHT. Second, T can be converted to estrogens by an aromatization process. In the first case, T is converted to more potent androgens, through the action of reductase enzymes, and the final product binds to androgen receptors. In the second case, T is converted to estrogens by the action of the enzyme aromatase, and estrogens then bind to estrogen receptors to exert their influences (Nelson, 1995). Both effects can be organizational and activational in the brain. Androgen receptors and aromatase activity are found in several areas of the brain especially in the hippocampus and the limbic system (Roselli, Handa, & Resko, 1985, 1989)

Role of T on Positive and Negative Emotional Responses

T plays an important role in the expression of positive and negative emotions affecting the reactivity of the amygdala and the orbitofrontal cortex, and their interaction, to threatening faces and sexual stimuli (Van Wingen, Ossewaarde, Backstrom, Hermans, & Fernandez, 2011). For example, Hermans, Ramsey, and Van Honk (2008) found that individuals with high T and low cortisol displayed higher activation of the amygdala, hypothalamus, and orbitofrontal cortex to angry and happy faces than to neutral faces. Van Wingen, Mattern, Verkes, Buitelaar, and Fernández (2010) reported that participants receiving a single nasal T dose also had a reduced functional coupling of the left amygdala with the orbitofrontal cortex when performing an emotional face-matching task.

The activation of brain areas associated with negative emotions is related to the idea that T could act as an anxiolytic preventing the expression of these emotions and promoting the expression of positive emotions. Consistent with this, Hermans, Bos, Osserwaarde, Ramsey, Fernández, and van Honk (2010) found that exogenous T positively correlated with the activation of the ventral striatum that occurred when participants performed a monetary incentive

delay task. In this task, participants who self-reported a lower intrinsic motivation exhibited larger T-induced ventral striatum activation. In addition, high levels of T are associated with low levels of depression and anxiety in men (Clark & Henderson, 2003). One physiological explanation of these effects assumes that T metabolites potentiate GABA-mediated brain processes that reduce anxiety (Fernandez-Guasti & Martinez-Mota, 2005). However T can have anxiolytic-like actions without the involvement of its metabolites (Fernandez-Guasti & Martinez-Mota, 2005). T can also have rewarding properties by itself.

Wood, Johnson, Chu, Schad, and Self (2004) reported increased levels of operant behavior after infusion of T into the lateral ventricle, compared to vehicle controls. Also, Arnedo, Salvador, Martinez-Sanchis, & Gonzalez-Bono (2000) conditioned a place preference in mice pairing T with a least preferred compartment. They found that in animals that preferred the clear compartment, the administration of T associated with this place, increased the time spent there. Other studies suggest that T could be linked to the opioid system (Bodnar & Kest, 2010; Nyber & Hallberg, 2012). The opioid system plays a key role in pain and rewarding processes (Grossmann, Diez-Guerr, Mansfield, & Dyer, 1987; Limonta, Dondi, Maggi, & Piva, 1991; McHenry, Carrier, Hull, & Kabbaj, 2014). Consistent with this idea, nandrolone decanoate, an anabolic androgenic steroid, increased the expression of opioid receptors in the hypothalamus, striatum, and periaqueductal gray (Johansson, Hallberg, Kindlundh, & Nyberg, 2000).

Incentive Upshift as a Model to Study Positive Emotions

A variety of animal models are useful to study emotional responses, but most research is designed to study negative emotions. The open field, for example, is used to induce anxiety-related behaviors and thus evaluate drug effects. Rats placed in the center of this enclosed arena

tend to stay close to the walls, avoiding the central area. This avoidance of an open area is used as a model of agoraphobia (Prut & Belzung, 2003).

Successive positive contrast (SPC), largely unexplored, is one potential area to study positive emotions. In the basic procedure, subjects have exposure to a small reward for several trials and then to a larger reward in subsequent trials (incentive upshift). When it occurs, SPC involves responding above that of an unshifted control always exposed to the larger reward (Flaherty, 1996). Such increased responding can be interpreted as reflecting a positive emotional state (e.g., elation; Crespi, 1942). SPC is unreliable in rats, which may lead to few studies using this procedure. The opposite procedure, incentive downshift (i.e., successive negative contrast, SNC), occurs more reliably and is extensively studied in connection to negative emotions related to frustration and anxiety (Flaherty, 1996; Papini, Wood, Daniel, & Norris, 2006). In SNC, animals have an exposure to a large reward for several trials and then to a small reward for subsequent trials. Typically, incentive downshifts lead to decrements in behavior, relative to unshifted controls, always receiving the small reward.

T affects SNC in several ways. For example, Justel, Ruetti, Bentosela, Mustaca, and Papini (2012a) injected T intracutaneously 30 min before each of the last five sessions with the large reward and with the subsequent small reward. T-treated rats exhibited a less pronounced SNC effect and recovered their response faster than vehicle-treated animals. This effect was also present when the animals were exposed to T before a similar behavioral training, but did not affect downshift situations previously reported to be insensitive to anxyolitics (Justel, Ruetti, Mustaca, & Papini, 2012b). Based on the effects of T on positive emotions reviewed above (e.g., place preference, rewarding self-administration, etc.), I hypothesize that this anxiolytic-like action of T on SNC is based on the induction of a positive emotion that counteracts the negative

emotion (frustration) caused by the incentive downshift. If this were correct, then it follows that T could be used to induce SPC in a situation in which animals respond to the incentive upshift without yielding a positive contrast effect.

There are two procedures to study incentive upshift: instrumental and consummatory. Instrumental successive positive contrast (iSPC) occurs if the anticipatory behavior is stronger, faster, or of greater strength than the behavior of animals exposed only to the large reward. Consummatory successive positive contrast (cSPC) occurs if consumption of the large reward is greater in upshifted animals than in unshifted controls always exposed to the large reward (Flaherty, 1996).

Crespi (1942) was interested in the effects of shifts of the magnitude of an incentive in the level of runway performance in the rat. He compared rats receiving 16 units of the incentive with other rats receiving 64 or 256 incentive units (1 unit was equivalent to 0.02 g) in terms of the speed of running in a runway. In another experiment, after rats received 16 incentive units for the acquisition of the running response, two thirds received 1 or 4 units, and then again 16 units, he found evidence of positive contrast since when the incentive was shifted from small to a large magnitude, rats increased the average speed in comparison to the animals that were always exposed to 16 units. Despite the fact that the animals increased their speed after the upward shift, the acceleration was lower than after a downward shift; that is, a shift from a large to a small reward led to an abrupt change in behavior. He posed that this asymmetry reflected a "greater difficulty of the rats in increasing their speeds when they are already approaching their physiological limit (Crespi, 1942, p. 491).

The problem of a physiological limit, or ceiling effect, was examined by Mellgren (1971). To reduce the speed of running of unshifted controls, Mellgren delayed the reward by 20

s. Using this procedure, he found evidence of positive contrast in animals exposed to 24, 48, and 72 preshift trials. Moreover, a more pronounced iSPC was found in the section of the runway closest to the goal box. Flaherty, Becker, and Checke (1983), using a consummatory paradigm, also investigated the role of the ceiling effect. In one of their experiments, one group of rats was exposed to a single alternation of sucrose concentrations across days (32% or 4% sucrose solution), while another group of rats was exposed to a 32% sucrose solution during the entire experiment. Although Flaherty et al. found significant differences in the first three upshifts between upshifted and unshifted groups (i.e., cSPC), this effect dissipated as the unshifted group increased its level of consummatory behavior. This result is consistent with a ceiling effect interpretation.

Pankseep and Trowill (1970) found evidence of iSPC when rats where responding to obtain rewarding electrical stimulation in the brain. Animals had a higher level of responding (or self-stimulation) when the initial current level was reinstated in successive replications. In this case the stimulation was in the medial forebrain bundle, an area that is part of mesolimbic reward system and carries dopaminergic information between the ventral tegmentum and nucleus accumbens. T could be a good inducer of positive contrast because androgen receptors were found in the mesolimbic reward system (Rosen, O'Bryant, Matthews, Zacharewski, & Wade, 2002). Also, having androgen receptors in this area has been found to be critical for the occurrence of certain behaviors. For example, when this system is affected, sexual behavior in males, but not in females, is disrupted (Hitt, Hendricks, Ginsberg, & Lewis, 1970). In addition, implants of T in the ventral tegmentum and medial preoptic area affect the occurrence of sexual behaviors (Sipos & Nyby, 1996) and self-administration of T into the lateral ventricles is also reinforcing. Together these data suggest that T could induce positive contrast because it is

involved in the regulation of forebrain areas that play a role in reward mechanisms and also have previously been shown to be associated to the occurrence of iSPC (Pankseep and Trowill, 1971).

Previous Research in our Laboratory

As stated before, cSPC is difficult to obtain: there is scanty evidence under standard circumstances (Experiment 2, Pecoraro, Timberlake, & Tinsley, 1999), positive evidence after previous experience with an incentive downshift (Maxwell, Calef, Murray, Shepard, & Norville, 1976), and positive evidence when the ceiling effect is reduced (Flaherty et al., 1983; Mellgren, 1971). The report by Pankseep and Trowill (1971) showed that it can be obtained by stimulating brain areas involved in rewarding mechanisms. T has rewarding properties by itself (Wood et al., 2004) and it has also been involved in the expression of positive emotions (Hermans et. al., 2008). The attenuation of cSNC after chronic administration of T suggests anxiolytic-like properties (Justel et al., 2012a, 2012b) that could also play a role in the expression of SPC. Several experiments done in this laboratory have helped discard some procedures and also identify potential conditions that will be used to test the hypothesis that T induces cSPC.

Single alternation with 48%, 32%, and 4% sucrose solutions. As suggested by Flaherty et al. (1983), a way to address the ceiling effect that obscures the cSPC effect is to expose the animals to single alternation between the low reward and high reward. Flaherty et al. exposed, a group of animals to a concentration of 32% sucrose solution throughout the entire experiment (unshifted control group) and another group to single alternations between 4% and 32% sucrose solution (experimental group). The observed variable in all the experiments described below was the goal tracking time, the time the animal spent in contact with the sipper tube. Figure 1 shows the results. We did not observed cSPC, suggesting that either the ceiling

effect was still an obscuring factor or the discrepancy between 32% and 4% was not enough for obtaining cSPC.

The second possibility led to Experiment 2, in which single alternations were scheduled between 4% and 48% sucrose solutions. The animals in the control group had exposure to a 48% during the entire experiment. During the first trial, as Figure 2 shows, there was no evidence of cSPC ruling out the possibility that the discrepancy between the small and the large reward (4% - 32% vs. 4% - 48%) in Experiment 1 was the obscuring factor and giving more support to the idea that the ceiling effect, or the control animals having really high goal tracking scores, is still interfering with the emergence of cSPC. Notice also that whereas the 32-4 alternation yielded patterns of goal tracking of consummatory behavior (Figure 1), the 48-4 alternation produced no clear evidence of an alternating pattern of behavior (Figure 2). The reasons for this lack of behavioral alternation are unclear.

Incentive upshifts with lower sucrose concentrations. Together both experiments suggest a different approach to the concentrations of sucrose to which the animals are exposed. In Experiment 3, animals had exposure to four different sucrose concentrations for 6 sessions (12%, 2%, 1%, and 0.5%). All the animals had then exposure to a 12% sucrose during the four postshift trials. In this experiment, upshifted animals drank less than unshifted animals, even during the first upshift trial (Figure 3).

Experiment 4 started a day after Experiment 3 and used the same animals. Unshifted animals were under the same conditions (exposure to 12% sucrose solution), but previously upshifted animals received single alternation training between the low concentration solution previously received (i.e., 0.5, 1, or 2% sucrose) and 12% sucrose as the control group. The results are presented in Figure 4. In this experiment, upshifted animals did not drink less than

control animals when exposed to 12% sucrose, but they did not exceed the goal-tracking time of unshifted controls. The results of Experiments 3 and 4 show that taste neophobia is a plausible interpretation. Taste neophobia is the tendency to ingest small quantities of novel substances (Domjan, 1976). Consistent with this, animals exposed to an incentive upshift may initially display taste neophobia to the large reward; then, when exposed again to the large reward, that taste neophobia would be attenuated thus leading to increased consummatory behavior. Although the animals are familiar with the sucrose taste, a particular high discrepancy (0.5% to 12%) could make them to experience a particular intense solution as a solution with a different taste. Still, no evidence of cSPC was obtained in these experiments.

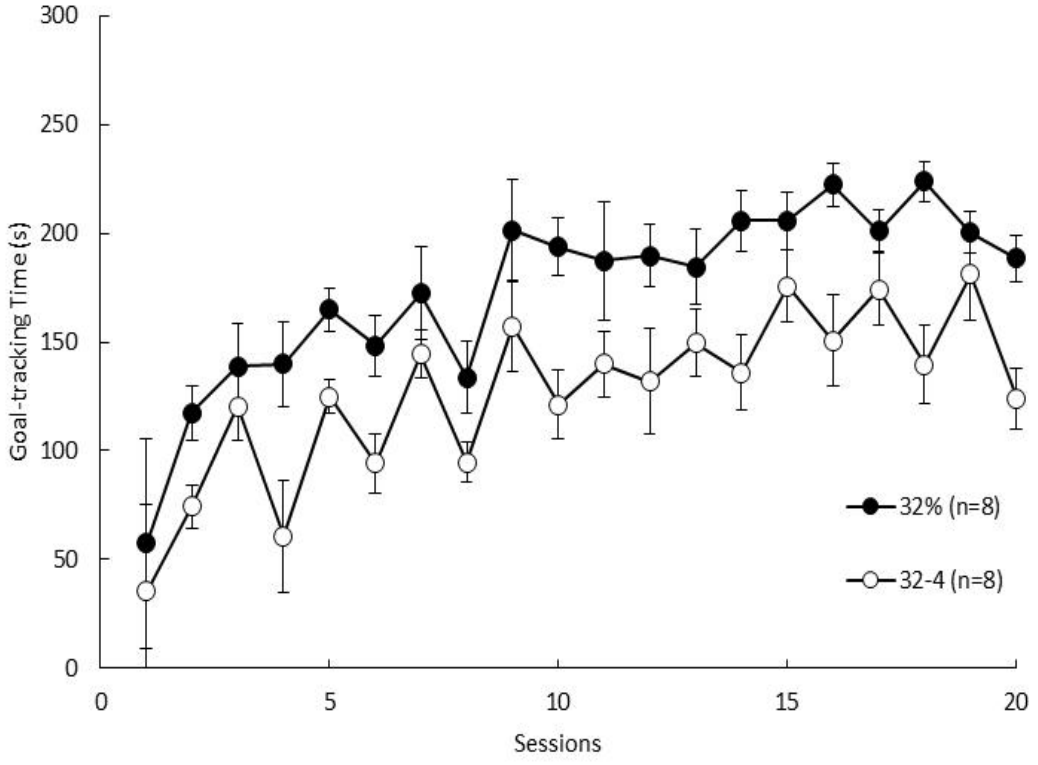

Figure 1. Single alternation of animals exposed either to 32% or 4% sucrose solution. Animals in the control group are exposed to 32% during the entire experiment. Goal-tracking time (s) is the dependent variable.

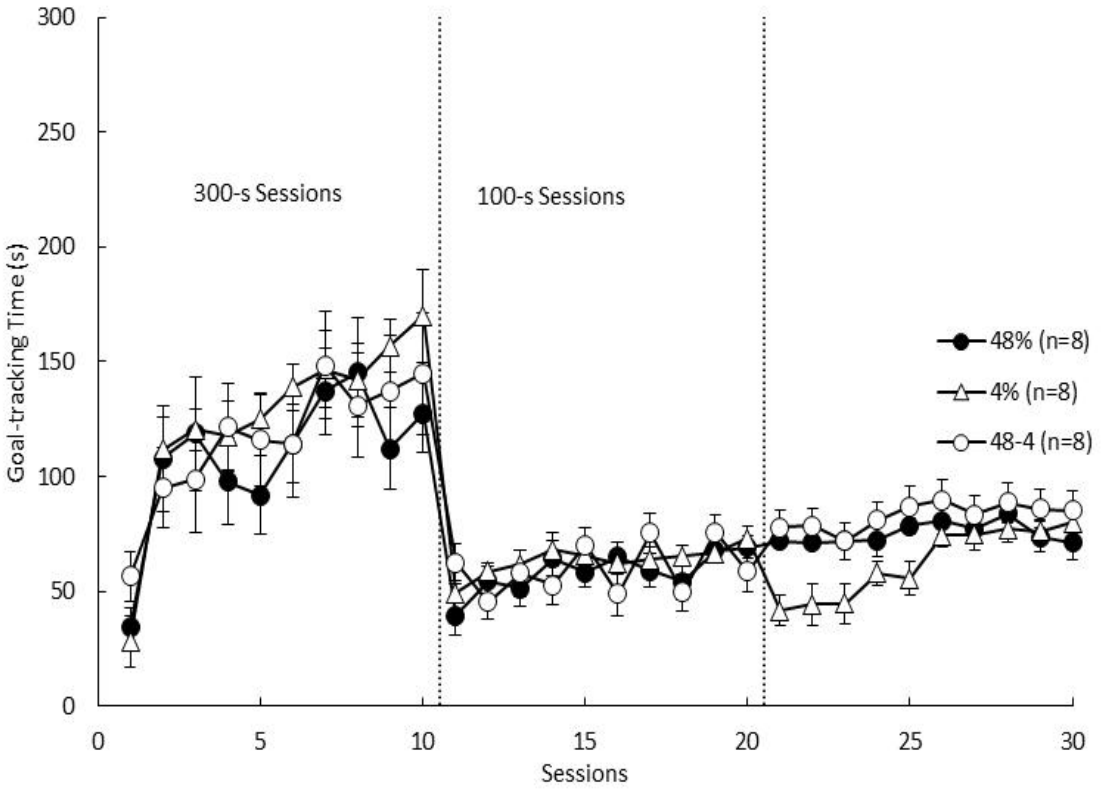

Figure 2. Single alternation of animals exposed either to 48% or 4% sucrose solution. Animals in the control group are exposed to 48% during the entire experiment. Goal tracking time is the observed variable. From trial 11 to 20 all animals are exposed to 100-s sessions. From trial 21 to 30, all animals are exposed to the 32% solution and to 100-s sessions

It is possible that it takes a relatively long time to detect the positive discrepancy between remembered and actual sucrose concentrations, thus leading to reduced drinking during a regular, 5-min session. This possibility was addressed in Experiment 5 by exposing the animals to the upshifted solution for a longer time and recording goal-tracking times in within-session bins of 100 s. The preshift trials consisted of 5 min each as in previous experiments, but the postshift trials consisted of 20 min each. It was expected that a longer postshift session would resolve the problem with the ceiling effect.

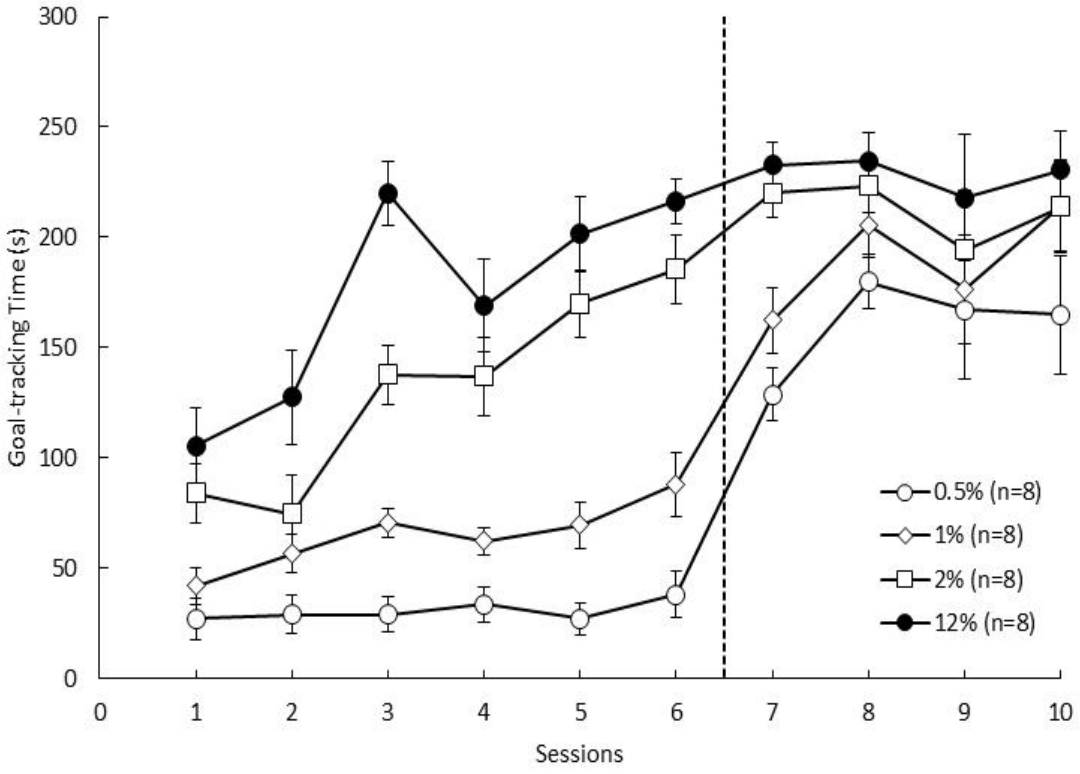

Figure 3. Goal tracking time in animals exposed in the preshift phase to 0.5%, 1%, 2% and 12% each. In the postshift phase all the animals were exposed to the 12% sucrose solution.

Experiment 6 was identical to Experiment 5, except that the animals were maintained on 100% of their ad libitum weight. Experiment 7 was also identical to Experiment 5, except that animals were preexposed to 10 ml of 12% sucrose in their home cage for three days before the beginning of behavioral testing.

In Experiments 5 (deprived) and 6 (nondeprived) we failed to find evidence of cSPC in any of the bins recorded during postshift sessions (Figures 5 and 6). The facts that the animals were exposed for a longer time and that the consumption was decreasing in both groups over time suggest that the ceiling effect is not an obscuring factor. In addition, upshifted animals were still drinking less than unshifted animals. Again, this results suggests a neophobia effect.

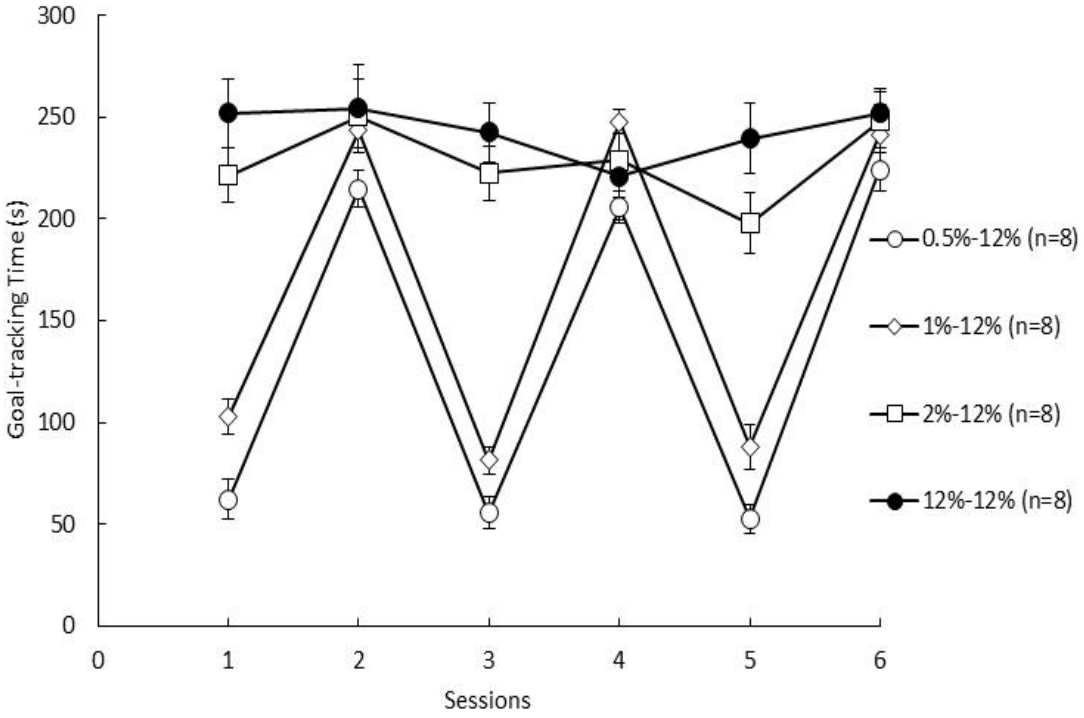

Figure 4. Single alternation of animals exposed either to a lower (0.5%, 1% or 2% sucrose solution) or the larger reward (12% sucrose solution. Control group is exposed to 12% sucrose solution during the entire experiment.

The results of Experiment 7 (Figure 7) were consistent with the idea of neophobia since preexposure to the larger sucrose concentration before behavioral testing led to as much drinking as in unshifted animals. Preliminary results in Experiment 8 using number of licks (Figure 8) seem to confirm that taste neophobia is an obscuring factor of cSPC.

In summary, the experiments suggest that experimental conditions to test for cSPC must control for the ceiling effect, for taste neophobia, and perhaps generalization decrement effects (an attentional tendency to respond less to stimuli not previously presented). I hypothesized that in a standard condition of inducing cSPC, experimental animals increased the consumption because a larger reward is presented (magnitude of reinforcement), and due to a suddenly exposition to that reward (positive contrast). On the other hand, experimental animals might not

increase above control animals because the large reward is new (taste neophobia) and because is a different solution (generalization decrement). A mildly anxiolytic drug could counteract the effect of neophobia and perhaps, may induce the cSPC effect. Testosterone is a good candidate, since it has anxiolytic properties and it has already been used in experiments studying incentive relativity. The T treatment selected was that used by Justel et al. (2012a) in their experiment on cSNC. In that case, the cSNC effect was attenuated in T-treated rats. The cSPC procedure proposed is that used in Experiment 7 involving preexposure to the large sucrose concentration as a way to attenuate neophobia.

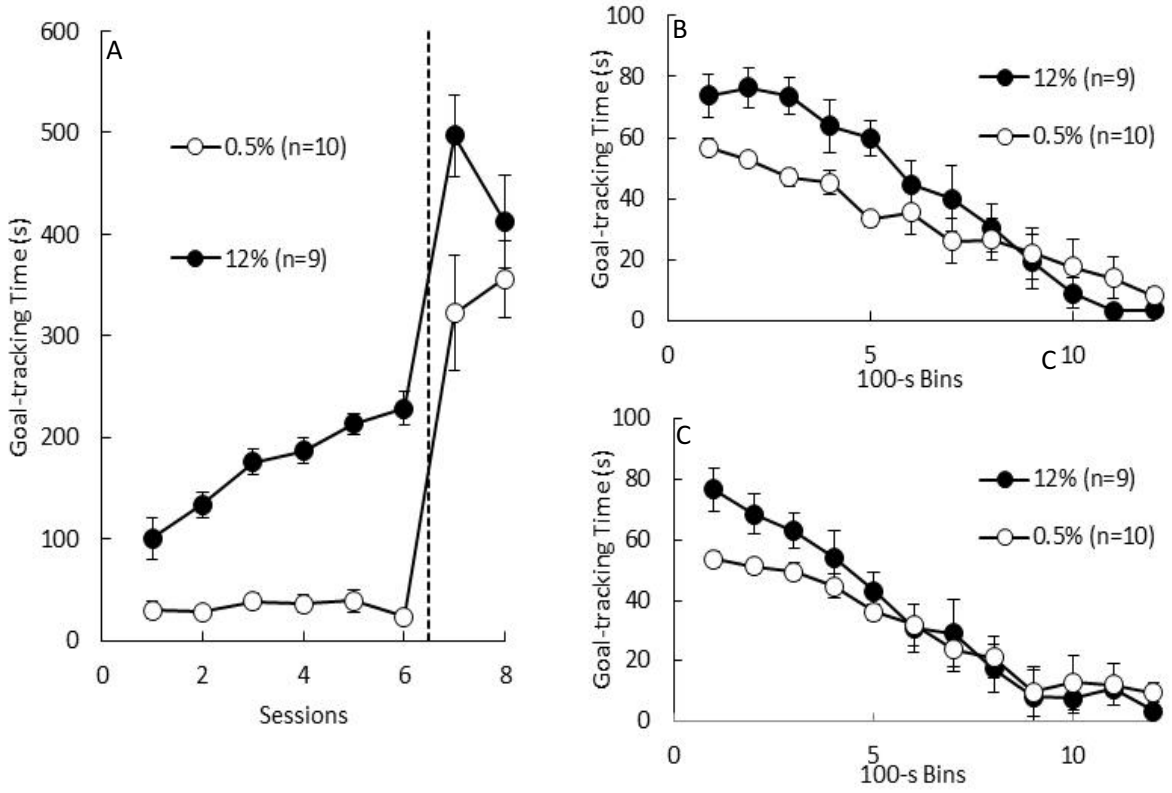

Figure 5. A. Total goal tracking time in preshift and postshift trials, 0.5% refers to animals exposed to 0.5% sucrose solution in the preshift phase, 12% refers to animals exposed to 12%. All of the animals received 12% sucrose solution in the postshift phase (trials 7 & 8). B. Goal tracking time of the animals in the experimental or control condition in trial 7 every 100-s bins.). B. Goal tracking time of the animals in the experimental or control condition in trial 8 every 100-s bins

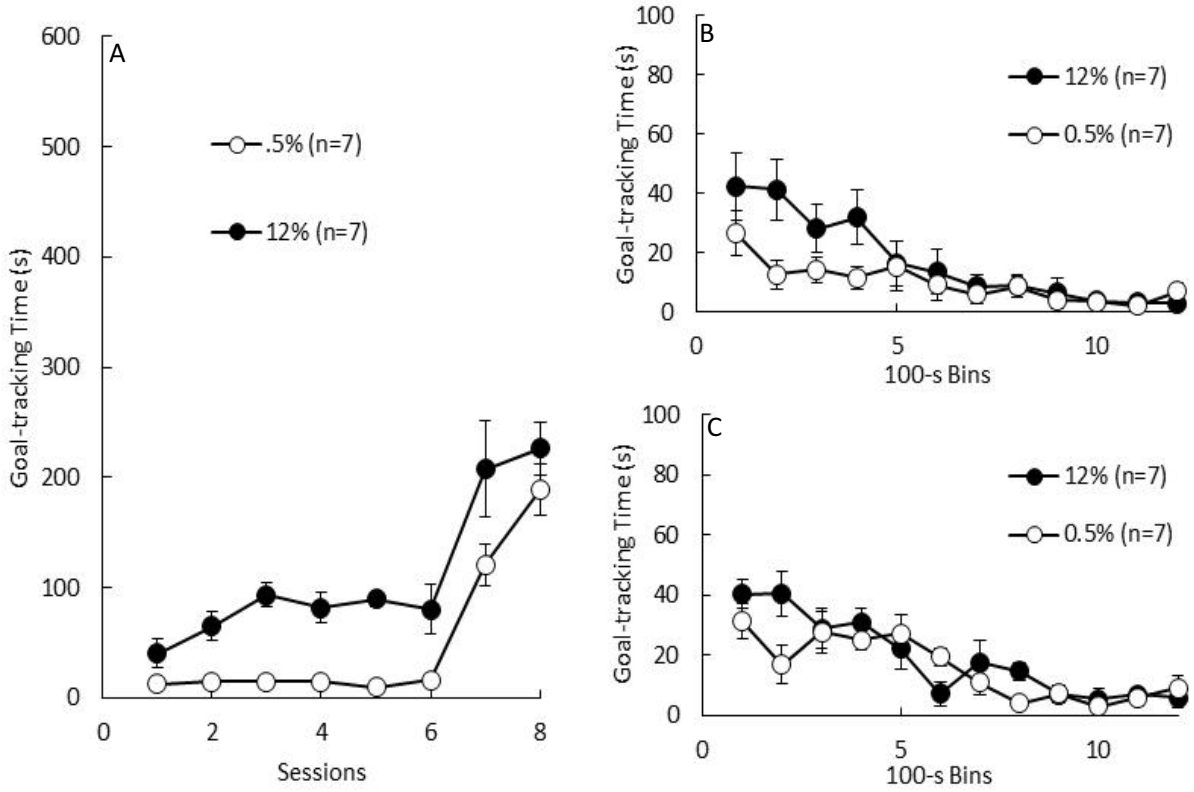

Figure 6. A. Total goal tracking time in preshift and postshift trials, 0.5% refers to animals exposed to 0.5% sucrose solution in the preshift phase, 12% refers to animals exposed to 12%. All of the animals received 12% sucrose solution in the postshift phase (trials 7 & 8). B. Goal tracking time of the animals in the experimental or control condition in trial 7 every 100-s bins.). B. Goal tracking time of the animals in the experimental or control condition in trial 8 every 100-s bins. Animals in this experiment were maintained at their ad-libitum body weight. Ad-libitum body weight was the average of 89 and 90 days old.

In summary, these experiments suggest that training conditions to test for cSPC must control for the ceiling effect, for taste neophobia, and perhaps generalization decrement effects. I hypothesized that in a standard condition of inducing cSPC, experimental animals increased the consumption because of a larger reward is presented (magnitude of reinforcement), because of a suddenly exposition to that reward (positive contrast). On the other hand, experimental animals might not increase above control animals because the large reward is new (taste neophobia) or because is a different solution (generalization decrement).

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Figure 7. A. Total goal tracking time in preshift and postshift trials, 0.5% refers to animals exposed to 0.5% sucrose solution in the preshift phase, 12% refers to animals exposed to 12%. All of the animals received 12% sucrose solution in the postshift phase (trials 7 & 8). B. Goal tracking time of the animals in the experimental or control condition in trial 7 every 100-s bins.). B. Goal tracking time of the animals in the experimental or control condition in trial 8 every 100-s bins. All animals were pre-exposed to 10ml of 12% sucrose solution for three days right before the experiment

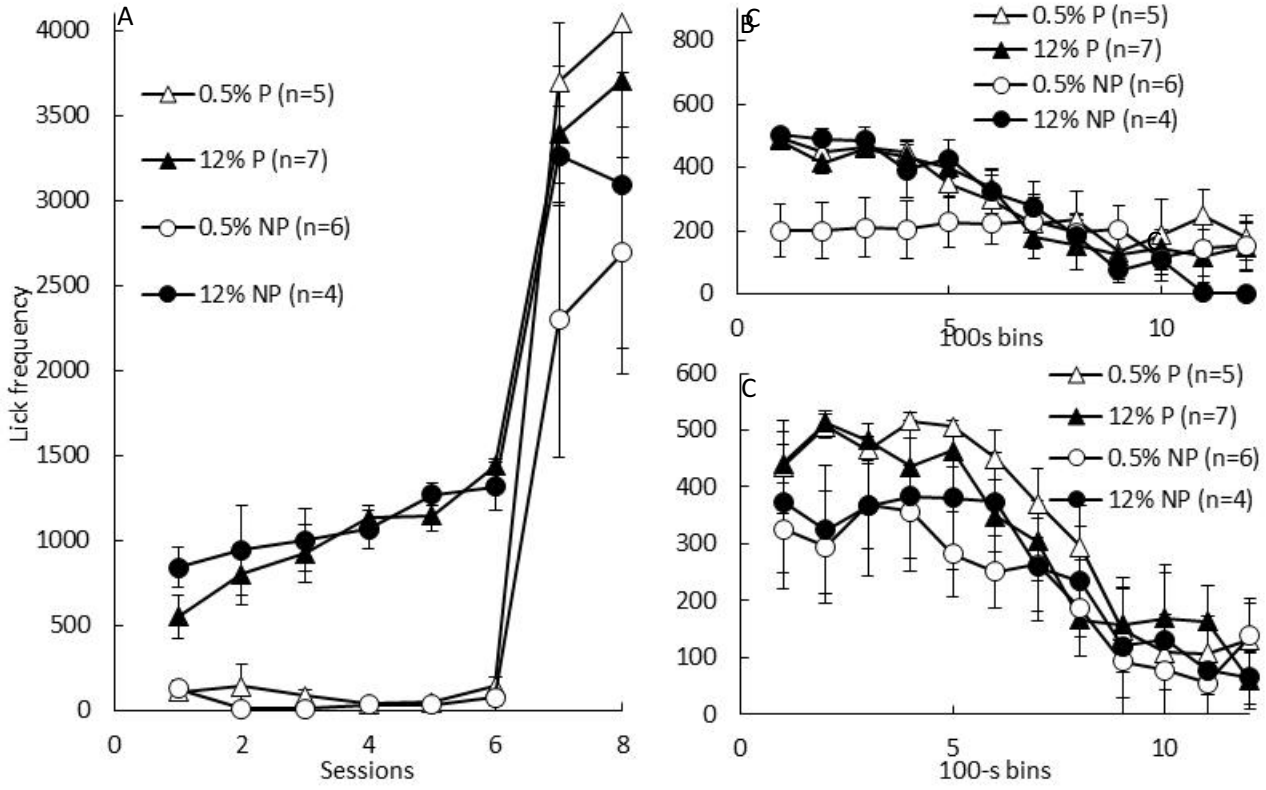

Figure 8. A. Licking frequency in preshift and postshift sessions, 0.5% refers to animals exposed to 0.5% sucrose solution in the preshift phase, 12% refers to animals exposed to 12%. All of the animals received 12% sucrose solution in the postshift phase (trials 7 & 8). B. Lick frequency of the animals in the experimental or control condition in trial 7 every 100-s bins.). C. Lick frequency of the animals in the experimental or control condition in trial 8 every 100-s bins. P= animals were pre-exposed to 10ml of 12% sucrose solution for three days before the experiment. NP= animals were pre-expose to 10ml of tap water for three days before the experiment

Experiment 1

The purpose of this experiment was to test the hypothesis that T can induce the cSPC effect. We selected the T treatment that Justel et al. (2012a) used in their experiment on cSNC. In that case, T-treated rats showed a less prominent cSNC. We used the cSPC procedure described in Experiment 7 involving preexposure to the large sucrose concentration as a way to attenuate neophobia and generalization decrement during the upshift.

Method

Subjects. Forty male rats, 90 days old were the subjects of this experiment. We housed them with their mothers for the first 3 weeks and afterward in groups of three to four for about 3 additional weeks. Around postnatal day 40, we housed them individually in wire-bottom cages until they reached postnatal day 90. Before beginning hormonal and behavioral testing, we food-deprived all rats to 81-84% of their ad libitum weight. The housing room had a 12:12 light:dark cycle, with lights on at 07:00 h. Beginning in postnatal day 90 and throughout the experiment we weighted animals daily.

Apparatus. We evaluated cSPC in 8 conditioning boxes (MED Associates, St. Albans, VT). Aluminum and Plexiglas were the materials for making the boxes (29.3 x 21.3 x 26.8 cm). Steel rods running parallel were the floor and the feeder wall parallel to them had one hole through which a sipper tube delivering the sucrose solution was inserted. The hole was located 4 cm from the floor and was 1 cm in diameter. During the first and second session, when the animals were exposed to the sucrose solution, the tube was flush with the wall; after the second session, the tube was inserted approximately 0.5 cm inside the hole. Bedding was located under the floor to collect urine and feces. In addition, a house light provided diffuse illumination and white noise masked unsystematic noises inside the box (80.1dB). A computer located in an

adjacent room controlled various aspects of the experiment, such as insertion of the sipper tube, the light and sound in the boxes, and the advancement of the feeder tube through the hole in the wall; it also recorded the lick frequency for a period of 5 min starting when the animal touched the tube for the first time. After this, we removed the animals and wiped the boxes clean.

Procedure. We administered the drug (T propionate) dissolved in olive oil (25 mg/kg) to half of the animals 30 min before the start of each training session, and just olive oil in the same amount but without T propionate to the other half of the animals in the time indicated by the other condition. Before starting the experiment all of the animals had access to 10 ml of 12% sucrose solution in their home cage during three days. For behavioral training we assigned the animals randomly to one of four different conditions:

(1) 12/T, animals with T injections during the preshift and 12% sucrose solution throughout the experiment;

(2) 12/O, animals in the same condition but receiving just the oil dissolvent but not T;

(3) 0.5/T, animals receiving 0.5 sucrose solution and T during the preshift phase, then 12% sucrose solution in the postshift days;

(4) 0.5/O, animals as the previous condition but instead receiving T, they received just the dissolvent oil.

A session started when the animal was placed in the conditioning box. The program started recording lick frequency when the animal touched the sipper tub. The session finished 5 min (preshift) or 20 min (postshift) after the initial contact. The program recorded the number of licks per session. On postshift Sessions 7-9, the software also recorded the lick frequency during 100-s bins for a total of 12 bins. In the preshift phase, the experiment had six sessions (1 session per day) in which animals had access to either 0.5 or 12% sucrose solution, for 5 min; in the

postshift phase, all animals had access to the 12% sucrose solution for 20 min during 3 sessions. We used the statistical software SPSS to analyze the data.

The variables involved were preshift (session 1 to 6), postshift (session 7 and 8), drug (oil or T), contrast (12% sucrose solution or .5%) and sessions.

Results and discussion

Figure 9 shows the results of Experiment 1. In the preshift, animals increased their consumption during the ($F_{5,170} = 4.29$, $p < 0.002$) and this was due to the increasing responding of the animals having access to the 12% sucrose solution, but not to the 0.5% sucrose solution, as it was detected by the statistical interaction between the preshift and contrast ($F_{5,170} = 5.09$, $p < 0.001$), and the additional post hoc analysis. Pairwise LSD indicated significant differences among several sessions at ps < 0.01 in 12% animals, but not significant differences among sessions in the 0.5% animals being ps ≥ 0.29). During all the sessions of the preshift animals having access to the 12% sucrose solution stayed more in contact with the sipper tube than the animals having access to the 0.5% sucrose solution ($F_{1,34} = 414.19$, $p < 0.001$). T-treated animals were not significantly different than Oil treated animals and I did not detect any additional interaction among the variables included.

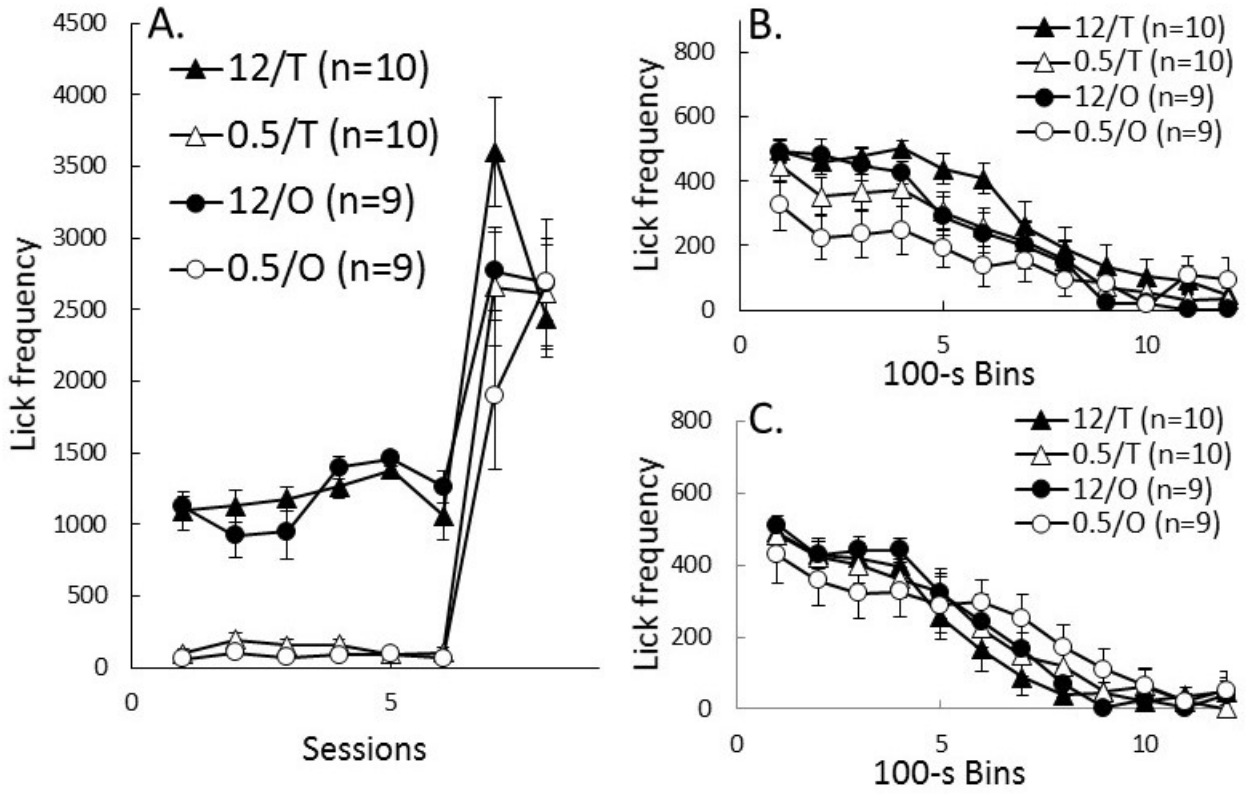

Figure 9. A. Licking frequency in preshift and postshift sessions, 0.5 refers to animals exposed to 0.5% sucrose solution in the preshift phase, 12 refers to animals exposed to 12% sucrose. All of the animals received 12% sucrose solution in the postshift phase (sessions 7 & 8). T refers to testosterone treated animals. O refers to the oil treated animals. B. Lick frequency of the animals in the experimental or control condition on session 7 every 100-s bins.). C. Lick frequency of the animals in the experimental or control condition on session 8 every 100-s bins.

In the postshift, when the experimental animals (T or oil treated) had access to a greater solution (12% as the control animals) and for a longer time (20 min), the statistical analysis did not detect a significant difference across sessions, but a double interaction between postshift sessions and contrast ($F_{1,34} = 9.02$, $p < 0.005$), and a double interaction between postshift and drug condition ($F_{1,34} = 8.38$, $p < 0.007$); pairwise LSD comparisons detected a significant difference between the animals 12% animals and 0.5% animals but in the opposite direction to our hypothesis ($p < 0.012$). Also, pairwise LSD comparison detected a marginal significant

difference between oil treated and t-treated animals in session one ($p = 0.06$); in this case T treated animals appear to consume more of the solution than oil treated animals regardless of the contrast condition.

Another way to look at the groups was to compare every session through bins of 100-s each for a total of 12 bins per one session. This would give us an indication of the pattern of responding over the 20 min that animals had access to the solution. Figure 10B and 10C show the bins in each session. In each session of the posthift, this pattern was decreasing, with the animals licking more during the first bins and gradually decreasing the level of consumption ($Fs_{11,374} \geq 50.46$, $p < 0.01$). The statistical analyses detected significant differences across bins in both sessions. In addition, in session 7, upshifted animal drank less during the first six bins but at the same level as the unshifted animals in the last six bins (interaction bins X contrast, $F_{11,374} = 3.21$, $p < 0.01$; LSD pairwise between upshifted vs. unshifted, $p < 0.01$ at bins 7 – 12). See Figure 10B.

Overall, this experiment suggest that T cannot induce successive positive contrast in a standard way, that is, by upshifting the animals to a larger reward. In addition, this experiment also indicates that neophobia is not the only factor deterring animals from drinking more, since even when the animals were preexposed to the solution, they were still drinking less of the solution. Finally, the fact that T-treated animals drank sucrose significantly more than Oil-treated animals after being upshifted is intriguing and suggest that perhaps a cSPC could be detected if instead of considering the solution, we consider duration of the session in which animals are exposed to a particular solution.

Experiment 2

The results of Experiment 1 suggest that T could induce a positive contrast if the duration of the session, rather than the concentration of sucrose, were manipulated. The purpose of Experiment 2 was to test this hypothesis.

Method

Subjects. Sixteen male rats, 90 days old were the subjects of this experiment. All the other details regarding housing, breeding, and characteristics of the control room and food deprivation procedure were as described in Experiment 1.

Procedure. The administration of T dissolved in olive oil or just olive oil were as described in Experiment 1. Half of the animals were T-treated and the other half oil-treated. Before starting the experiment all received access to 10 ml of 12% sucrose solution in their home cage in each of three days. For behavioral training all the animals had access to the same solution, 12% and we assigned them randomly to one of four different conditions:

(1) 20/T, animals with T injections during the preshift and postshift and access for 20 min to the solution;

(2) 20/O, animals in the same condition but receiving just the oil dissolvent but not T;

(3) 5/T, animals with T injections during the preshift and postshift and access for five minutes to the solution during the preshift, then 20 min in the postshift days;

(4) 5/O, animals as the previous condition but instead receiving T, they received just the oil.

A session started when the animal was placed in the conditioning box. The program started recording lick frequency when the animal touched the sipper tube. The session finished 5 min or 20 min after the initial contact depending on the group. The program recorded the

number of licks per session. On postshift sessions 7 and 8, the software also recorded lick frequency in 100-s bins for a total of 12 bins. In the preshift phase, the experiment had six sessions (1 session per day) in which animals had access for either 5 or 20 min to a 12% sucrose solution; in the postshift phase, all animals had access for 20 min to a 12% sucrose solution during 2 sessions.

We used the statistical software SPSS to analyze the data. The variables involved were preshift (session 1 to 6), postshift (session 7 and 8), drug (oil or T), contrast (5 or 20 min exposure) and sessions.

Results and discussion

Figure 10 shows the results of Experiment 2. For this experiment, statistical analysis detected a significant difference in preshift ($F_{5,60} = 7.63$, $p < 0.001$), contrast ($F_{1,12} = 89.94$, $p < 0.001$), and the interaction between preshift and contrast ($F_{5,60} = 3.81$, $p < 0.005$). T treated animals did not differ from oil treated animals ($F_{1,12} = 0.09$, $p = 0.77$). In the postshift when all the animals had access for 20 min to the 12% sucrose solution, the statistical analysis detected a significant difference across session ($F_{1,12} = 8.56$, $p < 0.05$) but not interactions between variables.

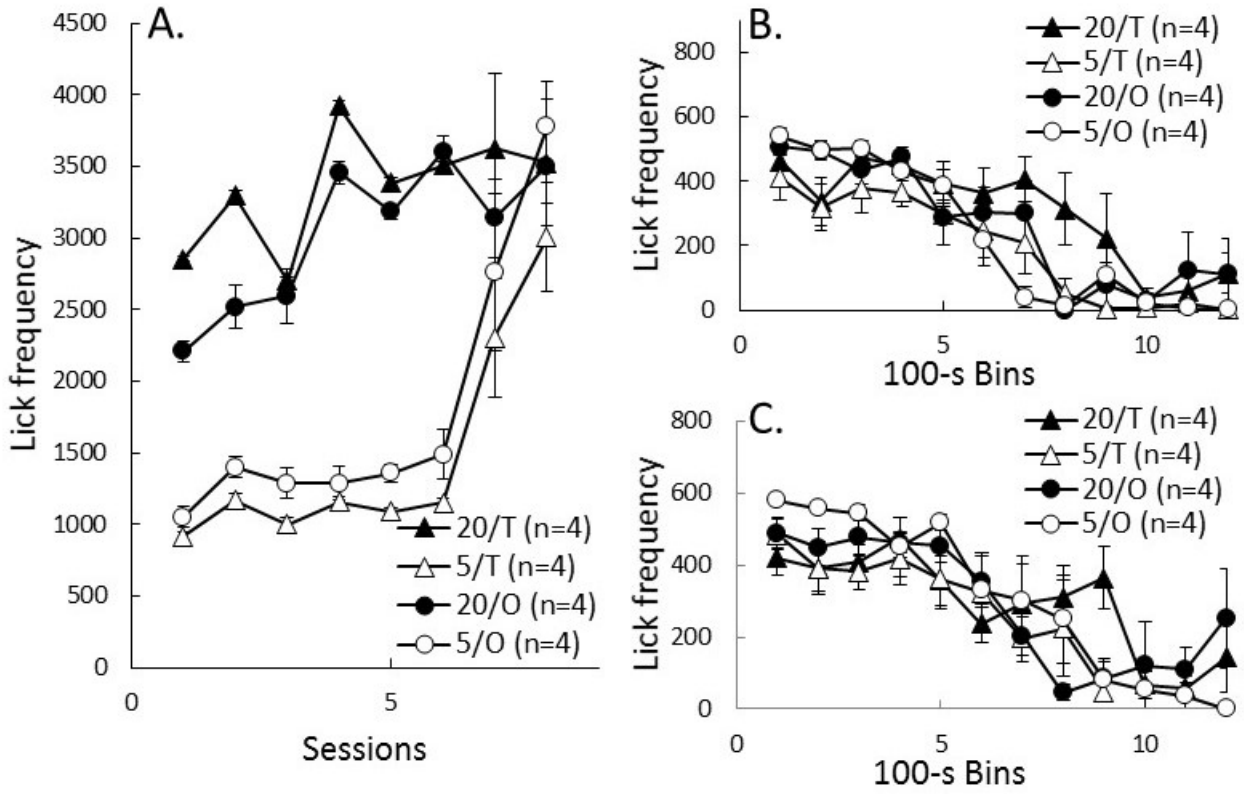

Figure 10. A. Licking frequency in preshift and postshift sessions, 20 refers to animals exposed to 12% sucrose solution for 20 min. 5 refers to animals exposed to 12% sucrose solution for 5 min. T refers to testosterone treated animals. O refers to oil treated animals. All of the animals received 12% sucrose solution for 20 min in the postshift phase (sessions 7 and 8). B. Lick frequency of the animals in the experimental or control condition in session 7 every 100-s bins.). C. Lick frequency of the animals in the experimental or control condition in session 8 every 100-s bins.

As in Experiment 1, there was a similar pattern of behavior, with a high frecuency of licks that gradually decreased ($Fs_{11,132} \geq 29.91$, $ps < 0.001$). On session 7, the statistical analysis detected a significant interaction between bins and drug ($F_{11,132} = 2.27$, $p < 0.014$); pairwise LSD detected significant difference between oil-treated animals and T-treated animals in bin 2 (oil-treated animals drank more of the solution, $p < 0.01$) and bin 8 (T-treated animals drank more of the solution, $p < 0.014$). In session 8, I did not detect any significant difference other than across bins in all animals. Figures 10B and 10C show the results across bins in sessions 7 and 8.

Overall, these results suggest that the administration of T affected the pattern of consumption, but this effect was not related to cSPC.

General Discussion

I ran two experiments to test the hypothesis that T could induce cSPC. I did not find any evidence of the phenomenon in oil-treated animals or in T-treated animals. Several reasons can account for these results. First, perhaps T does not have an effect on emotional responses; second, the activational effects of T depend on its organizational effects and our experiments did not reach the latter ones; third, cSPC is a very unreliable phenomenon that it is difficult to obtain even with the administration of drugs that have an effect on emotional responses; finally, the chosen parameters to test cSPC were not adequate to produce the phenomenon.

Perhaps T does not have an effect on emotional responses and all the effects described before are related to T's anabolic properties. For example, Wade and Gray (1979) found that that the administration of T increased food consumption. In my experiments I found that occasionally T affected the consumption of the 12% sucrose solution; this could be related to the fact that under T treatment animals are building muscles and one way to do it is to seek for higher calorie intake (Gray, Nunez, Siegel, & Wade, 1979; Joss, Zuppinger, & Sobel, 1963). The experiments in cSNC (Justel et al., 2012) could be interpreted in the same way, animals exposed to T would drink more of a sweet solution because of a need to increase calorie intake and not because of an emotional mechanism. Other experiments not involving consumption of caloric solutions have found that T appears to have antidepressant properties. In this sense, it would be implausible that T does not have any effect on emotional responses. Still it is possible that the antidepressant effects of T do not translate into particular positive emotions like elation.

Another alternative explanation would be that for T to have an effect it has to activate the system early in life. As I said previously, sexual hormones can have organizational and activational effects, the latter ones depending on the former ones. T "organizes" the brain making

it structurally different in areas related to the reproductive function; this is less evident in areas not related with this function. In line with this argument, T would have less "room" to affect the incentive upshift when administered in adult rats. If this were true, then it would imply that cSPC cannot be obtained under usual conditions, but could be present in some atypical individuals. Perhaps, this could explain why some researchers can find cSPC and others cannot; it is also consistent with Cuenya et al. (2015), who found that cSPC is obtainable in individuals that were isolated before adolescence. We did not test this idea in our experiments, so it remains an open question.

The fact that we did not find any effect of T on incentive upshift, and specifically T did not induce cSPC, raises questions about this hormone's involvement in the expression of positive emotions. Specifically, these results raise a question about the asymmetry of T's effects in the incentive relativity area. T would affect situations involving incentive downshift (Justel et al., 2012), but it would not affect situations involving incentive upshift (present experiments). Another way to look at the results would be to question the occurrence of cSPC (Annicchiarico et al., submitted).

cSPC is an unreliable phenomenon. Few researchers have reported it, replication studies are almost inexistent in the literature and no research program is devoted to the study of cSPC. Crespi (1942) and Zeaman (1949) found evidence of SPC, but that evidence was for iSPC, which appears to be less indicative of emotional responses than the consummatory ones; on the other hand, both studies lacked the proper control, an unshifted group always exposed to the large reward. Flaherty has been the researcher that most consistently has studied cSPC. Flaherty and Johnson (1975) addressed several problems regarding SPC but using a single-case design; Flaherty (1982) reviewed evidence on SPC and concluded that the scanty evidence was due to a

ceiling problem. Consistent with this, Flaherty (1983) found evidence of cSPC using reward upshift before the control group reached an asymptotic level in the responding; these results were replicated by Cuenya et al. (2015), but not replicated by Annicchiarico et al. (submitted).

As previously stated, cSPC is difficult to observe and, since Flaherty, the consensus was that the ceiling problem was the reason for that. Since control animals would acquire asymptotic level of responding, that would make it impossible for upshifted animals to surpass this level of responding; I started working with lower solutions as a way to counteract this ceiling problem. However, a 12% sucrose solution still elicited a high level of responding. Additional reports showed that cSPC is also not observed with control solutions of 2% sucrose. Could T induce the cSPC phenomenon with very low sucrose solutions? I hardly believe this would be the case, since other set of complex factors also act against positive contrast, such as generalization decrement and habit formation.

For cSPC to occur, a particular drug should act against perceptual basic abilities of the animals or against the formation of habitual behavior. A systematic approach of ten experiments by Annicchiarico et al. (submitted), found that these factors probably deter the phenomenon from occurring. These factors, like neophobia or generalization decrement, were one time used to explain SNC (see Flaherty, 1982, 1996) and it turns out they also explain why SPC is an elusive phenomenon. When the solution is new to the animals they tend to drink in less quantities (taste neophobia), although Rabin (1975) found the opposite in terms of iSPC; the sudden change of the solution appears to affect negatively the responding (generalization decrement); and when animals are exposed to more sessions of the small reward, they increase less their responding when they are upshifted in comparison with those animals exposed to the small reward in fewer sessions, and this even when the larger solution is relatively small (habitual behavior).

Perhaps I did not choose the right parameters to induce cSPC. This phenomenon is elusive but some researchers have obtained it under certain circumstances. One way would have been to follow the parameters under which the instrumental ones have been obtained. For instance, Shanab and Spencer (1979) found iSPC with repeated shifts and delaying the reward and Shanab, France, and Young (1976) found it altering the amount of reward given but keeping constant the concentration, 8% sucrose solution and repeating shifts. Repeated shifts, then, appears to produce more standard SPC and consistent with this, Flaherty (1983) found evidence of cSPC. Although this was replicated by Cuenya (2015), we did not replicate the Flaherty results in our own lab (Annicchiarico et al., submitted). In addition, we also delayed the reward in Experiment 2, but again did not obtain cSPC in oil- or T-treated animals.

Since factors induced by reward upshift counteract each other, it would be hard to find a drug that can neutralize only some of them. In most cases, researchers have used drugs to inhibit the cSPC that they found in another experiment like Rabin (1975) or Vacca and Phillips (2005). More interesting would be to find a drug which can induce the phenomenon because in a hypothetical scenario, that would be the base to study the neurobiological bases of positive emotions like elation. Suppose that an unshifted group and an upshifted group are under the same drug; both groups would neutralize other factors and the salient phenomenon (cSPC) would appear just in the experimental one.

Although my approach with T did not work, it is a good idea to look for drugs that can induce the phenomenon. For instance, benzodiazepines like chrlordiazepoxide and antidepressants like fluoxetine may counteract the aversive effects of having an incentive upshift. Fluoxetine is a good candidate because it helps prevent habit formation (Giasuddin, Nahar,

Morshed, Balhara & Sobhan, 2013; Monteiro & Feng, 2016). More research is needed to find a drug or a set of drugs that would induce cSPC.

Finally, I did find some effects of T on sucrose consumption. In Experiment 1 I found that T-treated animals had a positive contrast-like effect; when they had access to the large reward for 20 min, T-treated animals expressed a higher frequency of licks than oil-treated animals. I did not replicate the effect in Experiment 2, although I did find again isolated effects of T on consumption; while oil-treated animals tended to drink more the solution at the beginning, T-treated animals tended to energize the consumption later. This effect is consistent with Wade and Gray (1979), who found that T increased the consumption of food; since the effect did not happen under low solutions, but under a larger sucrose concentration, the high caloric content may explain the results.

In synthesis, T may not induce cSPC, although it affected the rate of consumption. Our negative finding should not discourage researchers to find an inducer drug of cSPC. The potential to understand the neurobiology of euphoria and elation, and the general effects of incentive upshift would be valuable.

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SKILLS

- Microsoft Office (Word, Excel, powerpoint, Outlook)
- SPSS (Statistical Package for Social Science)
- SIGMASTAT
- Experimental design
- M-Plus (Statistical package for Structural Equation Modeling)

EXPERIENCE

Texas Christian University - Fort Worth, TX. 2012 – 2016
Research Assistant

- Statistical analysis
- Experimental design
- Animal handling

Fundacion Universitaria Konrad Lorenz – Bogota, Colombia 2011
Teaching Instructor

- Teaching
- Virtual pedagogy

RESEARCH

Complex effects of reward upshift on consummatory behavior

- Supervisor: Mauricio Papini. Ph.D
- Collaborators: Amanda Glueck, Ph.D., Lucas Cuenya, Ph.D & Katsuyoshi Kawasaki, M.Sc.
- Texas Christian University – Fort Worth, TX, 2012 – 2015

Function of centromedial amygdala in reward devaluation and open field devaluation

- Supervisor: Mauricio Papini, Ph.D
- Collaborators: Amanda Glueck, Ph.D & Katsuyoshi Kawasaki, M.Sc
- Texas Christian University – Fort Worth, TX, 2013 – 2015

Effects of shifts in food deprivation on consummatory successive negative contrast

- Supervisors: Mauricio Papini, Ph.D & Alba Mustaca, Ph.D
- Collaborators: Lucas Cuenyas, Ph.D., Amanda Glueck, Ph.D & Matias Serafini
- Texas Christian University – Fort Worth, TX
- Instituto Lanaris – Buenos Aires, Argentina, 2012 – 2015

Latent class growth analysis of consummaotory successive negative contrast

- Collaborators: Lucas Cuenyas, Ph.Di
- Texas Christian University – Fort Worth, TX
- Instituto Lanaris – Buenos Aires, Argentina, 2012 - 2015

ABSTRACT

CAN TESTOSTERONE INDUCE CONSUMMATORY SUCCESSIVE POSITIVE CONTRAST?

by Ivan Annicchiarico, M.Sc. 2016

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Rats that have been exposed to an upshift from a small reward to a large one have shown patterns of inconsistent consummatory behaviors. First, it has been reported that they show a transient increased in their consummatory behaviors, being this phenomenon called Consummatory Successive Positive Contrast (cSPC); second, other researchers have reported no changes in their behavior.

cSPC could be a good model form studying positive emotion; however preliminary results in our lab indicate that this phenomenon is hardly to obtain. In this thesis, I explored the idea that cSPC is obtainable when testosterone (T) is administered. I did not find evidence of this. Factors like neophobia, generalization decrement and habitual behaviors are factors that probably prevent cSPC from happening.

Key words: Consummatory successive positive contrast, taste neophobia, generalization decrement, incentive relativity