Consummatory Successive Negative Contrast in Rats: Assessment Through Orofacial Taste Reactivity Responses

Lucas Cuenya1,2,3, Stefana Bura4, Matías Serafini1,2,3, Matías López4

1 Universidad de Buenos Aires, Facultad de Medicina, Instituto de Investigaciones Médicas A. Lanari, Buenos Aires, Argentina.

2 Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad de Buenos Aires, Instituto de Investigaciones Médicas (IDIM), Laboratorio de Psicología Experimental y Aplicada, Buenos Aires, Argentina.

3 Centro de Altos Estudios en Ciencias Humanas y de la Salud, Universidad Abierta Interamericana, Facultad de Psicología y Relaciones Humanas, Argentina.

4 Universidad de Oviedo, Oviedo, Spain.

Address for correspondence:

Lucas Cuenya, Ph.D.

Laboratorio de Psicología Experimental y Aplicada (PSEA)
Instituto de Investigaciones Médicas (IDIM - CONICET)
Combatientes de Malvinas 3150 1428 Bs. As., Argentina.
Tel: (+54-11) 4514-8702 int. 170
E-mail: lucascuenya@gmail.com

HIGHLIGHTS

The effect of successive negative contrast on taste palatability was examined in rats

A successive negative contrast procedure reduced appetitive taste reactivity responses

Incentive devaluation reduced intake and the hedonic value of the expected reward

ABSTRACT

Rats exposed to a downshift from a large reward (32% sucrose) to a small reward (4% sucrose) show an abrupt and transient reduction in consumption in comparison with animals that are always exposed to the small reward. This effect is called consummatory Successive Negative Contrast (cSNC) and involves negative affective consequences that lead to an aversive emotional, cognitive and behavioral state of frustration. There are few previous works that have investigated the hedonic alterations that undergo an unexpected incentive devaluation. The hedonic impact of fluids can be reliably assessed by examining the orofacial reactions of acceptance and rejection in the taste reactivity (TR) test. This study addressed in male adult Wistar rats the hedonic impact of incentive devaluation in an adapted cSNC protocol. Specifically, the orofacial responses to a sucrose solution infused into the oral cavity were measured. It was observed that animals exposed to reward devaluation, from a 32% to a 4% sucrose solution, showed a decrease in the duration of appetitive responses (tongue protrusions, mouth movements, paw licks) as compared with subjects which only experienced the low concentration of sucrose. The results are consistent with the hypothesis that incentive devaluation in a cSNC not only results in reduced intake, but also in a reduction in the hedonic value or palatability of the devalued reward.

Keywords: consummatory Successive Negative Contrast; Hedonic responses; Taste reactivity; Rats

**1. Introduction**

Affective responses to reward loss or the reduction in the perceived incentive value constitutes one of the most characteristic evolutionary advances in mammals. They are an important motivational source for behavioral adaptation to environmental changes, orientation of behavior and searching for lost reinforcement (Papini, 2003). These responses require not only prior learning of the physical aspects of rewarding stimuli (e.g., intensity and magnitude) but also learning of their motivational aspects and emotional states associated with reward loss (Papini, 2002; 2006). An extensive body of evidence shows that the incentive devaluation has negative affective consequences that lead to an aversive emotional, cognitive and behavioral state called frustration (Amsel, 1958, 1992). There are several experimental protocols used as animal models for evaluating frustration responses, including Successive Negative Contrast (SNC) (Flaherty, 1996). In its consummatory version (cSNC) in rodents, the animals are exposed to the unexpected devaluation of a preferred reward (e.g., 32% sucrose solution) to one of lower hedonic value (e.g., 4%). The negative contrast effect is observed in an abrupt and transient reduced intake compared to animals which only experience the low concentration of sucrose (e.g., Cuenya et al., 2015; Cuenya, Fosacheca, Mustaca, & Kamenetzky, 2012; Papini, Galatzer-Levy, & Papini, 2014).

 Different studies in rodents have shown that frustration state is closely related to the responses of fear and anxiety. For example, the incentive devaluation in a cSNC is accompanied by stress-related neuroendocrine responses such as increased release of corticosterone (Mitchell & Flaherty, 1998; Pecoraro, de Jong, & Dallman, 2009); the size of cSNC is diminished by the previous administration of benzodiazepines (e.g., Flaherty & Rowan, 1989; Mustaca, Bentosela, & Papini, 2000) and ethanol (Kamenetzky, Mustaca, & Papini, 2008); lesions in the corticomedial and central amygdala eliminate the negative contrast effect (Becker, Jarvis, Wagner, & Flaherty, 1984); and its size is reduced both by intra-amygdala infusion of diazepam (Liao & Chuang, 2003) and by inactivation of the centromedial amygdala (Kawasaki, Glueck, Annicchiarico, & Papini, 2015).

The response to incentive devaluation not only generates an aversive emotional state but also motivational changes around the devalued stimuli. The effect of cSNC is observed in rats even when the animals are trained under movement restriction at maximum, evidencing that the consumption decrease is not a mere by-product of searching behavior of the lost reward, but implies an active rejection of devalued reward (Lopez Seal, Cuenya, Suárez, & Mustaca, 2013). Motivational changes have also been reported around stimuli predicting frustration events, since rats learn to escape from cues previously associated with reward reduction (Daly, 1974). In this direction, Gray and McNaughton (2000) proposed a motivational equivalence between fear and frustration, according to which there would be a partial overlap between the responses generated by reward loss and punishment.

 However, motivation is not a unitary construct. It has different subsystems that contribute to regulate searching and consummatory behavior (Castro & Berridge, 2014). Two of these motivational components are ‘liking’ (hedonic impact) and ‘wanting’ (incentive salience). Liking essentially consists of the hedonic or affective impact of a reward, the brain reaction underlying the sensory pleasure produced by the presence of a reinforcer (e.g., a sweet taste). Wanting constitutes the incentive motivation, that without involving sensorial pleasure, configures the incentive salience and has a fundamental role in predicting pleasurable situations and behavioral orientation to recover the reinforcement. These components have different neuronal substrates and mechanisms. Dopaminergic circuits are predominant in wanting, while opioid, cannabinoids and gabaergic circuits are prevalent in liking (Berridge & Kringelbach, 2015; Peciña, Smith, & Berridge, 2006).

 In a cSNC protocol both components coexist, since the registration of the consumption response is determined by consummatory and preparatory behaviors (Konorski, 1967). However, there are appropriate experimental protocols to evaluate motivational subsystems in a differentiated way. The hedonic impact of taste stimuli can be reliably assessed by examining the animal’s orofacial reactions -stereotyped oral motor and somatic consummatory responses elicited by the fluid in the taste reactivity (TR) test (Grill & Norgren, 1978). In this test, rats are infused with a flavored solution via a cannula implanted in their oral cavity, and the orofacial taste reactivity responses are analyzed. These responses can be classified as appetitive reactions such as mouth movements, tongue protrusions, and paw licks (elicited, for example, by pleasant sweet tastes), or aversive (i.e., rejection reactions) such as gaping, chin rubbing, and paw treading (elicited, for example, by unpleasant sour or bitter tastes). Thus the assessment of TR behaviors provides information about why voluntary consumption has changed rather than merely assessing the size of the behavior modification (Berridge, 2000; Parker, 1998).

During the last decades, the theoretical discussions about the mechanisms involved in the cSNC were strongly focused on the weight attributed to the associative, cognitive and emotional components in this phenomenon (Amsel, 1992; Flaherty, 1996), but its hedonic consequences were neglected, leaving open the critical question of whether incentive devaluation results in the reduction of the hedonic value of the expected reward. There are few previous works in the literature that have investigated the hedonic shifts underlying an unexpected incentive devaluation (e.g., Grigson, Spector, & Norgren, 1993; Suárez, Mustaca, Pautassi, & Kamenetzky, 2013). If the negative contrast effect implies a decrease in the hedonic properties of reward, it is expected that the frustration response will be accompanied by a reduction in the orofacial indicators associated with appetitive solutions. In order to evaluate this hypothesis, an experiment was performed in which the animals were trained in an adapted cSNC protocol that analyzed the orofacial responses associated with the presentation of palatable solutions. Specifically, while the animals were exposed to reward devaluation, from a 32% to a 4% sweetened solution, we measured the orofacial taste reactivity responses associated with appetitive stimuli (mouth movements, tongue protrusions, paw licks). A decrease in these responses is expected in comparison to a control condition in which the animals were trained throughout the experiment to consume the less preferred reward.

**2. Method**

*2.1. Subjects*

Thirty male Wistar rats, approximately 90 days old and with a mean weight of 343 g (range: 315-393 g) at the start of the experiment, served as subjects. Upon arrival, they were housed individually in standard plastic cages (28 × 28 × 16 cm) in a colony room maintained on a 12-h light/dark cycle (lights on at 08:00) and at an ambient temperature of 21º C. All experimental manipulations took place during the light phase of the cycle. Throughout the experiment, the rats were maintained on a food-deprivation schedule as described below. All behavioral procedures were conducted in accordance with guidelines of the European Council Directive (2010/63/UE) and Spanish regulation RD53/2013 regarding the care and use of laboratory animals.

*2.2. Fluids and apparatus*

The fluid used was a sucrose solution of 32% or 4% (w/w; dissolved in water) depending on the experimental condition.

Pretraining took place in a dimly lighted room which contained 8 drinking boxes measuring 43 × 28 × 21 cm, with acrylic walls, steel mesh flooring and wire mesh lids. Fifty ml drinking bottles with metal spouts could be inserted at one end of each box. Consumption was measured by weighing bottles containing the fluid before and after each experimental session. Taste reactivity took place in a conditioning chamber located in the same room as the drinking boxes. The chamber was made of clear Plexiglas sides (26 × 23 × 14 cm) with a dark lid, and was placed on a table with a clear Plexiglas top. Two 50-Watt white lights on each side of the table provided a light illumination. A mirror beneath the chamber on a 45º angle facilitated viewing of the ventral surface of the rat during the intraoral infusion. The sucrose solution was administered to the rats through an infusion pump (KD Scientific) connected to an oral cannula which had been implanted prior to the experiment. While the rats were infused with the sucrose, their orofacial responses were recorded using a video camera (Sony Optical 20 X) connected to a computer. The videos were manually scored using the Observer XT 9.0 (Noldus Information Technology, Sterling, VA) event recording program. All the videos were analyzed by two independent raters.

*2.3. Cannulation surgery*

 In order to implant intraoral cannula, the rats were anaesthetized with an i.p. injection of ketamine (50 mg/kg) combined with medetomidine clorhidrato (0.15 mg/kg). Following surgery, the rats were administered ketoprofen (1.5 mg/kg, s.c.), an anti-inflammatory drug, and the antibiotic enrofloxacin (0.3 mg/kg, s.c.). In order to implant the cannula a thin-walled 15-gauge stainless steel needle was inserted at the back of the neck, directly subcutaneously around the ear and brought out behind the first molar inside mouth. A length of intramedic polyethylene tubing with an inner diameter of 0.86 mm and an outer diameter of 1.27 mm was then run through the needle after which the needle was removed. Two square elastic discs were placed over the tubing and drawn to the exposed skin at the back of the neck for the purpose of stabilizing the cannula. The tubing was held secure in the oral cavity by an O-ring, which was sealed behind the tubing prior to cannulation surgery. Following surgery, rats were monitored for three days and had their cannula flushed daily with chlorhexidine to prevent infection. For the purpose of fluid infusion, the cannula was connected to the infusion pump by slipping the tubing of the cannula inside a second polyethylene tubing (inner diameter 1.19 mm; outer diameter 1.70 mm) attached to the infusion pump.

*2.4. Procedure*

Three rats lost their cannula during training and were removed from the experiment. The remaining rats were randomly assigned to two groups: Group 32-4 (*n*=14) and Group 4-4 (*n*=13). After recovery of the surgery, the rats were placed on a food deprivation-schedule, reducing the daily amount of food until they reached approximately the 83-85% of their *ad libitum* body weight. This level of deprivation was maintained throughout the experiment by restricting the amount of daily food to which the animals had access. The food was supplied approximately 20 min after the experimental sessions. Throughout the experiment, this food deprivation regime was maintained. Water was always available in the home cages.

----INSERT TABLE 1 HERE----

Firstly, the rats received five pretraining sessions (one per day) in the drinking boxes (see Table 1). On each of these sessions, the rats were given access to the bottle containing the sucrose solution for 10 min, and the amounts consumed were measured (by weight). Rats in Group 32-4 received a 32% sucrose solution whereas those in Group 4-4 received a 4% sucrose solution. As many trials after surgery increases the chances of rats losing the cannula, the pretraining phase was introduced so that the animals had sufficient experience with the fluids previous to the intraoral administration. This allowed for a reduction in the number of trials after surgery. After recovery of the surgery, one habituation day in the taste reactivity (TR) chamber preceded the training phase. On this session, rats were given an intraoral infusion of water for 1 minute in order to habituate them to the apparatus and to the intraoral infusion method. Training consisted in a total of 7 daily sessions conducted in two phases, preshift (5 sessions) and postshift (2 sessions). In each session, the rats were placed in the TR chamber, and they were then infused with sucrose for 3 minutes (infusion rate 1ml/min). During the preshift phase, rats in Group 32-4 received a 32% sucrose solution whereas those in Group 4-4 were infused with a 4% sucrose solution. During the postshift phase all rats were given the 4% sucrose solution.

Based on the procedure followed by Parker (1998), and as previously used in our studies using the TR method (Dwyer, Gasalla, Bura, & López, 2017; Gasalla, Soto, Dwyer, & López, 2017; López et al., 2010), the appetitive orofacial reactivity responses scored were tongue protrusions (extension of the tongue out the mouth), mouth movements (movement of the lower mandible without opening the mouth), and paw licks (midline extension of the tongue directed to the forepaws). The total number of seconds that the rats displayed the responses was used as the appetitive response score. The aversive behaviors scored included the frequency of the responses of gaping (rapid, large-amplitude opening of the mandible with retraction of the corners of the mouth), chin rubbing (mouth or chin in direct contact with the floor or wall of the chamber and body projected forward), and paw treading (forward and backward movements of the forepaws in synchronous alternation). Forelimb flails (rapid horizontal movements of the forelimbs to remove fluid from the fur) and head shakes (rapid side-to-side head movements with the mouth open in order to remove the fluid out of the mouth) were also scored as aversive responses. These scores were added to provide a total aversive response score. Appetitive and aversive responses were scored on different scales (duration vs frequency) because they display very different properties: Appetitive responses are typically displayed over extended periods of time whereas aversive responses occur as isolated behavior (Berridge, 2000). In addition to the number of appetitive and aversive orofacial responses, the frequency of passive dripping (each occasion on which a drop of fluid was allowed to leak out of the mouth to the floor without other orofacial actions) was also scored. The inter-rater reliability for each behavior was computed analyzing the Pearson*´*s correlation coefficient. In all cases the inter-rater reliability was highly significant (*r*s > 0.91).

*2.5. Statistical analysis*

The data were analyzed using the SPSS 18.0 software. A 2 (group) × 5 (session) mixed ANOVA was used to examine the consumption data from the pretraining phase. The duration of the appetitive behaviors in the taste reactivity test during preshift and postshift sessions were analyzed by a mixed ANOVA with Group and Session as factors (2 x 7). A similar mixed ANOVA model was used to analyze the total frequencies of aversive behaviors displayed by the rats in the TR test including the last two sessions of the preshift phase and the postshift sessions. Whenever the sphericity assumption was not achieved, the Greenhouse-Geisser correction was used and the degrees of freedom were adjusted on the basis of this correction. The factor’s size effects and their interaction were computed using partial *eta* square (*ηp2*). The pairwise comparison method was used to assess specific differences as a *post hoc* test. An alpha level of *p* < .05 was used. Data are shown as mean ± *S.E.M.*

**3. Results**

Figure 1 shows the mean sucrose intake in grams by the groups during the pretraining phase. Group 32-4 had greater sucrose intake than Group 4-4, with this difference increasing over sessions. The 2 x 5 ANOVA conducted with these data revealed a main effect of Session, *F*(2.24, 56.06) = 89.10, *p* < .001, *ηp2* = .78, and a trend for significance in the Session by Group interaction, *F*(4,100) = 2.87, *p* = .059, *ηp2* = .1. The effect of group resulted nearest to the adopted significance value, *F*(1,25) = 3.96, *p* = .057, *ηp2* = .13. An analysis of simple effects revealed that consumption did not differ between groups during sessions 1 and 2 (*F*[1, 25] = 0.66, *p* = .424, *ηp2* = .02, and *F*[1, 25] = 1.271, *p* = .270, *ηp2* = .04, respectively, but Group 32-4 consumed significantly more sucrose than Group 4-4 in sessions 3 to 5 (lowest *F*[1, 25] = 4.43, *p* = .045, *ηp2* = .15, on session 3).

----INSERT FIGURE 1 HERE----

Figure 2 shows the mean duration in seconds of the appetitive taste reactivity responses (tongue protrusions, mouth movements, and paw licks totaled) displayed by the rats during preshift (left) and postshift (right) sessions. The ANOVA revealed significant main effects of Session, *F*(3.91, 97.91) = 6.8, *p* < .001, *ηp2* = .21, and a significant Session by Group interaction, *F*(3.91, 97.91) = 13.94, *p* < .001, *ηp2* = .35, while the effect of Group was not significant, *F*(1, 25) = 0.03, *p* = .84, *ηp2* = .001. An analysis of simple effects revealed no differences between groups in the mean duration of the appetitive responses elicited by the sucrose infusion in sessions 1 to 3 (largest *F*[1, 25] = 1.12, *p* = .3, *ηp2* = .04, on session 3). Group 32-4 displayed a longer duration of appetitive responses in sessions 4 and 5 than Group 4-4 (*F*[1, 25] = 8.94, *p* = .006, *ηp2* = .26, and *F*[1, 25] = 9.03, *p* = .006, *ηp2* = .26, respectively). Also, as shown in Figure 2, a reduction in total time of the appetitive orofacial responses can be observed when comparing the behavior of Group 32-4 between the last session of preshift and the first postshift session. The pairwise comparison showed a significant decrease between both sessions in Group 32-4, *F*(1, 25) = 78.67, *p* < .001, *ηp2* = .75, while the change of the response was not significant in Group 4-4, *F*(1, 25) = 2.48, *p* = .13, *ηp2* = .08. Finally, Figure 2 (right) shows that Group 32-4 displayed less appetitive responses to the sucrose solution than Group 4-4 during the postshift phase. The analysis revealed a significant difference in the mean duration of appetitive taste reactivity responses between the 32-4 and 4-4 groups in the postshift sessions 1 and 2, (*F*[1, 25] = 16.70, p < .001, *ηp2* = .40, and *F*[1, 25] = 4.91, *p* = .036, *ηp2* = .16, respectively). However, there was no evidence of passive dripping in any group of rats, suggesting that the reduction in appetitive responses reflects a reduction in positive affect rather than simply reduced consumption.

INSERT FIGURE 2 HERE

The ANOVA conducted with the orofacial aversive responses elicited by the sucrose infusion in the preshift sessions 4 and 5 and the postshift sessions 1 and 2 revealed a significant effect of Session, *F*(1.1, 27.66) = 5.13, *p* = .03, *ηp2* = .17, but not a significant effect of Group, *F*(1, 25) = 2.62, *p* = .11, *ηp2* = .09, nor a significant interaction between these two factors, *F*(1.1, 27.66) = 2.84, *p* = .10, *ηp2* = .10. It should be noted that although Group 32-4 displayed more aversive responses than Group 4-4 during the first postshift session (*M32-4*= 4, *SD* = 1.28 vs *M 4-4*= 0.84, *SD* = 1.32), the analyses did not reveal significant differences between the groups in any of the postshift sessions (largest *F*(1, 25) = 2.92, *p* = .1, *ηp2* = .10).

**4. Discussion**

The results obtained in this study showed that during pretraining the rats exhibited differences in consummatory drinking behavior that depended on the absolute rewarding properties of the sucrose solution employed, with animals in the 32% condition (Group 32-4) showing higher sucrose intake on sessions 3-5 than those in the 4% condition (Group 4-4). In addition, during the preshift phase, the duration of the appetitive responses was higher in Group 32-4 than in Group 4-4 in sessions 4 and 5, indicating that the effects on appetitive responses depended on the concentration of the sucrose solution. Most importantly, it was observed that a shift from a high sucrose concentration to a low concentration during the postshift phase (Group 32-4) caused a reduction in the mean duration of appetitive responses as compared with Group 4-4 which only experienced the low concentration of sucrose. Therefore, it can be concluded from this study that a devaluation of the rewarding properties of the sucrose caused a successive negative contrast effect as measured by orofacial responses in the taste reactivity test.

As previously showed by Kaplan, Roitman and Grill (1995) in ingestive taste reactivity analysis, the mouth movements observed during intraoral infusion of fluids ingested by rats can be linked to licking, since both are emitted in the same frequency range (5-8 Hz). It could be argued that the diminished time of these behavior in the downshift are attributable to a decrease in the muscular activity required for ingestion. However, if the effect reported in the taste reactivity test was due to a simply reduction in consumption, then we would expect to find differences regarding the passive dripping. On the contrary, there was not such behavior in any experimental condition.

Our data is in accordance with the hypothesis that incentive devaluation in a cSNC implies not only a reduction in consumption, but also a decrease in the hedonic value of the devalued reward. Specifically, it was observed that animals exposed to the incentive devaluation showed a decrease in the duration of orofacial responses associated with appetitive stimuli and a negative contrast effect in both the first and second postshift tests. The measurement of the orofacial responses during the intraoral infusion of the sucrose solution enabled an analysis of the affective consequences of a situation involving unexpected reward devaluation. This motivational component (hedonic impact of flavor) is not analyzed in the majority of works on cSNC since they record parameters of the consummatory response (e.g., total licks, goal tracking time, consumption) that do not allow the dissociation of the motivational subcomponents (Flaherty, 1996). The results are consistent with the few antecedents in which hedonic alterations were evaluated in cSNC. Suárez et al. (2013) evaluated orofacial responses with a TR in 18-day-old infant rats before incentive devaluation, in a protocol in which sweetened solutions were infused at 12% during the preshift phase and a devalued solution at 2% in postshift. They reported a significant increase in the number of orofacial responses associated with aversive patterns in the first postshift trial (head shaking, wall climbing and chin rubbing) compared to a control condition. This effect was not observed in the present study, suggesting that the hedonic alterations in cSNC are revealed mainly by a reduction in appetitive responses. Nevertheless, in the study by Suárez et al. (2013) the orofacial responses associated with appetitive patterns were not evaluated. There are several differences between the study by Suárez et al. (2013) and our own work: these authors used infant rats and reported only aversive orofacial responses. It should be noted that infant rats and adult rats show differences in frustration responses in several paradigms including SNC. For instance, the cSNC lasts only one day in infants, while it ranges from 2 to 4 days in adult rats, showing different components in terms of the emotional responses involved in the initial phase (the first postshift trial) and subsequent ones (Amsel, 1992). Our study represents the first examination of the appetitive hedonic expressions in adult rats, confirming that incentive devaluation in a cSNC affected the hedonic value of the expected reward as showed by a reduction in the duration of appetitive responses.

Another method that allows assessment of the hedonic value of sapid solutions consists of analyzing the temporal microstructure of the consumption response (see Dwyer, 2012). Previous studies have shown that when rats drink palatable solutions, the number of licks per cluster increases monotonically as a function of concentration (e.g., Spector, Klumpp, & Kaplan, 1998), while it equally decreases when the concentration of an aversive solution such as quinine increases (e.g., Spector & St John, 1998). Using this method, Grigson et al. (1993) found in a cSNC that adult rats show changes in the microstructure of drinking behavior, indicative of a decrease in the hedonic value of the less preferred reward. Both in the first and second postshift session, they found a decrease in the overall number of licks and the number of licks per burst, while they

reported an increase in the number of bursts in both trials and also an increase in the inter-burst interval in the first test trial. A recent study in mice has confirmed that animals shifted from a high sucrose concentration (32%) to a low sucrose concentration (4%) made smaller lick clusters than a control group (Austen, Strickland, & Sanderson, 2016).

Two different interpretations have dominated the theoretical debate about the mechanisms underlying the cSNC effect. According to the multi-stage model proposed by Flaherty (1996), two consecutive phases would happen during the postshift. In the first, when the animal finds the new solution, a cognitive evaluation (the comparison between the new reward with the previous one) and a search for the lost reward would be performed. In a second phase, stress or anxiety responses would appear. This would occur because the animal experiences a conflict between a tendency to consume the devalued solution because of its absolute value, and a tendency to withdraw and search for the expected and more preferred reward. This model was proposed to account for certain results, such as the non-effect of chlordiazepoxide (Rosen & Tessel, 1970), flurazepan (Flaherty, 1990), midazolam (Becker, 1986; Flaherty, 1990) and ethanol (Becker, & Flaherty, 1982) in the first day of postshift, in contrast with the effects found in the following days. To the contrary, Amsel's theory (1958; 1992) confers an emotional mechanism to both stages of the phenomenon. Amsel discriminated two types of emotional responses: an unconditioned aversive response to the reward loss (primary frustration) and an anticipatory response (secondary frustration).The latter is expressed in subsequent trials in which the animal experiences a conflict caused by the anticipation of the expected reinforcement and the frustration associated with the devalued reward.

Overall, the results reported here and the background reported by Suárez et al. (2013) and Grigson et al. (1993) form a body of evidence consistent with Amsel's frustration theory. If the initial suppression of the response to reward devaluation was due only to the animals’ detection of change and searching behaviors, no variations in affective or hedonic parameters should be observed in the first trial. On the contrary, both the present study and the mentioned antecedents reflect a decrease in the hedonic value of devalued reward from the initial moment of change. This is consistent with an unconditioned response of frustration that would induce an active rejection of the less preferred reward. As in previous studies (e.g., Arias, Pautassi, Molina, & Spear, 2010; Suárez et al., 2013), the results of the present experiment also support the hypothesis proposed by Berridge (2000) which states that orofacial responses to taste stimuli do not reflect their sensory value, but rather their hedonic or affective value. Future studies could contribute to understanding the affective and motivational mechanisms of expected food reward devaluation or loss, which states that orofacial responses to taste stimuli do not reflect their sensory value, but rather their hedonic or affective value which may be significant psychological factors for understanding the difficulty in establishing and maintaining healthy eating patterns.

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Authors’ contribution: LC and ML both developed the study concept, performed the data analysis and drafted the original manuscript. SB and MS both contributed to data collection and data analysis. All authors approved the final version of the manuscript. The authors declare that they have no conflict of interest related to this work.

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Table and Figure legends

Table 1. Experimental design. During pretraining, the rats were given access to sucrose for 10 min in the drinking boxes and the consumption measured. During training (preshift and postshift sessions), the rats were intraorally infused with sucrose for 3 min in the taste reactivity apparatus. The numbers 32% and 4% refer to the concentration of sucrose solution received by rats in each phase.

Figure 1. Mean (± *SEM*) amount of sucrose solution (g) consumed by Group 32-4 and Group 4-4 during the pretraining phase.

Figure 2. Mean (± *SEM*) duration of appetitive taste reactivity responses displayed by the groups during the preshift and postshift sessions, separated by a dashed vertical line. The black bars represent the Group 32-4 and the white bars represent the Group 4-4. \*\*: significant at *p* < .01; \*: significant at *p* < .05.

**Table 1**

|  |  |  |  |
| --- | --- | --- | --- |
| Group | Pretraining (Drinking boxes)5 sessions |  | SNC (TR chamber) |
| Preshift5 sessions | Postshift2 sessions |
| 32-4 (*n*=14) | 32% | Cannulation surgery | 32% | 4% |
| 4-4 (*n*=13) | 4% | 4% | 4% |

**Figure 1**

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**Figure 2**

