Andrea B. Suárez[1]
Alba E. Mustaca[1]
Ricardo M. Pautassi[2]
Giselle V. Kamenetzky[1]

[1]*Laboratorio de Psicología Experimental y Aplicada*
IDIM
CONICET - UBA—Centro de Altos Estudios en Ciencias Humanas y de la Salud (CAECIHS-UAI)
Universidad Abierta Interamericana
Buenos Aires, Argentina
E-mail: yoselevich@hotmail.com

[2]*Instituto de Investigación Médica Mercedes y Martín Ferreyra, CONICET*
Universidad Nacional de Córdoba
Córdoba, Argentina

Ontogeny of Consummatory Successive Negative Contrast in Rats

ABSTRACT: *Consummatory successive negative contrast (cSNC) occurs when organisms repeatedly exposed to a high-magnitude reward are suddenly given a low-magnitude reward. This results in a significant reduction in the consumption of the devalued reinforcer, at a level even below that of a group which had been always exposed to the low-magnitude reinforcer. A scarcity of animal studies assessed the expression of this phenomenon during early development. Three experiments assessed age of cSNC onset in preweanling rats. Percent body weight gained (%BWG) and taste reactions associated with reinforcement devaluation were measured. A reduction in %BWG and a significant increase in emission of aversive hedonic behaviors, indicative of cSNC, occurred on postnatal day 18 (PD 18; Experiments 1 and 2), but not on PD 14 or PD 17 (Experiments 3a and 3b). The neurobiological mechanisms underlying these effects and theoretical implications are discussed.* © *2013 Wiley Periodicals, Inc.* Dev Psychobiol 56: 989–998, 2014.

Keywords: *ontogeny; consummatory contrast; disgust; rats*

INTRODUCTION

The paradoxical reward effect (PRE) or frustration effect comprises a set of learning phenomena involving surprising reward loss or downshift (Amsel, 1958). Successive negative contrast (SNC), an example of such an effect and the focus of the present work, occurs when subjects are repeatedly exposed to a high-magnitude reward and are then suddenly given a low-magnitude reward. This results in a significant reduction in the consumption of the devalued reinforcer, at a level even below that of a group which had been always exposed to the low-magnitude reinforcer. Several theories consider that SNC constitutes an aversive situation that produces similar effects to those induced by the expectation or presentation of exteroceptive nociceptive stimuli (Papini, Wood, Daniel, & Norris, 2006). According to Amsel (1958, 1992), animals learn to anticipate a reward when faced with cues previously associated with reinforcement. Reactions to a lower-than-expected reinforcer are referred as "primary frustration." Primary frustration is an unconditioned reaction similar to those provoked by aversive stimuli like exteroceptive nociceptive stimulation. After this first reaction, animals anticipate devaluation and "secondary frustration" mechanisms are triggered. Secondary frustration is theorized to be an internal state resembling that of primary frustration and evoked by the anticipation of the downshifted solution.

SNC occurs in a wide variety of species, including humans infants (e.g., Kobre & Lipsitt, 1972; Mast, Fagen, Rovee-Collier, & Sullivan, 1980), monkeys (e.g., Tinklepaugh, 1928), dogs (e.g., Bentosela, Jakovcevic, Elgier, Mustaca, & Papini, 2009), sheep (e.g., Catanese, Freidin, Cuello, & Distel, 2011), rats (e.g., Crespi, 1942), mice (e.g., Mustaca, Bentosela, & Papini, 2000), and birds (e.g., Freidin, Cuello, & Kacelnik, 2009). Most studies, however, used adult rats and consummatory procedures to measure reinforcer consumption, licking, or access time to a sipper tube containing liquid or solid reinforcers (e.g., Cuenya, Fosacheca, Mustaca, & Kamenetzky, 2012; Justel, Ruetti, Mustaca, & Papini, 2011; López Seal, Pellegrini, & Mustaca, 2010; Mustaca, Bentosela, & Papini, 2000;

Manuscript Received: 20 March 2013
Manuscript Accepted: 4 October 2013
Correspondence to: G.V. Kamenetzky
Article first published online in Wiley Online Library (wileyonlinelibrary.com): 14 November 2013
DOI 10.1002/dev.21178 • © 2013 Wiley Periodicals, Inc.

Pellegrini & Mustaca, 2000; Papini, Mustaca, & Bitterman, 1988), or instrumental procedures to estimate running speeds in a straight-alley runway or lever-pressing in a conditioning box (Chen, Gross, & Amsel, 1981; Cuenya, Sabariego, et al., 2012). Although numerous studies analyzed factors associated with the expression of negative contrast effect in adult rats, very few assessed this phenomenon in infant rats, which is rather striking, as the second week of life in the rat is a critical window for the study of aversive learning (Arias, Pautassi, Molina, & Spear, 2010). The subsequent evidence illustrates learning an aversion versus a preference depending on age. Up to postnatal day (PD) 8 or 10 animals are much more prone than older animals to learn appetitive than aversive learning. Arias and Chotro (2006) observed that animals intoxicated with ethanol on PDs 7–8 later exhibited enhanced ethanol intake and ethanol palatability, but animals given ethanol a few days later, on Days 10–11 exhibited reduced ethanol intake and enhanced aversion to ethanol's flavor. Similarly, Upton and Sullivan (2010) observed that, before PD 10, conditioned odor preference occurs even when employing exteroceptive nociceptive stimulation. After PD 10 and associated with increasing corticosterone levels and a functional switch in the role of opioid kappa receptors (from appetitive to aversive), the expression of aversive and appetitive learning is similar to that commonly observed in adult subjects. Moreover, during the second week of life the ability of the rat to express aversive learning is critically refined and unconditional stimulus-dependent responses start to emerge. Five-day-old animals exhibit similar behavioral responsiveness when stimulated with a lithium-chloride or footshock paired CS, but this lack of differentiation disappears in 15-day-old rats (Hoffmann, Hunt, & Spear, 1990).

The ontogeny of SNC has been studied through instrumental procedures (e.g., iSNC). Chen, Gross, and Amsel (1981) found iSNC in 34- to 35-day-old rats but not in 25- to 26-day-old rats, when evaluated in a straight-alley runway with solid food in the goal box. However, a robust contrast effect was observed when using milk as a reinforcer on PD 25–26. According to Amsel's hypothesis, the expression of iSNC (and of paradoxical effects in general) requires complex associative processes that depend on the maturity of certain brain regions, particularly the septo-hippocampal system (Amsel & Stanton, 1980).

Suárez, Mustaca, Pautassi, and Kamenetzky (2012) showed that 2-week-old infant rats discriminate between different concentrations of a sweet reinforcer but do not exhibit consummatory successive negative contrast (cSNC). These results suggest that at this age behavior is driven by the absolute, instead of relative, value of reinforcers. It seems that the ability to discriminate reinforcer magnitude is not enough to allow the expression of cSNC. Instead, several skills may be needed for the emergence of this ability. Specifically, the subject should be capable of (1) discriminating between the values of the different rewards; (2) remembering previously received rewards; (3) developing reward expectations; (4) comparing previous and current rewards; and (5) responding differentially based on previous experience.

To our knowledge, there are no studies on the ontogeny of cSNC. Studies involving brain lesions in adult rats suggest a partial overlap between brain areas underlying cSNC and iSNC. Sastre and Reilly (2006) reported that bilateral lesions of the gustatory thalamus eliminate cSNC but not iSNC. Furthermore, hippocampal lesions eliminate iSNC (Salinas & White, 1998) but not cSNC (see Flaherty, 1996). The same occurs with electrolytic lesions of the nucleus accumbens (Leszczuk & Flaherty, 2000). On the other hand, the cSNC effect was found to be attenuated by lesions of the lateral regions of the amygdala (basolateral, lateral, and basomedial nuclei) and eliminated by lesions of the medial regions of the amygdala (corticomedial and central nuclei; Becker, Jarvis, Wagner, & Flaherty, 1984). Likewise, lesions of the basolateral/lateral complex of the amygdala reduced the duration of the iSNC (Salinas, Parent, & McGaugh, 1996). Differences could also be exhibited in connection with neurodevelopment. Previous work indicates that differentiation of granule cells in the dentate gyrus (septo-hippocampal system) is rapid between PD 12–14, and attains adult levels around PD 25–30 (Amsel & Stanton, 1980). On the other hand, morphometrical analysis revealed that neural development in the amygdaloid complex reaches its highest level around PD 14 (Berdel, Moryś, & Maciejewska, 1997). These results suggest that the age of appearance of SNC may vary depending on the experimental paradigm used (e.g., consummatory or instrumental). Also, it seems that the neural structures underlying cSNC mature earlier than those responsible for iSNC. Therefore, cSNC is likely to occur prior to iSNC (Chen et al., 1981).

Frustration has been defined as an aversive affective state provoked by the devaluation or omission of an appetitive reinforcer (Amsel, 1958). This aversive state might be reflected in disgust reactions that accompany the incentive downshift. Berridge (2000) points out that the taste reactions exhibited by subjects reflect hedonic value rather than specific sensory qualities of the reinforcer. Indeed, it has been indicated that taste reactions to gustatory stimuli are plastic and sensitive to learning. Arias et al. (2010), for instance, observed that rats given pairings of saccharin and lithium

chloride or ethanol emitted conditioned disgust reactions when subsequently stimulated with saccharin. Previous studies conducted on adult subjects have shown two main patterns of orofacial responsiveness: *Positive or hedonic*, induced by sweet tastes like sucrose or milk (e.g., mouth movements, tongue protrusions and tongue lateral movements) and *Negative or aversive*, elicited by bitter tastes like quinine (e.g., gapes, chin rubbing, head shaking, face washing, forelimb flailing, and paw pushing—Blass, Ganchrow, & Steiner, 1984; Ganchrow, Steiner, & Daher, 1983; Grill & Norgren, 1978; Steiner & Glaser, 1984; Steiner, Glaser, Hawilo, & Berridge, 2001; Ueno, Ueno, & Tomonagac, 2004). Salty or acidic flavors also trigger negative reactions but more moderate than bitter tastes (Berridge, 2000). Reactions typically accepted as disgust in adult rats may differ from those exhibited by infant rats. Several studies, however, report wall climbing and head shaking as conditioned disgust reactions in 2-week-old rats (Arias et al., 2010; Hoffmann, Hunt, & Spear, 1991; Pautassi, Arias, Molina, & Spear, 2008). Under this framework, it should be possible to infer the aversive state induced by incentive downshift in an SNC paradigm based on the emission of disgust reactions.

The main goal of this work was to assess cSNC in 14-, 17-, and 18-day-old rats using an intraoral infusion paradigm. This paradigm allows measuring percentage of body weight gained (%BWG) and disgust reactions toward the devalued reinforcer. To our knowledge, orofacial responsiveness during incentive downshift has not been assessed in infant rats. The present study analyzed wall-climbing (WC), head-shaking (HS), and chin-rubbing behaviors associated with reinforcement devaluation. The hypotheses were as follows: (1) cSNC will occur at an earlier age than that reported for iSNC (PD 25), since the former is determined by neural structures attaining their highest level of maturity at an earlier age. (2) Subjects experiencing cSNC will exhibit disgust reactions.

MATERIALS AND METHODS

Experimental Designs

A 2 (Group: 12-2, 2-2) × 2 (Trial) factorial design was employed in Experiment 1, with 10 animals in each group. In Experiments 2 and 3, a 2 (Group: 12-2, 2-2) × 4 (Trial) factorial design was employed, with 16 animals in each group. Group nomenclature refers to sugar concentrations (expressed as percentage) received during pre- and postshift phases, respectively (e.g., group 12-2 received a 12% sugar solution at preshift and a 2% sugar solution at postshift phase).

Subjects

One hundred sixteen Wistar rat pups were used. These animals were derived from 30 litters born and reared in the vivarium of the Instituto de Investigaciones Médicas Dr. Alfredo Lanari (IDIM-CONICET, Argentina). The vivarium had a 12 hr/12 hr light/dark cycle, with lights on at 7 am, and controlled temperature (22–24°C) and humidity. The day of parturition was considered postnatal day 0 (PD0). The pups were housed with their dams and had ad libitum access to water and lab chow (Cooperación, Buenos Aires, Argentina). Number of pups and litters in each experiment was as follows: Experiment 1 (20 females; 9 litters), Experiment 2 (30 animals; 8 litters; 15 females and 15 males), and Experiment 3 (64 animals; 13 litters; 33 females and 31 males). Mean and SD for ad libitum weight (g) of the subjects was 27.05 ± 1.91 and 27.07 ± 3.43 (Experiment 1), 21.84 ± 2.41 and 20.88 ± 2.27 (Experiment 2), 22.77 ± 2.02 and 22.43 ± 1.72 (Experiment 3, PD13 animals) and 17.77 ± 1.72 and 17.50 ± 2.07 (Experiment 3, PD10 animals); in experimental and control groups; respectively. Experiments were run in squads of animals composed by similar number of experimental and control subjects, and all procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Apparatus

An infusion pump (KD Scientific, Holliston, MA) was used with eight Prexajet 5-ml syringes. The total infusion volume delivered during each 10 min trial was equal to 2.5% of each animal's body weight (Pautassi et al., 2008). The syringes contained either a 12% or 2% sucrose solution. These concentrations were selected based on experiments conducted in our lab, which revealed significantly greater consumption of 12% sucrose than 2% sucrose solution (Suárez et al., 2012). The concentrations were prepared by diluting commercial sugar (Ledesma, San Luis, Argentina) in tap water until obtaining 100 ml of total solution. Twelve and two grams of sugar were diluted to prepare the 12% and the 2% solution, respectively. Each syringe was connected to PE 50 polyethylene tubing, which in turn was attached to a PE10 cannula (Clay Adams, Parsippany, NJ) previously inserted through the animal's cheek. During the deprivation period, pups were group-housed in a black acrylic box (24.5 cm × 20 cm × 22 cm) equipped with a heating pad. In Experiment 1, 2 clear acrylic boxes (28 cm × 15 cm × 15 cm) were used. Each box had four partitions (7 cm × 15 cm × 15 cm). A sheet of paper towel was placed underneath the boxes. In Experiments 2 and 3, two boxes were used of the following size: 54.5 cm front width tapering to 28 cm rear width, 23 cm (placed at roughly 45 degrees), and 24.5 cm high. Each box, in turn, had two partitions of the following size: 27 cm front width, 13.5 cm rear width, 23 cm, and 18.5 cm of partition wall. The side and rear walls and the floor were built of mirrored glass panels. The front wall was made of clear glass and the compartment partition panel of tinted glass.

Trials were recorded with two cameras (Sony, DCR-SR47). The JWatcher software (version 1.0) was used by two

independent observers, who were blind to treatment assignment, for behavioral recording and analysis.

Incentive Downshift Procedure

Three hours before starting each trial, pups were separated from their dams and intraorally cannulated, as described in Pautassi et al. (2008). Cannulas (PE 10 polyethylene tubing, length: 5 cm, Clay Adams) were made using a heat source to flatten one of the ends. They were subsequently inserted inside the animal's cheek. A dental needle was attached to the non-flanged end of the cannula and was used to place the cannula in the middle portion of the animal's intraoral mucosa, with the flattened end inside. This cannulation procedure requires no more than 10 s per subject and does not induce major stress in preweanling rats (Spear, Specht, Kirstein, & Kuhn, 1989). Alternate cheeks (i.e., right and left) were cannulated in each trial for animal tissue preservation. After 3 hr, the anal/genital region was gently stimulated with cotton wool to induce urination and/or defecation. Subsequently, the weight was recorded, and immediately after the cannula was attached to a PE50 cannula, which in turn was connected to the infusion pump. Training consisted of 4 days. During this pre-shift phase animals were given two daily trials. Testing (i.e., post-shift phase) consisted of 2 days. During each testing day animals were given two trials. Two daily trials were conducted to minimize the amount of cannulations and prevent tissue damage. Animals did not experience handling before downshift procedure. Each trial lasted 10 min with a 3-hr interval between trials. The interval between sessions was 24 hr. In the pre-shift phase, pups were intraorally infused with a continuous 12% (Group 12-2) or 2% (Group 2-2) sucrose solution. In Experiment 1, Group 12-2 received a 12% sucrose solution in Trial 1 and a 2% sucrose solution in Trial 2, during the post-shift phase. This procedure was repeated on the following day (post-shift trial 2). Group 2-2 was infused with a 2% sucrose solution throughout the experiment. At the end of each trial, the body weight of each animal was recorded again. In Experiments 2 and 3, a 2% sucrose solution was given throughout the four post-shift trials, for all age groups (i.e., 14-, 17-, and 18-day-old rats). The training boxes were cleaned with a damp cloth at the end of each trial. Training was conducted during the light cycle, between 10 am and 5 pm. The dependent variable was % BWG [(post-weight − pre-weight)/pre-weight × 100]. During the post-shift phase, the frequency and duration of wall climbing, and frequency of head shaking (Experiments 1, 2, and 3), and chin rubbing (Experiments 2 and 3) was measured. Wall climbing was defined as standing on hind legs with forepaws leaning against the box wall for support. Head shaking was defined as a rapid movement of the head from side to side. Chin rubbing was defined as bringing the mouth or chin in direct contact with the floor and projecting the body forward.

Statistical Analysis

The percentage of body weight gained during each phase was separately examined by analyses of variance (ANOVAs). A two-way ANOVA was employed to analyze the %BWG during Phase 1 trials. The between-subjects factor was Group (12-2 and 2-2) and Trials was the within-subjects factor (8 repeated measures). In the post-shift phase the between-subjects factor was Group (12-2, 2-2) and Trials was the within-subjects factor (2 repeated measures for Experiment 1 and 4 repeated measures for Experiments 2 and 3). Data were collapsed across sex since this factor exerted no significant main effect nor interacted with the remaining variables.

The loci of significant main effects or significant interactions were subsequently analyzed using *t*-test. In Experiment 2, and guided by a priori hypotheses, planned comparisons were conducted between the 12-2 and the 2-2 group in each trial of the post-shift phase.

A mixed ANOVA was used to analyze each behavior (i.e., wall climbing, head shaking, and chin rubbing), with the Group Factor as a between-subjects measure and the first two post-shift trials as a repeated measure. Reliability between the independent observers was calculated as follows: [(Total frequency of coded behaviors agreed + Total frequency of coded behaviors disagreed)/Total frequency of coded behaviors agreed] × 100. Across experiments and behaviors measured, reliability was between 91% and 98%. The loci of significant main effects or significant interactions were analyzed through follow-up ANOVAs. Across data, normality assumptions were corroborated via Kolmogorov–Smirnov tests. p Values $<.05$ were considered significant.

RESULTS

Experiment 1

Figure 1A shows the %BWG during pre- and post-shift trials in Groups 12-2 and 2-2. Visual inspection of this figure suggests that during the pre-shift phase both groups gradually increased their sucrose intake throughout the trials, with the 12-2 group exhibiting the highest level of consumption. The ANOVA for the pre-shift phase indicated a main effect of Group, F (1, 17) = 12.60, $p = .002$, and of Trial, F (7, 119) = 16.02, $p = .0001$, and a Group × Trial interaction, F (7, 119) = 5.15, $p = .0001$. Subsequent independent—samples *t*-test for each session (comparative factor: Group) revealed significantly greater %BWG in animals given 12% than 2% sucrose solutions in trials 1, 3, 5, and 7.

In the post-shift phase, animals in the experimental, 12-2, group seemed to exhibit a sudden drop in sucrose acceptance, and in Trial 10 they apparently fell below the levels of the control group. These impressions, suggestive of a negative contrast effect, were confirmed by the ANOVA, which indicated a main effect of Trial, F (3, 51) = 5.73, $p = .001$ and a Group × Trial interaction, F (3, 51) = 6.12, $p = .001$. Subsequent independent—samples *t*-test were run for each of the four trials in phase 2 (comparative factor: Group). The *t*-test for

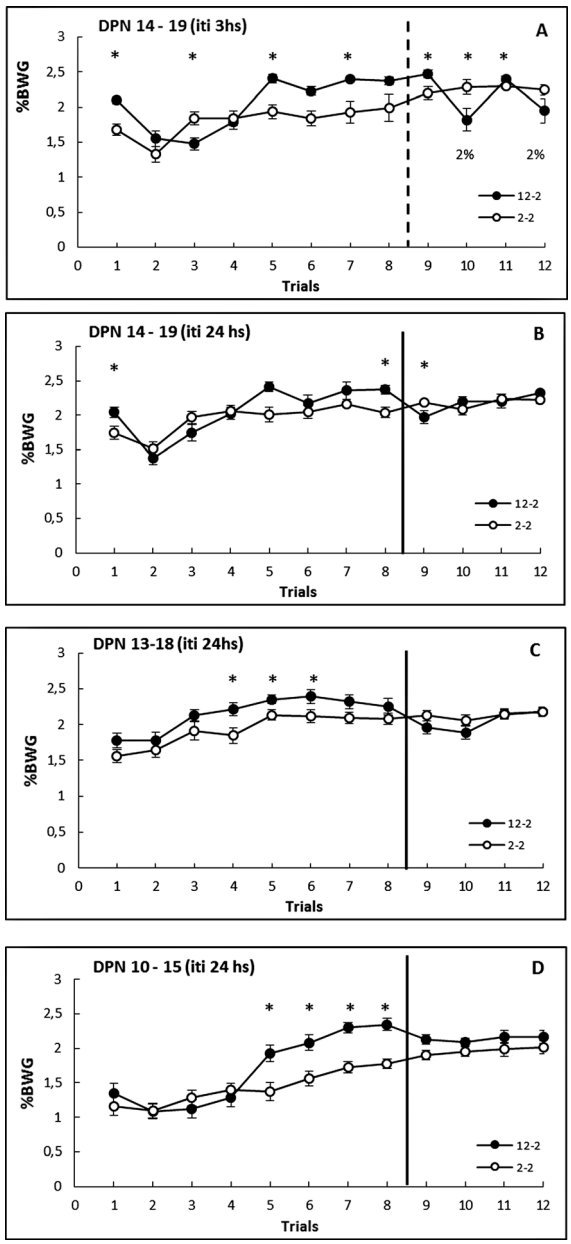

FIGURE 1 Mean percentage of body weight gained (% BWG) in pre-shift (1–8) and post-shift (9–12) trials in Group 12-2 and Group 2-2, in Experiments 1 (A), 2 (B), and 3 (C, D). Trials consisted of 10 min of continuous infusion of sucrose (total infusion: 2.5% of body weight). Means and standard error of the means are shown; $^{*}p < .05$.

trials 9 and 11 (in which groups 12-2 and 2-2 were given 12% and 2% sucrose, respectively) revealed significantly greater sucrose intake in group 12-2 than group 2-2; $t(17) = 2.53$, $p = .021$, $t(17) = 2.60$, $p = .018$. Perhaps more important, the *t*-test for Trial 10 (i.e., the first trial in which all subjects were given the less preferred 2% sucrose solution) indicated that sucrose acceptance in Group 12-2 dropped significantly below the level of Group 2-2, $t(17) = -2.51$, $p = .022$.

An analysis of disgust responsiveness only yielded a main effect of Group in the HS measure, with subjects in Group 12-2 emitting more HS than counterparts in Group 2-2, F (1, 15) = 9.09, $p = .009$. The Group × Trial interaction was not significant. Guided by our a priori hypotheses, planned comparisons were conducted between the devalued and non-devalued groups, for each measure on each downshift trial. These analyses revealed significantly greater HS scores in Group 12-2 than in control counterparts in Trial 10, F (1, 18) = 9.89, $p = .006$. During the second devaluation trial the difference between the groups only neared significance, F (1, 16) = 4.09, $p = .06$ (see Fig. 2A). Planned comparisons for wall climbing scores on Trial 10 yielded a marginal difference in frequency of wall climbing [F (1, 18) = 3.38, $p = .08$], and an statistically significant difference in duration of wall climbing behavior [F (1, 18) = 5.08, $p = .03$]. Frequency and duration of WC was higher in Group 12-2 than in the control condition (see Figs. 2A and 3A).

Results indicate the expression of cSNC in 18-day-old rats. In other words, it seems that during the third week of life animals already regulate consummatory responses based on the expected value of reinforcers. Furthermore, the decrease in sucrose consumption in rats receiving the downshifted solution was associated with a significant increase in disgust reactions (i.e., head shaking and wall climbing).

Experiment 2

A cSNC effect was obtained in Experiment 1 by 18-day-old rats giving pups high-magnitude (12%) and low-magnitude (2%) sucrose solutions during the same session, with a 3-hr interval between trials. An alternative explanation could be, however, that animals drop sucrose consumption merely due to the persistence of the previously perceived taste. Although the 3-hr interval seems to be long enough to rule out this sensory explanation, another experiment was conducted in 14- to 19-day-old rats. In Experiment 2 the post-shift phase was run 24 hr after completing the pre-shift phase. Moreover, the 2% sucrose solution was given throughout the four post-shift trials.

Figure 1B shows the percentage of body weight gained during pre- and post-shift trials as a function of Group (i.e., 12-2 and 2-2). The ANOVA for the pre-shift phase showed a significant main effect of Trial, F (7, 196) = 15.84, $p = .0001$ and a significant Group × Trial interaction, F (7, 196) = 2.96, $p = .005$. Subsequent *t*-test for each trial indicated statistically significant

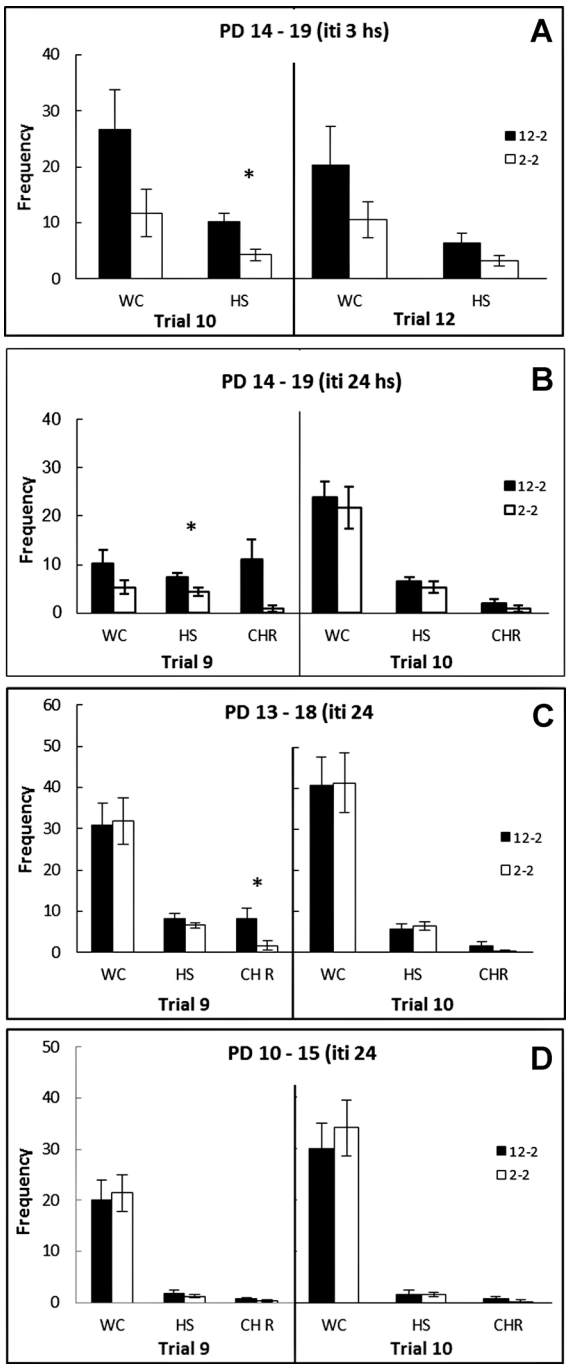

FIGURE 2 Mean frequency of wall-climbing (WC) and head-shaking (HS) in Trials 10 and 12 of Experiment 1 (A), and WC, HS and Chin Rubbing (CHR) in Trials 9 and 10 in Experiments 2 and 3 (B–D). In these trials, the animals in Group 12-2 received a 2% sucrose solution. Means and standard error of the means are shown; $^{*}p < .05$.

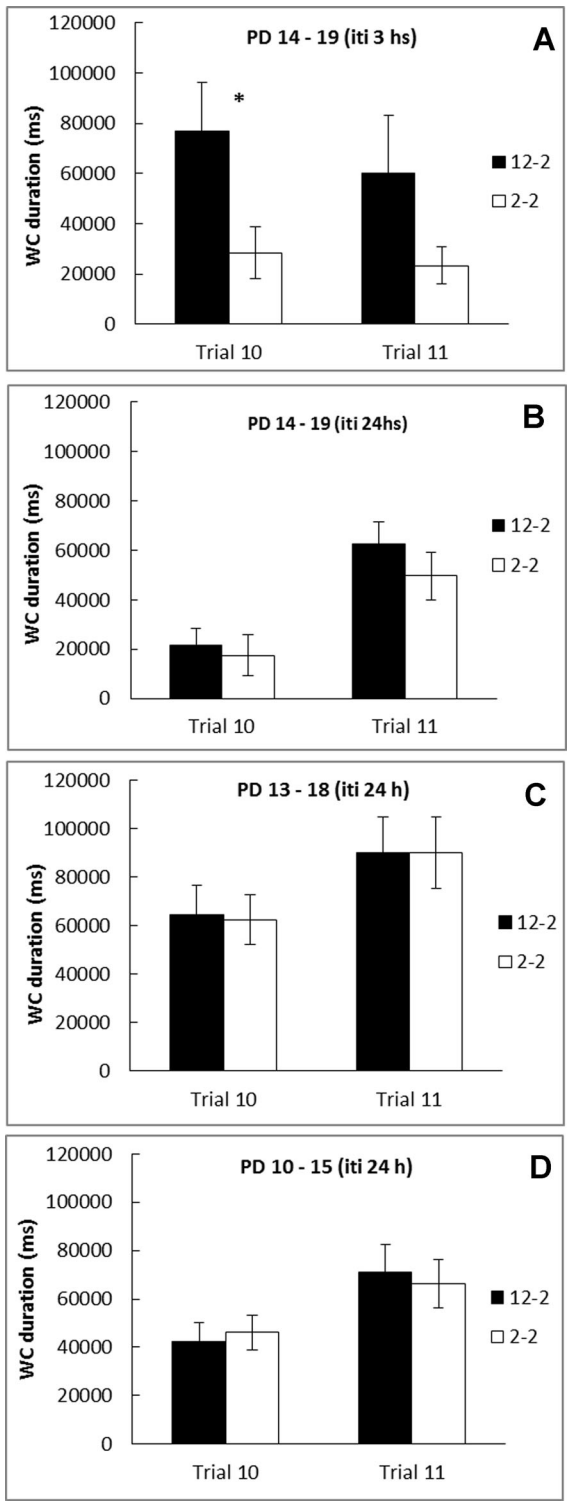

FIGURE 3 Mean duration in milliseconds of wall-climbing (WC) in Trials 10 and 12 of Experiment 1 (A), and Trials 9 and 10 in Experiments 2 and 3 (B–D). In these trials, the animals in Group 12-2 received a 2% sucrose solution. Means and standard error of the means are shown; $^{*}p < .05$.

greater sucrose intake in Group 12-2 than in group 2-2 in Trials 1 and 8.

The post-shift phase revealed a sudden drop in the % BWG of Group 12-2 compared to Group 2-2. A

mixed factor ANOVA (within factor: trials 1, 2, 3, and 4) revealed a marginally significant effect of Trial, F (1, 28) = 3.41, $p = .07$. Planned comparisons revealed significantly less sucrose intake scores in Group 12-2 than in control counterparts, during the first post-shift trial [F (1, 29) = 5.54, $p = .02$] but not during the subsequent trials.

The analysis of WC frequency and duration scores revealed a significant main effect of Trial, F (1, 27) = 33.58, $p = .0001$ and F (1, 27) = 16.65, $p = .0001$, respectively (Figs. 2B and 3B). The ANOVA for HS, on the other hand, yielded a significant Group effect, F (1, 26) = 5.92, $p = .02$. The Group × Trial interaction was not significant. Planned comparisons indicated statistically significantly greater frequency of head shaking in Group 12-2 than in Group 2-2, in Trial 9, F (1, 27) = 6.11, $p = .02$. Chin rubbing analysis showed a significant effect of Trial, F (1, 26) = 8.98, $p = .006$, Group F (1, 26) = 9.57, $p = .005$, and a significant Trial × Group interaction, F (1, 26) = 7.50, $p = .011$. Subsequent independent samples t-test indicated statistically significant greater emission of chin-rubbing behavior in Group 12-2 than in Group 2-2 in Trial 9, t (12,34) = 2.83, $p = .01$ (see Fig. 2B).

The results indicate that animals discriminate between sweet solutions with different concentrations (pre-shift phase) and that cSNC occurred during the first trial of the post-shift phase on PD 18. This effect, which disappeared in subsequent trials, was expressed 24 hr after the last pre-shift trial. It is therefore unlikely that sensory processes play a significant role in its expression. Behavioral reactivity suggests that reinforcement devaluation in 18-day-old rats produces an aversive affective state. Duration of these disgust responses coincided with the drop in consumption in downshifted animals. In other words, cSNC appeared exclusively during the first post-shift trial, and was associated with a relative enhancement of disgust reactions.

Experiment 3

The goal of Experiment 3 was to determine developmental boundaries (i.e., age of first appearance and disappearance) of cSNC. The rats were between 13 and 18 days old (post-shift trials occurred on PD 17 and 18 in this group) or between 10 and 15 days old (post-shift phase on PD 14–15). These animals were exposed to the same protocol that have indicated the emergence of sSNC in 18-day-old rats in Experiment 2.

Figure 1C (PD 13–18) and 1D (PD 10–15) illustrates that, during the pre-shift phase, pups in both age groups increased sucrose intake across trials, and that intake was higher in animals assigned to Group 12-2 than in animals in Group 2-2.

During the pre-shift phase, significant main effects of Group [F (1, 30) = 11.31, $p = .002$] and Trial [F (7, 210) = 29.006, $p = .0001$] were found in the 10- to 15-day-old group. The Trial × Group interaction also achieved significance, F (7, 210) = 5.39, $p = .00001$. t-Test conducted for each session revealed significantly greater %BWG in animals given 12% than 2% sucrose solutions in trials 5, 6, 7, and 8 (all $ps < .05$). Similarly, 13- to 18-day-old pups in Group 12-2 showed a higher %BWG than Group 2-2. At this age, the ANOVA revealed significant main effects of Group and Trial, F (1, 30) = 7.36, $p = .011$; F (7, 210) = 16.37, $p = .0001$; respectively.

During the post-shift phase, the ANOVA for 10- to 15-day-old rats yielded neither significant main effects nor significant interactions. Planned comparisons indicated that Group 12-2 decreased consumption gradually. The experimental, 12-2 group, exhibited significantly greater sucrose intake than the control group in Trial 9, F (1, 31) = 4.86, $p = .03$. Both groups exhibit similar %BWG in Trial 10.

During the post-shift phase, the behavior of 13- to 18-day-old pups was similar to the younger animals' (i.e., they showed a gradual adjustment in consumption toward the downshifted solution). A significant main effect of Trial was observed, F (3, 90) = 5.47, $p = .002$. No significant main effect of Group nor a significant interaction between Group and Trial was observed. Planned comparisons did not reveal statistically significant differences between both groups in any of the post-shift trials.

The analysis of behavioral responsiveness in 10- to 15-day-old animals yielded a significant effect of Trial for both WC frequency and duration scores, F (1, 30) = 17.14, $p = .0001$ and F (1, 30) = 12.09, $p = .002$, respectively. During Trial 10, both groups showed significantly more WC behavior than in Trial 9. The Group × Trial interaction was not significant. Planned comparisons revealed the lack of significant differences across behaviors and trials (Figs. 2D and 3D). The analysis for WC behavior in 13- to 18-day-old animals showed a significant main effect of Trial, both in frequency, F (1, 30) = 6.72, $p = .015$, as in duration of this behavior, F (1, 30) = 10.57, $p = .003$. Planned comparisons failed to show any difference in both measures (see Figs. 2C and 3C). The ANOVA for chin-rubbing yielded a significant main effect of Trial, F (1, 30) = 8.50, $p = .007$, Group, F (1, 30) = 5.83, $p = .02$, and a marginal effect for the interaction of these factors, F (1, 30) = 3.57, $p = .07$. Planned comparisons showed that Group 12-2 exhibited a higher frequency of chin-rubbing behavior in Trial 9, F (1, 31) = 5.11, $p = .03$ (see Fig. 2C,D).

Results show that, when exposing 14- to 17-day-old rats to a downshifted solution, they gradually adjust consumption toward the levels of the control group. These results help to understand the ontogeny and developmental boundaries of cSNC. It is striking that animals in the experimental 12-2 group exhibited a higher frequency of chin-rubbing behaviors, yet similar sucrose acceptance, than control, unshifted animals. This may suggest a dissociation between disgust reactions and consummatory responses (see Fig. 2D).

GENERAL DISCUSSION

The main new findings are the expression of cSNC in 18-day-old rats exposed to a downshift in reinforcement quality. These results were observed with a 3-hr interval between the pre-shift and post-shift phases (Experiment 1) and with a 24-hr interval between both phases (Experiment 2). On the other hand, 14- and 17-day-old pups given the same downshift protocol as that employed in Experiment 2 exhibited no cSNC effect (Experiment 3).

In Experiments 1 and 2, the negative contrast effect extended only over 1 trial. This is in contrast with experiments conducted in adult rats, in which cSNC lasted two to four trials (Justel et al., 2011; López Seal et al., 2010). This difference could be explained by pups having limited mnesic abilities, a more limited emotional response, or any combination between these factors (Amsel, 1992; Campbell & Spear, 1972; Rovee-Collier, 1999).

It could be argued that reduced acceptance of 2% sucrose, instead of indicating cSNC, is due to habituation or satiation. This seems, however, unlikely. Animals did not exhibit decrease sucrose acceptance during the second trial of each session of the preshift. If there had been satiety, fluctuations in the acquisition curve would have been expected. Furthermore, animals were deprived of food since home cage removal.

To our knowledge, this is the first evidence of cSNC in infant rats. The ontogeny of the phenomenon had been studied only through instrumental procedures. Chen et al. (1981) suggested that the age of first appearance of iSNC is on PD 25. cSNC seems to appear earlier than iSNC, a fact that may be accounted for by differential involvement of brain regions in each phenomena. Moreover, the amygdala attains relative maturity by PD 14 (Berdel et al., 1997), while the hippocampus completes its development by PD 25–30 (Amsel & Stanton, 1980). In the present study cSNC was observed on PD 18, yet the experiment started on PND 14. Therefore, it can be suggested that the emergence of cSNC depends on the amydgala reaching its maximum development during the pre-shift phase. Furthermore, it cannot be ruled out that the earlier appearance of cSNC, relative to iSNC, obeys to the development of inhibitory control. Under this assumption, younger animals may detect the downshift but simply not be able to express due to immature motor inhibitory abilities.

Another possible explanation for why cSNC appears earlier than iSNC might be that, although both phenomena require expectation regarding upcoming reinforcers, consummatory procedures involve recognition memory, while instrumental procedures need evocative memory. In the first case the animal has to come in contact with the reinforcer and compare it with a previously received reinforcer; in the second case, the animal has to anticipate a downshift in reinforcement in order to adjust its response, such as its running speed in a straight-alley runway (Papini & Pellegrini, 2006).

Emotional responses provoked by reinforcement omission could be detected by observing the taste reactions elicited by the intraoral infusion of the reinforcer. The present work studied, to our knowledge for the first time, taste reaction emitted in a SNC situation. Results obtained during the first devaluation trial revealed enhanced emission of aversive responses (i.e., head shaking and chin rubbing) in the experimental compared to in the control group. Seventeen-day-old animals also increased their chin-rubbing behavior during the first reinforcement devaluation trial, although these animals did not exhibit cSNC. This could suggest that, under certain circumstances, consummatory and taste reactivity are dissociated. Previous studies on conditioned taste aversion revealed that, although antinausea agents did not interfere with the LiCl-mediated consumption response, they did so with conditioned taste responses (Limebeer & Parker, 2000; Pautassi et al., 2008). Another possibility is that disgust reactions are a more sensitive index of cSNC than consumption at this age.

Results of the present study support Berridge's proposal (2000) that taste responsiveness reflects hedonic rather than sensory value. In conditioned taste aversion studies, sweet tastes are followed by lithium chloride or other malaise-inducing agents. This procedure usually results in rats suppressing hedonic reactions to sweet tastes and instead emitting aversive responses (Berridge, 2000). In the present study subjects were given a sucrose solution which typically elicits tongue protrusions and lateral mouth movements. Yet, HS, wall climbing and chin rubbing were observed after the sudden incentive downshift. It could be argued that cSNC is yet another case of psychological manipulation eliciting negative hedonic responses to an appetitive reinforcer. The results also support theories

postulating that reinforcers have a relative value and that cSNC involves aversive emotional processes (Flaherty, 1996).

In summary, the main new finding of this study was the early emergence of consummatory successive negative contrast during the third-week of life in the rat, as reflected in reinforcer intake and in disgust reactions evoked by the devalued reinforcer. Further investigation is necessary to determine which brain mechanisms are responsible for these forms of learning in infant rats, as well as to assess if it similarly expressed with other rewards. Milk acceptance, for instance, probably has a greater adaptive importance than sucrose acceptance. Therefore the age of appearance of cSNC may be different if milk had been used. The study of expectation-mediated learning seems to serve as an adequate model for assessing mechanisms involved in loss situations, even early in life.

NOTES

This study was supported by CONICET and Universidad Abierta Interamericana. We are grateful to Noelia González for her cooperation.

REFERENCES

Amsel, A. (1958). The role of frustrative nonreward in noncontinuous reward situation. Psychological Bulletin, 55, 102–119.

Amsel, A. (1992). Frustration theory. Cambridge, UK: Cambridge University Press. Appleton. Traducción al castellano en Madrid Alianza, 1984.

Amsel, A., & Stanton, M. (1980). Ontogeny and phylogeny of paradoxical reward effects. Advances in the Study of Behavior, 11, 227–267.

Arias, C., & Chotro, M. G. (2006). Ethanol-induced preferences or aversions as a function of age in preweanling rats. Behavioral Neuroscience, 120, 710–718.

Arias, C., Pautassi, R. M., Molina, J. C., & Spear, N. E. (2010). A comparison between taste avoidance and conditioned disgust reactions induced by ethanol and lithium chloride in preweanling rats. Developmental Psychobiology, 6, 545–557.

Becker, H. C., Jarvis, M. F., Wagner, G. C., & Flaherty, C. F. (1984). Medial and lateral amygdalectomy differentially influences consummatory negative contrast. Physiology & Behavior, 33(5), 707–712.

Bentosela, M., Jakovcevic, A., Elgier, A., Mustaca, A. E., & Papini, M. R. (2009). Incentive contrast in domestic dogs (Canis familiaris). Journal of Comparative Psychology, 2, 125–130.

Berdel, B., Moryś, J., & Maciejewska, B. (1997). Neuronal changes in the basolateral complex during development of the amygdala of the rat. International Journal of Developmental Neuroscience, 15, 755–765.

Berridge, K. C. (2000). Measuring hedonic impact in animals and infants: Microstructure of affective taste reactivity patterns. Neuroscience and Biobehavioral Reviews, 24, 173–198.

Blass, E. M., Ganchrow, J. R., & Steiner, J. E. (1984). Classical conditioning in newborn humans 2–48 hours of age. Infant Behavior and Development, 7, 223–235.

Campbell, B. A., & Spear, N. E. (1972). Ontogeny of memory. Psychological Review, 79, 215–236.

Catanese, F., Freidin, E., Cuello, M. I., & Distel, R. A. (2011). Devaluation of low-quality food during early experience by sheep. Animal, 6, 938–942.

Chen, J., Gross, K., & Amsel, A. (1981). Ontogeny of successive negative contrast and its dissociation from other paradoxical reward effects in preweanling rats. Journal of Comparative and Physiological Psychology, 95, 146–159.

Crespi, L. P. (1942). Quantitative variation in incentive contrast studies involving discrete-trial procedures. American Journal of Psychology, 55, 467–517.

Cuenya, L., Fosacheca, S., Mustaca, A. E., & Kamenezky, G. (2012). Effects of isolation in adulthood on frustration and anxiety. Behavioural Processes, 90, 155–160.

Cuenya, L., Sabariego, M., Donaire, R., Fernández-Teruel, A., Tobeña, A., Gómez, M. J., Mustaca, A. C., & Torres, M. C. (2012). The effect of partial reinforcement on instrumental successive negative contrast in inbred Roman High- (RHA-I) and Low- (RLA-I) Avoidance rats. Physiology & Behavior, 105, 1112–1116. (00319384).

Flaherty, C. F. (1996). Incentive relativity. Cambridge, UK: Cambridge University Press.

Freidin, E., Cuello, M. I., & Kacelnik, A. (2009). Successive negative contrast in a bird: Starlings' behaviour after unpredictable negative changes in food quality. Animal Behavior, 77, 857–865.

Ganchrow, J. R., Steiner, J. E., & Daher, M. (1983). Neonatal response to intensities facial expressions in different qualities and of gustatory stimuli. Infant Behavior and Development, 6, 189–200.

Grill, H. J., & Norgren, R. (1978). The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Research, 143, 263–279.

Hoffmann, H., Hunt, P., & Spear, N. E. (1990). Ontogenetic differences in the association of gustatory and tactile cues with lithium chloride and footshock. Behavioral and Neural Biology, 53(3), 441–450.

Hoffmann, H., Hunt, P., & Spear, N. E. (1991). Ontogenetic differences in CS palatability following conditioned taste aversion training. Learning and Motivation, 22, 329–352.

Justel, N., Ruetti, E., Mustaca, A. E., & Papini, M. (2011). Effects of pretraining treatment with testosterone on successive and anticipatory negative contrast. Physiology & Behavior, 105, 933–937.

Kobre, K. R., & Lipsitt, L. P. (1972). A negative contrast effect in newborns. Journal of Experimental Child Psychology, 14, 81–91.

Leszczuk, M. H., & Flaherty, C. F. (2000). Lesions of nucleus accumbens reduce instrumental but not consummatory negative contrast in rats. Behavioural Brain Research, 116, 61–79.

Limebeer, C. L., & Parker, L. A. (2000). The antiemetic drug ondansetron interferes with lithium-induced conditioned rejection reactions, but not lithium-induced taste avoidance in rats. Journal of Experimental Psychology: Animal Behavior Processes, 26, 371–384.

López Seal, F., Pellegrini, S., & Mustaca, A. (2010). Respuestas de elección durante el contraste negativo sucesivo consumatorio en ratas. Avances en Psicología Latinoamericana, 28, 219–225.

Mast, V. K., Fagen, J. W., Rovee-Collier, C. K., & Sullivan, M. W. (1980). Immediate and long-term memory for reinforcement context: The development of learned expectancies in early infancy. Child Development, 51, 700–707.

Mustaca, A. E., Bentosela, M., & Papini, M. R. (2000). Consummatory successive negative contrast in mice. Learning & Motivation, 31, 272–282.

National Research Council. (1996). Guide for the care and use of laboratory animals. Washington, DC: National Academic Press.

Papini, M. R., Mustaca, A. E., & Bitterman, M. E. (1988). Successive contrast in the consummatory responding of didelphid marsupials. Animal Learning and Behavior, 16(1), 53–57.

Papini, M. R., & Pellegrini, S. (2006). Scaling relative incentive value in consummatory behavior. Learning and Motivation, 37, 357–378.

Papini, M., Wood, M., Daniel, A., & Norris, J. (2006). Reward loss as psychological pain. International Journal of Psychology and Psychological Therapy 6, 189–213.

Pautassi, R. M., Arias, C., Molina, J. C., & Spear, N. E. (2008). Domperidone interferes with conditioned disgust reactions but not taste avoidance evoked by a LiCl-paired taste in infant rats. Developmental Psychobiology, 50, 343–352.

Pellegrini, S., & Mustaca, A. (2000). Consummatory successive negative contrast with solid foods. Learning and Motivation, 31, 200–209.

Rovee-Collier, C. (1999). The development of infant memory. Psychological Science, 8, 80–85.

Salinas, J. A., Parent, M. B., & McGaugh, J. (1996). Ibotenic acid lesions of the amygdala basolateral complex or central nucleus differentially effect the response to reductions in reward. Brain Research, 742, 283–293.

Salinas, J. A., & White, N. M. (1998). Contributions of the hippocampus, amygdala, and dorsal striatum to the response elicited by reward reduction. Behavioral Neuroscience, 112, 812–826.

Sastre, A., & Reilly, S. (2006). Excitotoxic lesions of the gustatory thalamus eliminate consummatory but not instrumental successive negative contrast in rats. Behavioural Brain Research, 170, 34–40.

Spear, L. P., Specht, S. M., Kirstein, C. L., & Kuhn, C. M. (1989). Anterior and posterior, but not cheek, intraoral cannulation procedures elevate serum corticosterone levels in neonatal rat pups. Developmental Psychobiology, 22, 401–411.

Steiner, J. E., & Glaser, D. (1984). Differential behavioral responses to taste stimuli in non-human primates. Journal of Human Evolution, 13, 709–723.

Steiner, J. E., Glaser, D., Hawilo, M. E., & Berridge, K. C. (2001). Comparative expression of hedonic impact: Affective reactions to taste by human infants and other primates. Neuroscience and Biobehavioral Reviews, 25, 53–74.

Suárez, A., Mustaca, A., Pautassi, R., & Kamenetzky, G. (2012). Discriminación de sabores en procedimientos de cambios sorpresivos del reforzador durante la temprana ontogenia de la rata. Suma Psicológica, 19, 19–31.

Tinklepaugh, O. L. (1928). An experimental study of representative factors in monkeys. Journal of Comparative Psychology, 8, 197–236.

Ueno, A., Ueno, Y., & Tomonagac, M. (2004). Facial responses to four basic tastes in newborn rhesus macaques (*Macaca mulatta*) and chimpanzees (*Pan troglodytes*). Behavioural Brain Research, 154, 261–271.

Upton, K. J., & Sullivan, R. M. (2010). Defining age limits of the sensitive period for attachment learning in rat pups. Developmental Psychobiology, 52, 453–464.