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BRIEF REPORT

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Consummatory succesive positive contrast produced by the downshift of an aversive solution in infant rats

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Abstract

Subjects trained in successive positive contrast are usually given an appetitive stimulus of relatively low quality during a pre-shift, followed by exposure to a significantly greater quality of the same stimulus. Enhanced responsiveness to the high-quality stimulus during the post-shift phase, compared to a control group that receives the superior reward in both phases, is taken as an index of successive positive contrast. Successive positive contrast reports are rare, probably due to performance limitations inherent to the experimental protocols available. We exposed infant rats (14 days old at the start of training) to .1% or .01% quinine during 4, 10 min, trials (pre-shift phase). All animals were then given two trials of exposure to .01% quinine (post-shift phase). During the pre-shift the level of intake was greater in pups stimulated with the relatively less aversive .01% quinine solution. These animals also exhibited, compared to those stimulated with .1% quinine, lower emission of the aversive response paw treading. During the post-shift phase, the group that had been exposed to .1% quinine exhibited significantly greater intake of .01% quinine, along with a reduction in the emission of paw treading and an enhancement in paw licking, an ingestive, appetitive response. Altogether, the evidence is suggestive of the emergence of consummatory successive positive contrast during the second week of life of the rat. To our knowledge, this is the first evidence of positive contrast using an aversive solution.

KEYWORDS
infant rats, positive contrast, qunine, taste reactivity responses

1 | INTRODUCTION

Paradoxical effects of reinforcement occur after a significant variation in the magnitude of an expected reward (Amsel, 1958). Animals exposed to a sudden downshift of an expected reward exhibit responses (e.g., reward avoidance, anxiety related behaviors) indicative of frustration (Papini, Wood, Daniel, & Norris, 2006; Phelps, Mitchell, Nut, Marston, & Robinson, 2015). On the other hand, when the value of the reward exceeds what is expected the animals can show greater reward seeking or intake (i.e., "euphoria") than un-shifted counterparts (Cuenya, Mustaca, & Kamenetzky, 2015; Cuenya, Serafini, Mustaca, & Kamenetzky, 2015; Hall, Humby, Wilkinson, & Robbins, 1997). These effects are referred to as negative and positive contrast, respectively, and indicate that animals encode information on the absolute and relative magnitude of expected rewards. In other words, behavioral responses are controlled not only by the absolute value of a reward, but also by the relationship between what is expected and what is actually received (Flaherty, 1996).

Reports of successive negative contrast abound, yet finding successive positive contrast (Flaherty, 1982) has proven a much more difficult task (also see Spence, 1956). It is possible that this obeys to methodological or performance limitations inherent to the experimental protocols available. Subjects trained in successive positive contrast are usually given an appetitive stimulus of relatively low quality (e.g., access to a 4% w/v sucrose solution), for several sessions. After this pre-shift phase, they are suddenly exposed (i.e., "shifted") to a significantly greater quality of the same stimulus (e.g., access to a 32% w/v sucrose solution). Enhanced intake of the high-quality solution during the second phase, compared to a control group that receives the superior reward in both phases, is taken as

an index of consummatory successive positive contrast. A caveat is that the control group may be responding near the upper, functional limit of the dependent variable. Under these "ceiling effect" conditions, it would be very difficult, if not impossible, for the upshifted group to overshoot the control.

Cándido, Maldonado, Rodríguez, and Morales (2002) described consummatory successive positive contrast in an aversive situation involving a nociceptive stimulus. The authors placed rats in a compartment in which they received a warning signal followed by an electric shock. The animals learnt to anticipate the shock and were eventually able to avoid it by escaping to a safe, adjacent compartment. During a pre-shift phase, the experimental group was allowed to stay only 1 s in the safe compartment, whereas in the post-shift phase this period was extended to 30 s. Control animals were allowed 30 s stays in the safe compartment in both phases. During the post-shift, experimental animals exhibited significantly lower escape latency than control counterparts. The authors proposed that the safe place is a positive reward whose incentive value becomes relative due to the relationship between what was experienced in the pre-shift and post-shift phases.

To our knowledge, however, there has been no attempt to assess consummatory successive positive contrast using aversive, sapid solutions. A possibility would be to expose experimental animals to a .1% of quinine solution for several trials (pre-shift phase), followed by exposure to a much less aversive (.01%) quinine solution. Enhanced intake of the latter solution in the experimental group, relative to a control group given .01% quinine throughout trials, would reveal consummatory successive positive contrast. This protocol should prevent the occurrence of a ceiling effect.

Paradoxical effects of reinforcement in the rat emerge between postnatal days (PD) 10 and 63, albeit most of these studies have employed only instrumental procedures (Amsel, 1992). We, however, recently reported consummatory successive negative contrast in 2 week-old rats (Suárez, Mustaca, Pautassi, & Kamenetzky, 2014). We observed reduced consumption and a significant decrease of orofacial disgust reactions, as assessed in a taste reactivity test, in response to a sweet solution that had been devaluated. Taste reactivity involves appetitive (e.g., paw licking, tongue protrusions) and aversive (e.g., paw treading, chin rubbing, gaping) responses towards sweet and bitter tastes, respectively (Berridge, 2000; Grill & Norgren, 1978; Steiner, Glaser, Hawilo, & Berridge, 2001), that are amenable to experience (Arias & Chotro, 2005; Arias, Pautassi, Molina, & Spear, 2010; Díaz-Cenzano & Chotro, 2010a; Lin, Arthurs & Reilly, 2013; Parker & MacLeod, 1991). Pautassi, Arias, Molina, and Spear (2008) reported taste avoidance and conditioned disgust reactions (e.g., head-shaking) in rats stimulated with saccharin paired with lithium chloride (LiCl). This is, the taste-LiCl pairings resulted in a palatability shift.

The main aim of this work was to test the occurrence of consummatory successive positive contrast in preweanling rats exposed to a highly aversive solution (.1% quinine), followed by exposure to a lower quinine concentration (.01%). The hypothesis was that this reduction or shift in the hedonic value of the taste would result in a greater quinine intake, and a hedonic-like pattern of taste responses towards this bitter solution.

2 | MATERIALS AND METHODS

2.1 | Experimental design

A two-group (.1, –.01) design was employed, with 9–10 animals in each group. Group nomenclature refers to quinine concentrations (%) received during pre-shift phase (i.e., group .1 was exposed to .1% quinine during the pre-shift phase, which was composed by four trials). All groups were then given two trials of exposure to .01% quinine (post-shift phase).

2.2 | Subjects

Nineteen Wistar infant rats (14–19 PD) were used, 10 females and 9 males. These animals were born and reared at the vivarium of the Instituto de Investigaciones Médicas Dr. Alfredo Lanari (IDIM-CONICET, Argentina). The vivarium had a 12/12 hr light/dark cycle, with lights on at 7 a.m., 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 which had *ad libitum* access to water and lab chow (Cooperación, Buenos Aires, Argentina). The experiment was run in squads composed by similar number of experimental and control subjects. All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

2.3 | Procedure

The training (i.e., pre-shift phase, PDs 14–17) consisted of four daily trials (10 min length), in which the animals were intraorally stimulated with .1% (Group .1) or .01% (Group .01) quinine. These solutions were prepared by diluting 100 mg or 10 mg quinine (Sigma–Aldrich, Buenos Aires, Argentina), respectively, in 100 ml of distilled water.

At the beginning of each trial pups were separated from the dam for 90 min. They were then intraorally cannulated, as described in Pautassi et al. (2008). The cannulas (PE 10 polyethylene tubing, 5 cm length) were made using a heat source to flatten one of the ends. A dental needle, attached to the non-flanged end, was used to place the cannula in the middle portion of the mucosa, with the flattened end inside. This procedure requires ±8 s per subject and does not induce major stress (Spear, Specht, Kirstein, & Kuhn, 1989). Alternate cheeks were cannulated in each trial.

After the cannulation, pups were group-housed for 90 min in a black acrylic box ($24.5 \times 20 \times 22\ cm^3$) kept warm with a heating pad. The urogenital region of each animal was then gently stimulated with a cotton wool to induce urination and defecation. Animals were weighted and placed in a trapezoid-shaped chamber with a front clear wall (34 cm wide) made of glass. The side and rear (18 cm) walls and the floor were made of mirrored glass panels. The chamber (18 cm high) was divided into two halves with a partition panel made of tinted glass. The glass panels were intended to facilitate the analysis of taste responses regardless of the location of the pup. Two pups were stimulated at a time, one per section of the chamber. Each intraoral cannula was attached to a PE50 length, which in turn was connected to

an infusion pump (APEMA, Buenos Aires, Argentina), equipped with four Prexajet 5 ml syringes. The pump delivered the corresponding quinine solution at a continuous rate, and the volume was adjusted to deliver 2.5% of the pup's body weight. This procedure combines forced administration of the solution with voluntary intake. Previous studies (e.g., Pautassi et al., 2008) indicate that the animals can regulate the amount of liquid consumed by deliberately emitting ingestive (e.g., tongue protrusions) or rejective (e.g., head shaking) taste reactivity responses. At the end of each trial the body weights were recorded again and the trapezoid box was cleaned with a damp cloth.

During the test (i.e., post-shift phase: two trials of 10 min each, conducted in PDs 18–19) both groups were stimulated with .01% quinine, following the procedures and parameters described for the training. The last pre-shift and the two test trials were recorded (Sony DCRSR47, Minato, Tokyo, Japan) for subsequent analysis (Jwatcher software, version 1.0, Sydney, Australia and Los Angeles, CA), by two independent observers who were blind to treatment assignment. The reliability between the independent observers {calculated as follows: for frequency, [Total frequency of coded behaviors agreed/(Total frequency of coded behaviors disagreed + Total frequency of coded behaviors agreed)] *100; for duration, minor total time/major total time} was >93%.

The percentage of body weight gained [% BWG: (post-infusion weight—pre-infusion weight)/preinfusion-weight *100] was calculated in each trial. Appetitive (i.e., frequency and duration of paw licking) and aversive (i.e., frequency of head shaking and chin rubbing, frequency and duration of paw treading) measures, were analyzed. Head shaking was recorded each time that a rapid movement of the head from side to side occurred. Chin rubbing was defined as bringing the mouth or chin in direct contact with the floor and projecting the body forward. Paw treading was recorded when the animal vigorously rubbed its forelimbs against the floor. Finally, paw licking was defined as licking of forelimbs (Díaz-Cenzano & Chotro, 2010b; Grill & Norgren, 1978; Pautassi et al., 2008).

2.4 | Data analysis

Separate, 2-way analyses of variance (ANOVAs) were employed to analyze the %BWG achieved during pre-shift and post-shift trials. The between-subjects factor was Group (.1 and .01), whereas Trials was the within-subjects factor (i.e., training trials 1–4 for pre-shift scores; test trials 1 and 2 for post-shift scores). Separate *t*-tests were used to analyze behavioral or taste reactions responses towards quinine (i.e., head shaking, chin rubbing, paw treading, and paw licking) during the last training day; whereas separate mixed ANOVAs were used to analyze these behaviors during the post-shift phase.

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 through follow-up ANOVAs. Planned comparisons were conducted between shifted and unshifted groups, when justified by our *a priori* hypotheses. A previous study conducted in our laboratory indicated that rats—of the same age as those employed in the present study—expressed negative contrast only in the first post-shift trial (Suárez et al., 2014). Therefore, we expected that rats exposed to the consummatory successive positive contrast procedure would show greater quinine acceptance than controls only in the first post-shift trial. The alpha value was kept at .05.

3 | RESULTS

The ANOVA for %BWG scores during the pre-shift indicated a significant main effect of Group [F (1, 17) = 16.22, p < .0001] and Trial, [F (3, 51) = 13.77, p < .0001], and a significant Group x Trial interaction [F (3, 51) = 3.55, p < .021]. Subsequent one-way ANOVAs for each session revealed significantly less %BWG in animals given .1% than in those given .01% quinine, in trials 2 [F (1,17) = 15.24, p < .001], 3 [F (1,17) = 17.20, p < .0007], and 4 [F (1,17) = 21.67, p < .0002—see Figure 1A]. The corresponding ANOVA for post-shift scores yielded a main effect of Trial [F (1, 17) = 6.87, p < .018], and a Group × Trial interaction [F (1, 17) = 14.83, p < .001]. Subsequent one-way ANOVAs revealed that Group .1 consumed significantly more .01% quinine than Group .01 in the first post-shift trial [F (1, 17) = 5.26, p < .035—see Figure 1A].

A *t*-test revealed that, during the last pre-shift trial, animals given .1 quinine exhibited significantly greater frequency [t (17) = 4.12, p < .001] and duration [t (17) = 3.92, p < .001] of paw treading than those in Group .01. Group assignment did not significantly modulate the expression of the other taste reactivity responses, during this trial. Figure 1B and C show the results of those responses that yielded significant differences.

The ANOVAs for taste reactivity responses in the post-shift trials revealed a significant main effect of Trial for duration of paw licking [F (1, 17) = p < .01]. Planned comparisons revealed significantly greater paw licking during the first post-shift trial in the group .1 than in group .01 [F (1, 17) = 6.22, p < .024—see Figure 1C]. The ANOVA for paw treading showed a significant Group × Trial interaction [F (1, 17) = 5.20, p < .036]. Subsequent analysis revealed that, in the first post-shift trial, the Group .1 performed paw treading for a significantly less time than the Group .01 [F (1, 17) = 5.06, p < .04—see Figure 1C]. The ANOVAs for chin rubbing and head shaking indicated the lack of significant main effects or significant interactions. Planned comparisons, however, indicated significantly less emission of chin rubbing in group .1 than in group .01 [F (1, 17) = 5.01, p < .04], during the first post-shift trial (see Figure 1B).

4 | DISCUSSION

The main result was that preweanling rats given a sudden reduction in the magnitude of an aversive stimulus exhibited behaviors suggestive of consummatory successive positive contrast. This apparent paradoxical effect of reinforcement was observed in intake and taste reactivity responses. Pups given .01% quinine after a history of exposure to .1% quinine exhibited, when compared to an un-shifted group continuously exposed to .01 quinine, enhanced intake and an altered pattern of behavioral response towards the solution.

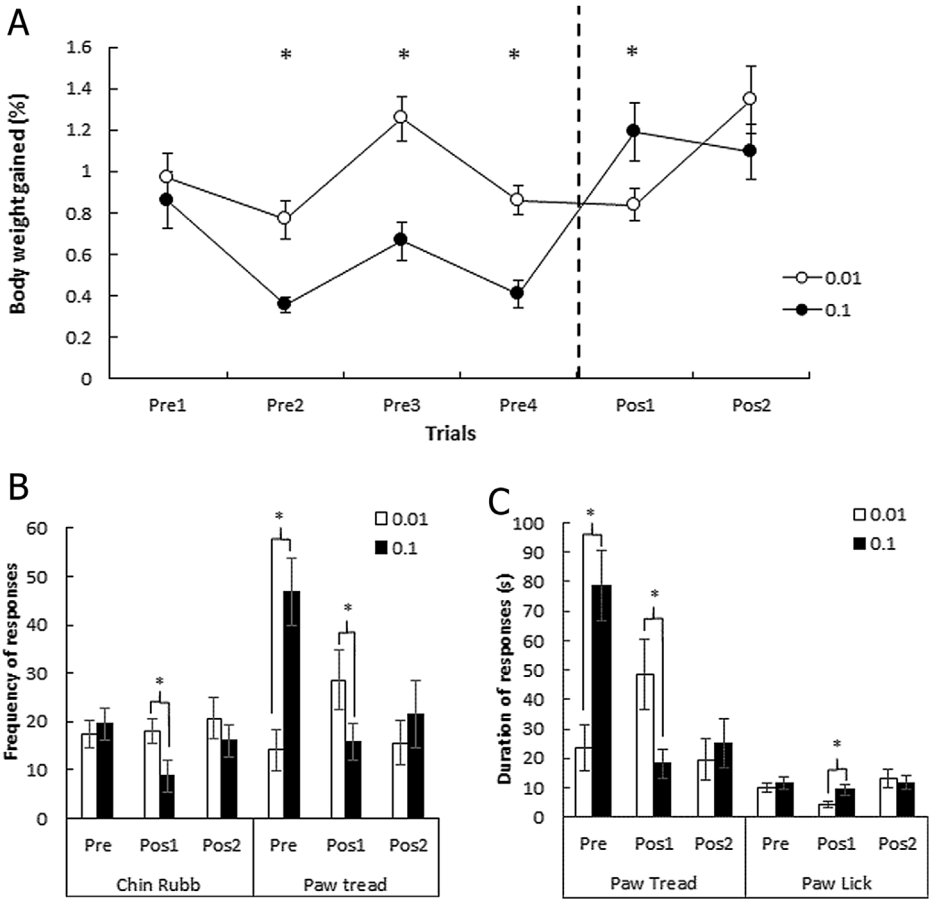

FIGURE 1 (A) Mean percentage of body weight gained (%BWG) as a function of trials, for each group. Nomenclature .01 and .1 refers to quinine concentrations (%) received during pre-shift phase. The white circles represent the control group (.01) and black circles, the experimental group (.1). Both groups received .01 at post-shift phase. Trials consisted of 10 min of continuous infusion of a quinine solution. The dotted line indicates where the shift phase occurred. (B) Mean frequency of aversive responses (i.e., chin rubbing and paw treading) in the last pre-shift trial and in the two post-shift trials, for each group. (C) Mean duration (seconds) of paw treading and paw licking (aversive and hedonic responses, respectively) in the last pre-shift trial and in the two post-shift trials, for each group. The white bars represent the control group (.01) and black bars, the experimental group (.1). *Indicates p values <.05

During the pre-shift the level of intake was—as expected—greater in pups stimulated with the relatively less aversive .01% quinine solution. These animals also exhibited, compared to those stimulated with .1% quinine, lower emission of paw treading, a behavior usually clustered among aversive taste reactivity responses (Berridge, 2000; Hoffmann, Hunt, & Spear, 1991). This pattern reversed during the post-shift phase: the upshifted group exhibited significantly greater intake, a reduction in the emission of paw treading and an enhancement in paw licking, an ingestive, appetitive response. Altogether, the evidence is suggestive of the emergence of consummatory successive positive contrast during the second week of life of the rat. Moreover, to our knowledge this is the first evidence of positive contrast using an aversive solution. In other words, the results suggest that the upshift changed the hedonic value of the quinine solution. Conditioned changes in ingestive and hedonic patterns of response to flavors have been reported in adult (Parker, 1995) and preweanling rats (Arias et al., 2010; Pautassi et al., 2008; Suárez et al., 2014). For instance, Arias et al. (2010) exposed rat pups to pairings of saccharin and the aversive, post-ingestive consequences of ethanol or lithium chloride. When re-stimulated with saccharin at a test, these animals exhibited saccharin avoidance, as shown by reduced consumption of the sweet solution and increased amounts of grooming, general activity, head shaking, and wall climbing.

The findings of the present study can also be explained by the development of expectative and associated internal representations of reward. Under this framework, continuous exposure to an extremely bitter solution resulted in the development of an expectative of reinforcement. This expectative was violated (i.e., not met) during the up-shift, which in turn caused euphoria, operationalized through greater quinine intake and an altered pattern (i.e., more appetitive, less aversive) of responses towards the tastant (Cuenya, Mustaca, & Kamenetzky, 2015; Cuenya, Serafini, Mustaca, & Kamenetzky, 2015; Suárez et al., 2014).

Some studies, that altered reward value, could also be framed under a "euphoria" interpretation. As already mentioned, adult rats exposed to a one-way avoidance task showed evidence of instrumental successive positive contrast. Animals that used to spend only 1 s in a safe place after an electric shock, escaped faster (i.e., show shorter latencies) when

allowed 30 s to stay, as compared to controls always trained with 30 s (Cándido et al., 2002). Further research is needed to determine whether the mechanisms underlying this result (found via a conditioned fear protocol) are similar to those underlying the result found in the present study through the aversive solutions. It is also unknown whether the reduction of the concentration of quinine results in relief (i.e., negative reinforcement), or if the aversive solution actually becomes appetitive. The protocol put forward in the present work may represent a psychological manipulation promoting positive "liking" reactions towards an aversive solution (i.e., quinine), similar to those observed in experiments that manipulated a physiological sodium deficiency state (Tindell, Smith, Peciña, Berridge, & Aldridge, 2006). Future research are needed to elucidate these important questions.

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