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ABSTRACT: *Adolescence is a time involving a series of changes in the use of appetitive reinforcers like food, as well as neuroendocrine changes like those taking place in the mesolimbic dopamine function. Social isolation from postnatal day 21 to 36 in rats leads to behavioral and neurophysiological alterations such as increased consumption of appetitive reinforcers. The work is focused on studying how exposure to chronic stress induced by social isolation during adolescence can have a long-lasting effect on responses to reinforcement shifts in adulthood. Two experiments were performed in rats in order to analyze the effect of adolescent isolation on the responses to unanticipated shifts in reinforcement during adulthood, in reinforcement devaluation (32–4% of sucrose solution), increase (4–32% of sucrose solution), and extinction (32–0% of sucrose solution) procedures. Adolescent isolation intensified the intake response resulting from a reinforcement increase (i.e., greater positive contrast), but had no effect on the response to reinforcement devaluation and omission. The implications of this procedure are discussed, along with the underlying behavioral and neurochemical mechanisms.* © *2015 Wiley Periodicals, Inc.* Dev Psychobiol

Keywords: *adolescence; isolation; reward; contrast; rat*

INTRODUCTION

Adolescence is a time of transition between childhood and adulthood when behavioral changes occur, including an increased focus on peer-directed social interactions; novelty seeking and the pursuit of new sensations (see Spear, 2000); changes in the use of appetitive reinforcers like food (e.g., Neumark-Sztainer et al., 2006; Patton, Coffey, & Sawyer, 2003; vanStrien, van der Zwalum, & Engels, 2010) and drugs of abuse (e.g., Chen & Jacobson, 2012; Cuenya, 2006; Krank et al., 2011); and close proximity to incentive situations (see Ernst, Romeo, & Andersen, 2009). In rats, it is characterized by behavioral and neurochemical changes from postnatal day 20 (PND 20) to PND 55 (see Spear, 2000). There is an increase in social interaction and play behaviors with peers (Vanderschure, Niesink, & Van Ree, 1996), exploratory behaviors (e.g., Douglas, Varlinskaya, & Spear, 2003; Fox, Sterling, & Van Bockstaele, 2009), release of gonadal hormones (see Sisk & Foster, 2004), and changes in the mesolimbic dopamine function (see Wahlstrom, Collins, White, & Luciana, 2010).

Early post-weaning social isolation in rats is one of the protocols producing behavioral and neurophysiological alterations in adulthood. Usually, the procedure consists in socially isolating animals for a specific time period with as little manipulation as possible for home-cage cleaning only; however, the subjects keep visual, auditory, and olfactory contact with their littermates in the room (e.g., Weiss, Domeney, Heidbreder, Moreau, & Feldon, 2001). The behavioral and neuroendocrinological changes produced as a result of adolescent isolation are mainly observed when treatment is applied since the first day of weaning (PND 21) until PND 30. Within the wide range of alterations noted during adulthood can be found a decrease in social interactions, impairment of

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inhibitory mechanisms and sensorimotor gating, shown in low latent inhibition and prepulse inhibition, hyperresponsivity to novel environments, cognitive inflexibility, dopaminergic hyperactivity in the nucleus accumbens (NAcc) and in the ventral striatum, and glutamatergic and dopaminergic hypoactivity in the prefrontal cortex (see Fone & Porkess, 2008).

Several studies have shown that adolescent isolation produced alterations in the consumption of appetitive reinforcers during adulthood, like hyperphagia (Fiala, Snow, & Greenough, 1977; Jahng, Yoo, Ryu, & Lee, 2012), higher preference for sucrose solutions (Hong et al., 2012), and changes in responsivity to novelty in food (Hall, Humby, Wilkinson, & Robbins, 1997), albeit these effects might depend on experimental parameters and relevant variables such as sex (Hall et al., 1997; Hellemans, Benge, & Olmstead, 2004; Hong et al., 2012). These studies were focused on exploring the effects on the absolute value of reinforcements. However, the effect that a reinforcer has upon behavior is determined not only by its absolute value but also by its relative value, which in turn depends on the animal's previous experience with reinforcers of different quality and quantity (Flaherty, 1996). The Successive Negative Contrast (SNC) and Successive Positive Contrast (SPC), as well as Extinction are all models of wide use when investigating the response to an unexpected shift in reinforcement, the violation of expectation, and the euphoria or frustration responses entailed by such situations (Amsel, 1992; Crespi, 1942; Flaherty, 1996). Briefly, SNC in rats involves a decrease in the consummatory behavior of a low valued reward or in the instrumental response required to obtain it, after being trained with a higher valued reward. Whereas SNC is triggered by the presentation of an unexpectedly reduced reward, Extinction consists of the complete omission of rewards in a situation previously asociated with them. Meanwhile, SPC consists of an increased consummatory behavior or instrumental response when the animal finds a reward with a higher hedonic value than expected. Shanab and Ralph (1979) compared adult male rats that were group-housed or isolated from weaning to adulthood in an instrumental SNC (iSNC) and in the Instrumental Partial Reinforcement Extinction Effect (iPREE), using a straight-alley maze. They found out that housing conditions had no effect on the iSNC, but that only isolated animals showed iPREE, which the authors interpreted as the expression of a higher level of emotionality or anxiety under such conditions. However, this study did not make a distinction between the specific effect of adolescent isolation (AI) and the possible effect of adult isolation. Adult isolation is also considered a stressor generating alterations in mammals, although its effects cannot be matched to AI as each one produces different patterns of deleterious consequences (see Morgan & Tromborg, 2007).

This same methodological problem is seen in the study by Hall et al. (1997). The authors compared adult male Lister hooded rats reared in isolation after weaning for 8 weeks with rats housed always in groups, in connection with the consumption of sucrose solutions of different concentrations (0.7%, 2.1%, 7%, 21% and 34%) presented in an ascending and descending order during 5 min trials. The authors concluded that the consummatory positive contrast was increased in isolated animals, while no differences were noted in the negative contrast. Not only did the study fail to differentiate between the effect of AI and adult isolation, but also had yet another problem-consumption trials were run every 25 min instead of every 24 hr like in standard incentive contrast procedures (e.g., Cuenya, Fosacheca, Mustaca, & Kamenetzky, 2011; Cuenya, Fosacheca, Mustaca, & Kamenetzky, 2012; Flaherty, 1996) so that differences might be due to a sensory after-effect and not to a violation of expectation of central origin. Finally, the design used did not feature groups receiving the same incentive value throughout the training so as to determine the contrast effect. Therefore, these results do not allow for conclusive interpretations on how AI affects the animals' response to shifts in reinforcements.

The purpose of this paper is to analyze the specific effect of exposure to chronic stress induced by social isolation during adolescence on responses to an unexpected shift in reinforcements during adulthood using sucrose solutions, in reinforcement devaluation, increase, and extinction procedures.

EXPERIMENT 1

Considering that AI treatment generates greater anxiety (e.g., Bledsoe, Oliver, Scholl, & Forster, 2011) and heightened locomotion in adulthood (e.g., Meng, Li, Han, Shao, & Wang, 2010), and that responses to an unexpected shift in reinforcement are related to anxiety and locomotion levels (Amsel, 1992; Flaherty, 1996), in Experiment 1 animals were evaluated in an elevated plus maze (EPM), with the purpose of studying the effects on these variables during free exploration in order to confirm the efficacy of the treatment. Experiment 1 was also intended to record possible alterations in a situation involving a series of reward shifts-devaluation, effect of partial reinforcement on devaluation, omission, and incentive increase.

Materials and Methods

Subjects. Thirty-two naïve male Wistar rats (weighing 246–432 g at the start of the experiment), representative of 5 L were employed (5–9 males per litter). The animals were housed in groups of 4 in stainless steel cages measured 44 cm in length, 32 cm in width, and 22 cm in height. When they were 75 days old they were food deprived until their weights were lowered to 85% of individual ad libitum weights. The animals were individualized for 3 hr daily in stainless steel cages measured 28 cm in length, 26 cm in width, and 22 cm in height, where a restricted amount of food was administered according to their weights. This procedure does not affect responses to reward changes, as previous data of our laboratory showed that full isolation during adulthood does not affect the frustration and euphoria responses in consummatory SNC and consummatory partial reinforcement on incentive devaluation effect (Cuenya et al., 2011; Cuenya et al., 2012). During training, the rats were fed daily at least 20 min after completion of the training trial and animals were kept in a daily light–dark cycle of 12 hr (lights on at 07:00 h). The housing and testing rooms were maintained at a relatively constant temperature (around 22 °C) and humidity. All testing sessions were performed between 12:00 and 16:00 h. All procedures were in accordance with the Guide for Care and Use of Laboratory Animals (1996).

Apparatus.

Elevated plus maze. Rats were tested in a maze constructed in Plexiglas of four arms forming a cross that extended from a central square platform. The two closed arms were surrounded by walls of black acrylic on the perimeter of 40 cm high. Each arm had a length of 50 cm and a width of 10 cm, and the maze floor was elevated to 50 cm. The two open arms had a base of 1.5 cm high at the sides and the front wall. A SONY Video Camera was used to film the behavior of animals in the EPM.

Conditioning boxes. Rats received training in four similar conditioning boxes enclosed in a sound-attenuating cubicle (MED Associates, East Fairfield, VT). Each box measured 24.1 cm in length, 29.2 cm in width, and 21 cm in height. The floor was made of aluminum bars (0.4 cm in diameter, 1.1 cm apart). In the center of one of the lateral walls there was a 5 cm hole, 3.5 cm deep, 1 cm above the floor level, through which a sipper tube could be introduced from the outside. When fully inserted into the hole, the sipper tube protruded 2 cm. A diffuse house light was located above the sipper tube, 18 cm above the floor. The goal-tracking time (GTT) in 0.01 s units was the main dependent variable, and it was measured by detecting the insertion of the rat's head into the hole by means of a photocell. Goal-tracking time correlates positively and significantly with the amount of fluid intake during 5 min trials (Mustaca, Freidin, & Papini, 2002). Sucrose solutions (in weight per volume) were prepared by mixing the appropriate quantity of commercial sugar (320 or 40 g) in 1 L of tap water.

Procedure.

Postweaning isolation. In PND 21, animals were assigned to two housing conditions, either in groups or in isolation. Both treatments were represented in each litter. The grouped subjects were housed in metal cages (44 cm long, 32 cm wide, and 22 cm high) in groups of 5 or 6, while isolated rats were placed in individual cages (28 cm long, 20 cm wide, 15 cm high), with black plastic walls and floor and metal bar roof, until PND 36. In both cases the floor was covered with wood shavings, and the isolated animals were allowed to have visual, auditory, and olfactory contact with other rats. From PND 36 to PND 60, the grouped and isolated subjects were regrouped between animals of the same condition in cages holding 5–6 animals each. Prior to regrouping, the animals were weighed and no significant differences were found between isolated and grouped treatments, $T(30) = 0.03$, $p < .9$. At two months of age and throughout the experiment, the animals were kept in large metal cages housing 4 animals each.

EPM test. At PND 75, the animals were assessed with EPM tests. Each subject was placed in the center of the maze facing the closed arm. The test was administered before training in procedures of reward change because previous studies and unpublished data from our laboratory indicate that prior manipulation influences results in EPM test (e.g., Da Cunha et al., 1992; Doremus-Fitzwater, Varlinskaya, & Spear, 2009). The test lasted 5 min, and was filmed with a video camera placed approximately 1 m above the maze. At the time of the test, the experimenter was out of the room, which was illuminated with a central red light. Feces and urine were removed with a paper towel between trials and the device was wiped with a damp cloth to mix the scents. The behaviors were then analyzed with the program JWatcherV1.0 for ethological observation. The number of entries and the time spent by the animals in the closed and open arms was measured, counting as an entry each time the animal placed its two front paws into the arm. Entries and the time spent in the open arms were regarded as indicators of anxiety, and entries to the closed arms as a measure of

locomotor activity. The behaviors were measured twice by one blind observer. The reliability coefficient of both measures was obtained by dividing the number of agreements between observations by the total number of observations and then multiplying the value by 100. Reliability was above 90%.

Consummatory partial reinforcement on incentive downshift effect (cPRIDE). This procedure started on PND 90 and consisted of a total of twenty-one 5 min daily trials, during which the animal had free access to a sipper tube in the conditioning boxes. The preshift phase extended over 14 trials. The continuous reinforcement group-housed (GC, $n = 8$) and isolation-housed (IC, $n = 8$) groups had access to a 32% sucrose solution throughout the trials, while the partial reinforcement group-housed (GP, $n = 8$) and isolated-housed (IP, $n = 8$) groups experienced 50% of reinforced trials (R, 32% solution), and 50% of non-reinforced trials (N, empty sipper tube). The R and N trial sequence was the same for all the subjects in the partial reinforcement program: R-N-R-R-N N-R-N-R-N-N-R-N-R. In the postshift phase all the animals had access to a 4% sucrose solution during 7 trials with a continuous reinforcement program. Table 1 illustrates the experimental design used.

Data Analysis. A statistical SPSS 17 package was employed. The data obtained from the EPM were analyzed using an ANOVA with two between-subjects factors—Isolation (Isolated vs. Grouped) and Reinforcement (Continuous vs. Partial). The goal-tracking time (GTT) data from each trial were analyzed using a three-factor analysis of variance with two between-subjects factors—Isolation (Isolated vs. Grouped), Reinforcement (Continuous vs. Partial), and Trials as within-subjects factor (14 in the preshift phase, and 4 in the postshift phase). Two separate ANOVAs were run for the preshift and postshift data, and the preshift data were analyzed separately for the reinforced and non-reinforced trials. When the data violated the sphericity assumption, the Greenhouse–Geisser correction was applied. Pairwise comparison analysis was performed using Bonferroni adjustment for multiple post-hoc comparisons. The alpha significance level was set at .05 for all comparisons.

Results

EPM. To rule out biased assignment of animals the factor Reinforcement (Continuous vs. Partial) was included. As expected, the Reinforcement factor did not show any effect, therefore further analysis were conducted comparing Isolated versus Grouped animals. Figure 1 shows that isolated subjects had significantly more amount of entries to the closed arms than the grouped ones, $F(1,28) = 4.45$, $p < .05$, entered more to the open arms though the result was not statistically significant. Both conditions did not differ regarding the time spent in open and closed arms ($Fs < 1.71$, $ps > .2$). These results indicate that isolated animals were more active than grouped animals, but they did not show differences in their levels of anxiety.

cPRIDE.

Preshift phase: non reinforced trials. The GTT is plotted in Figure 2. It was higher in subjects who received the reward, and also there was not difference regarding the response to reward omission between subjects with or without AI. The ANOVA yielded a main effect of Reinforcement, $F(1,23) = 248.92$, $p < .001$, and Trial × Reinforcement interaction, $F(6,138) = 3.12$, $p < .01$. The Isolation factor and the rest of possible interactions were not significant.

Preshift phase: reinforced trials. Consummatory behavior was acquired along the preshift phase in both conditions. In subjects without AI there was no difference in GTT between partial and continuous reinforcement groups, whereas in subjects with AI it was found in the last four reinforced trials with partial reinforcement that the animals increased their GTT to higher levels compared to those receiving continuous rein-

Table 1. Experimental Design Used in Experiment 1

			cPRIDE PND 90	
Group	PND 21–36	PND 75	Preshift 14 trials	Postshift 7 trials
GC $n = 8$	Grouped	EPM	32% sucrose solution	4% sucrose solution
GP $n = 8$	Grouped	EPM	50% trials 32% sucrose solution 50% trials empty tube	4% sucrose solution
IC $n = 8$	Isolated	EPM	32% sucrose solution	4% sucrose solution
IP $n = 8$	Isolated	EPM	50% trials 32% sucrose solution 50% trials empty tube	4% sucrose solution

Note: GC, grouped–continuous reinforcement; GP, grouped–partial reinforcement; IC, isolated–continuous reinforcement; IP, isolated–partial reinforcement.

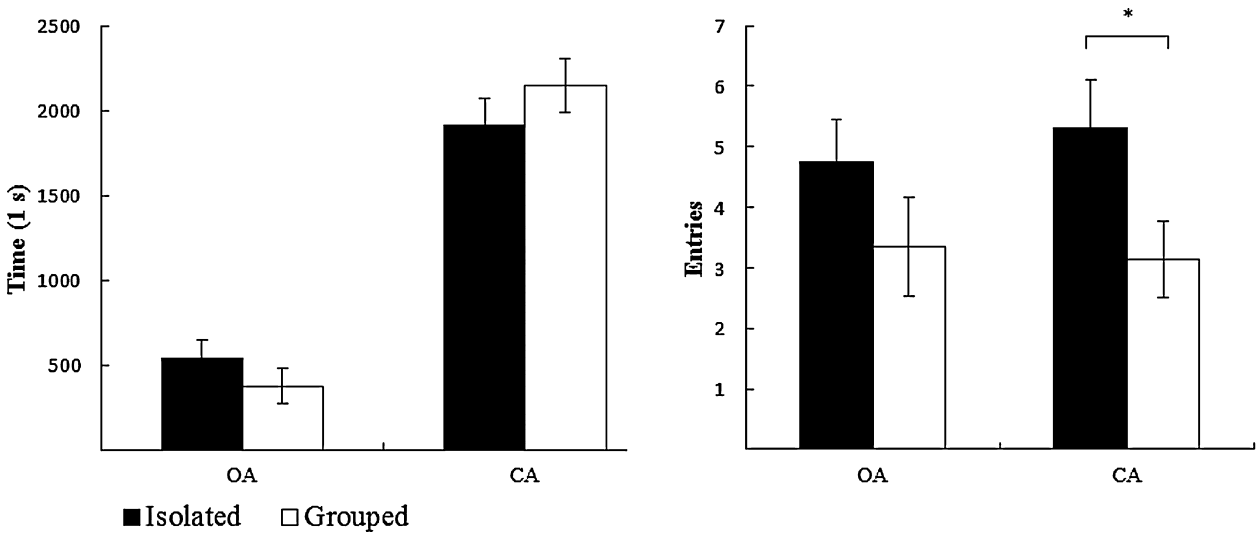

FIGURE 1 Behaviors on the EPM with animals with AI (black bars) and without AI (white bars). Left panel: Average time of permanence ($\pm ETM$) in the open arms (OA) and the closed arms (CA). Right panel: Average number of visits ($\pm ETM$) in the open arms (OA) and in the closed arms (CA). $^{*}p < .05$.

forcement. This suggests the existence of a positive contrast effect in subjects exposed to AI.

The analysis revealed a main effect of Trial, $F(6,162) = 20.77$, $p < .001$, and the interactions Trial × Reinforcement, $F(6,162) = 3.7$, $p < .01$, Trial × Isolation, $F(6,162) = 2.97$, $p < .01$, Reinforcement × Isolation, $F(1,27) = 9.3$, $p < .01$, and a marginal significance in the triple interaction Trial × Reinforcement × Isolation, $F(6,162) = 1.99$, $p < .07$, while no significant main effect was detected regarding Isolation, $F(1,27) = .005$, $p > .9$. The pairwise comparison revealed that GC and GP groups did not differ in any trial, $ps > .09$, while IP showed a significantly higher GTT than IC in the last four reinforced trials during preshift (trials 7, 9, 12, and 14; $ps < .01$).

Postshift phase. As the GTT during the preshift phase was not similar between grouped and isolated animals, statistical analysis of the postshift phase focused on the rate of change: the GTT of the last trial of the preshift phase/(the GTT of the last trial of the preshift phase + postshift trial) of each animal. In this measurement, values range from 0 to 1, where 0.5 indicates no change; above 0.5, lower consumption and below 0.5,

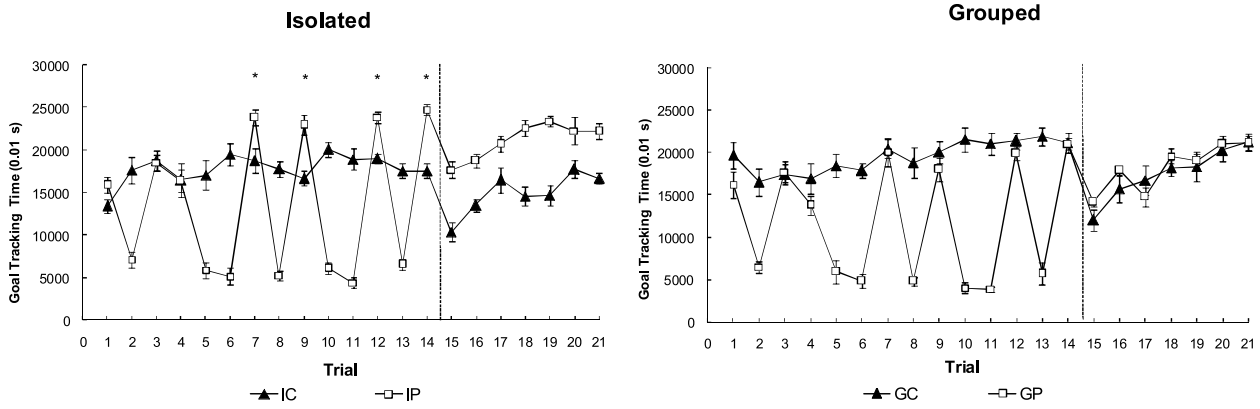

FIGURE 2 Average GTT ($\pm ETM$) as a function of preshift and postshift trials on subjects with a history of AI (left panel) and with no history of AI (right panel). $^{*}p < .01$ in the comparison between the GTT of the group with partial reinforcement and the group with continuous reinforcement when the partial reinforcement group shifts from 0% to 32% sucrose solution.

increased consumption during the postshift phase compared to the preshift phase. Thus, we studied the increase or decrease in consumption during the postshift phase in each of the animals compared to the preshift phase.

The rate of change is plotted in Figure 3. The ANOVA yielded a main effect of Trial, $F(3.73, 96.87) = 27.01$, $p < .001$, Reinforcement, $F(1,26) = 4.26$, $p < .05$, and Trial × Reinforcement interaction, $F(3.73, 96.87) = 3.06$, $p < .03$, while the Isolation factor did not show statistical significance, $F(1,26) = 0.01$, $p > .9$, neither the rest of the interactions.

When change ratio in each postshift trial was analyzed, a main effect of Reinforcement was found in the first, $F(1,26) = 9.25$, $p < .01$, and fourth trial, $F(1,27) = 6.68$, $p < .02$, showing that subjects with partial reinforcement had a lower rate of change in both trials. Isolation factor only showed main effect in the last postshift trial, $F(1,27) = 5.70$, $p < .03$, while the Reinforcement × Isolation interaction was not significant in any. Results derived from these analyses indicate expression of cPRIDE (i.e., higher response persistence to incentive devaluation in subjects with partial reinforcement, comparing to animals that had continuous reinforcement) in postshift trials 1 and 4, regardless the treatment that animals received during the adolescence.

Discussion. In this experiment, AI generated higher levels of locomotor activity, without affecting anxiety on the EPM. In this sense, previous literature shows variable data; some studies demonstrate hyperlocomotion for AI in rodents (e.g., Meng et al., 2010; Naert, Callaerts-Vegh, & D'Hooge, 2011), although such effect is not evident in others (e.g., Brenes, Padillaa, & Fornaguera, 2009). The same applies to anxiety; while previous work shows that rats with AI displayed an anxiogenic profile both on the EPM (e.g., Bledsoe, 2011) and in other behavioral tests (e.g., Hermes, Li, Duman, & Duman, 2011). In other studies, as in the present experiment, no anxiogenic effects caused by AI have been detected on the responses to the EPM (e.g., Fone, Shalders, Fox, Arthur, & Marsden, 1996; Jahng et al., 2012).

The most remarkable results of this experiment lie in the differences found in subjects isolated during adolescence in the cPRIDE preshift phase. When the AI subjects exposed to a partial reinforcement program switched from a non-reinforced trial to a reinforced one with a 32% solution, they showed an increased consummatory response above the levels of the group that was always receiving trials with a 32% solution (continuous reinforcement). This phenomenon was not observed in the subjects with no history of AI. These data demonstrate that, in a similar situation of increased incentive in adulthood, the AI subjects manifested a possible successive positive contrast or elation effect which is not observed in the subjects in the control group. These data are consistent with a study by Hall et al. (1997); as they show an increased positive contrast effect in subjects with AI, and manage to eliminate variables that confound interpretation: 1) the results of our experiment suggest that the positive contrast effect has a central rather than a sensory origin, as there is a 24 hr interval between trials; 2) there is an adequate control group that was never exposed to incentive shifts, which allows for a proper comparison; and 3) isolation was used exclusively during adolescence (PND 21–36), which indicates that said effect is the result of manipulation during this ontogenetic stage.

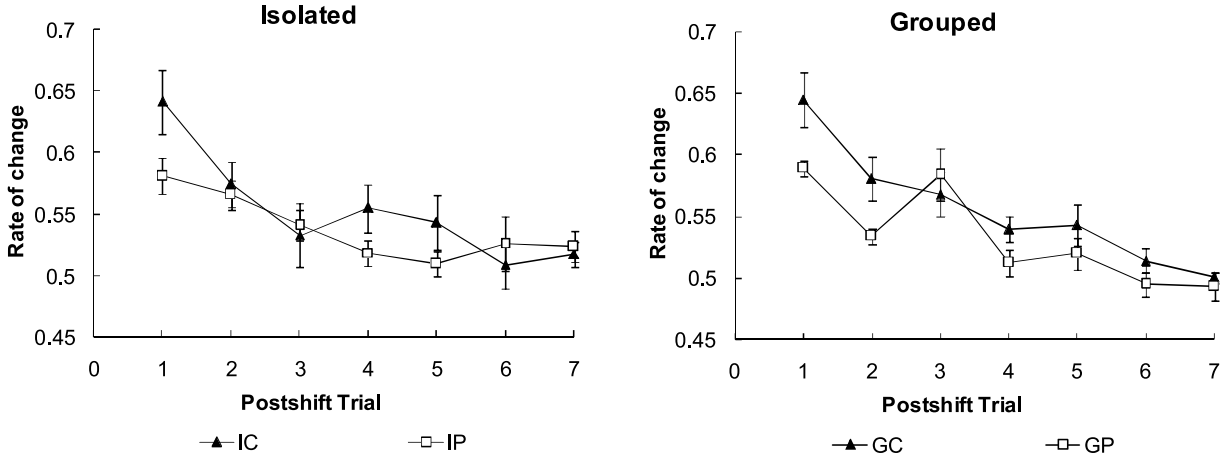

FIGURE 3 Average shift rate (±*ETM*) of postshift trials on subjects with a history of AI (left panel) or with no history of AI (right panel).

Although AI may lead to hyperphagia (e.g., Jahng et al., 2012), it should be noted that no differences were found in the solution consumption level among subjects with or without AI in the animals exposed to continuous reinforcement, so that the positive contrast effect may not be attributed to differences in the reinforcement consumption level per se. Finally, it was noted that the treatment did not alter responses following reward omission or devaluation since no differences were found between both conditions in the non-reinforced trials run either in the preshift or in the postshift phases.

In the postshift phase a cPRIDE was observed in the first and fourth trial, expressed as a lower shift rate in the partial reinforcement compared to the continuous reinforcement condition. However, the early-life isolation experience did not seem to alter either the intensity of consummatory suppression following reinforcement devaluation or the expression of cPRIDE. Shanab and Ralph (1979) found that only animals that were isolated from adolescence exhibited a partial reinforcement extinction effect in an instrumental task; however, it is important to note that a significant dissociation is apparent in consummatory and instrumental tasks (Morgan & Tromborg, 2007), and that in Shanab and Ralph's study the animals were isolated from weaning, both during adolescence and adulthood.

In short, these results show that subjects with a history of exposure to a chronic stressor during adolescence, such as isolation, evidenced a specific alteration in adult response to increased reinforcement, while no alterations were evident in reinforcement devaluation or omission situations. This observation is consistent with data obtained on the EPM, where no differences in anxiety were detected, although such differences were observed in locomotor activity. Possibly, AI did not generate differences in anxiety levels in adulthood, but it may have altered other temperamental features like impulsivity or greater sensitivity to unexpected appetitive reinforcers, which could facilitate the expression of positive contrast phenomena related to euphoric emotional states.

EXPERIMENT 2

Experiment 2 assessed behavioral differences during adulthood between subjects with or without AI in a standard procedure of consummatory Successive Positive Contrast (cSPC) (Flaherty, Becker, & Checke, 1983), and consummatory Extinction (cE). Indeed, if AI specifically alters the mechanisms involved in euphoric responses, a greater cSPC effect is expected to be found in subjects undergoing this experience, and no differences in responses in cE. The aim was to evaluate whether AI also generates a more pronounced elation effect in adulthood, even when the difference between reinforcements decreases (sucrose solution from 4% to 32% instead of an empty sipper tube to a 32% solution). With regard to cE, the goal was to evaluate whether the AI groups differ using a protocol showing a greater difference between both phases.

Materials and Methods

Subjects. Thirty-nine naïve male Wistar rats (weighing 270 to 405 g at the start of the experiment), representative of 11 litters were employed (2–4 males per litter). The general isolation method and housing conditions were the same as employed in Experiment 1, representing all litters in each housing condition. All testing sessions were performed between 12:00 hr and 16:00 hr.

cSPC and cE. The cSPC started on PND 90 and consisted of a total of fifteen 5 min daily trials, during which the animals had free access to a sipper tube in the conditioning boxes. The animals were randomly assigned to four experimental conditions. Groups differed in terms of their early-life treatment (Isolated vs. Grouped) and their manipulation in the cSPC procedure (32-4-32 vs. 32-32-32). In the 32-4-32 condition, rats received alternation trials of access to a 32% and 4% sucrose solution, while the 32-32-32 group had access only to a 32% solution throughout the trials. In the first trial, both groups were allowed access to a 32% solution. The day after completing the cSPC, during trial 16, the cE phase started, which comprised two trials and consisted in exposing all animals to an empty sipper tube for 5 min. Thus, the experimental design (see Table 2) was made up of four groups; the subjects in the experimental group with or without AI (I 32-4-32, $n = 10$; G 32-4-32, $n = 10$), and the subjects in the control group with or without AI (I 32-32-32, $n = 10$; G 32-32-32, $n = 9$).

Data Analysis

The GTT data from each trial were analyzed using an ANOVA with two between-subjects factors and one within-subject factor—Isolation (Isolated vs. Grouped) and Contrast (Experimental vs. Control), and Trials as within-subject factor (15 in cSPC, and 2 in cE). cSPC data was analyzed with two separate ANOVAs, one for the trials in which all the animals received 32% and the other one for the trials in which a half received 32% and the other half, 4%. A separated ANOVA was carried out in cE data, and another within-subject factor was introduced: Minutes (5 min). Pairwise comparison analysis was performed using Bonferroni adjustment

Table 2. Experimental Design Used in Experiment 2

Group	PND 21–36	cSPC 15 trials	cE 2 trials
G 32-32-32 $n = 9$	Grouped	32% sucrose solution	Empty tube
G 32-4-32 $n = 10$	Grouped	50% trials 32% sucrose solution 50% trials 4% sucrose solution	Empty tube
I 32-32-32 $n = 10$	Isolated	32% sucrose solution	Empty tube
I 32-4-32 $n = 10$	Isolated	50% trials 32% sucrose solution 50% trials 4% sucrose solution	Empty tube

for multiple post-hoc comparisons. When the data violated the sphericity assumption, the Greenhouse–Geisser correction was applied.

Results

cSPC. The GTT in cSPC in animals with or without AI are depicted in Figure 4. When analyzing the data from trials where all the animals received 32%, the repeated measures analysis yielded an effect of Trial, $F(4.45, 147.01) = 57.56$, $p < .001$, Contrast, $F(1,33) = 15.09$, $p < .001$, and Trial × Isolation interaction, $F(4.45, 147.01) = 2.37$, $p < .05$. The performed pairwise comparison revealed that G 32-4-32 showed a significantly higher GTT than G 32-32-32 in the first and second upshift (trial 3, $p < .04$; trial 5, $p < .04$). These differences were also found when comparing the I 32-4-32 versus I 32-32-32 conditions (trial 3, $p < .001$; trial 5, $p < .001$). Nevertheless, in this case the higher consumption of I 32–4-32 during the upshift trials lasted much longer, since a significantly higher GTT was detected in trials 7, 9, and 13 ($ps < .05$), and a trend in the same direction in trial 11, $p < .06$. These results show a positive contrast effect in animals with or without AI in the first two trials where the incentive was increased. In turn, subjects with AI showed such effect in almost every trial where incentive was increased (trials 3, 5, 7, 9, and 13).

The tests also showed that in trials 11 and 13 the I 32-4-32 group had a significantly higher level of GTT than the G 32-4-32 ($ps < .05$). Groups I 32-32-32 and G 32-32-32, which always consumed 32%, did not differ in any trial ($ps > .15$), demonstrating that the reason why positive contrast was more persistent in animals with AI is not related to differences in general level of sucrose solution consumption.

When contrast groups received 4% solution (trials 2, 4, 6, 8, 10, 12, and 14), we observed that animals with or without AI showed a significant lower GTT than animals receiving 32% ($ps < .05$), but differences between groups IE and GE were not found ($ps > .1$), which involves that response to incentive downshift was not affected by the experience of AI.

In sum, the AI seemingly intensified the euphoria reaction caused by the upward change in reinforcement conditions. However, the same was not true for the frustration reaction caused by the unexpected downward shift in rewards.

cE. Fig. 4 shows the GTT in the cE phase. Subjects extinguished the consummatory response in equal

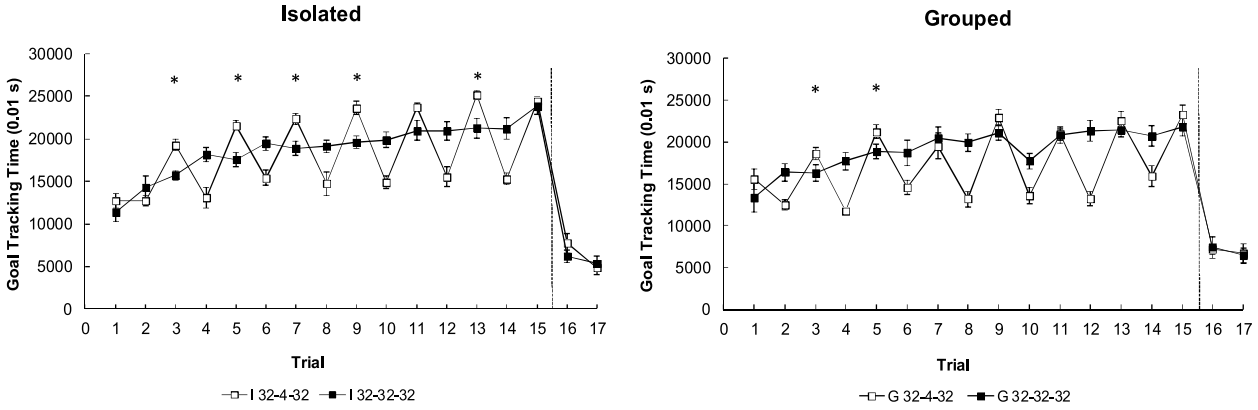

FIGURE 4 Average GTT (±*ETM*) as a function of cSPC and cE trials on subjects with a history of AI (left panel) and with no history of AI (right panel). $^{*}p < .05$, in the comparison between the 32-4-32 and 32-32-32 conditions in trials where both groups received a 32% solution.

velocity, regardless the early treatment and the previous experimental condition during the cSPC. The ANOVA performed yielded a main effect of Trial, $F(1,140) = 21.02$, $p < .001$, Minute, $F(1.96, 72) = 175.66$, $p < .001$, and the Trial × Minute interaction, $F(2.05, 72) = 4.46$, $p < .02$. Effects in between-subject factors were not found, neither other interactions ($ps > .1$).

Discussion. In the first training trial of cSPC, when all the animals were exposed to a 32% solution for the first time, the GE group exhibited longer GTT than the IC group. Considering that the animals had not been previously exposed to sucrose solutions, this might demonstrate a greater initial neophobia reaction to a sucrose solution in animals with a history of AI. Earlier studies have shown that this type of treatment increases the neophobia response to novel food during adulthood (e.g., Holson, 1986).

Just like in the study of Flaherty et al. (1983), the subjects with no history of AI had a 2 day cSPC effect. AI resulted in an effective early stressor. Evidence for this came from the fact that the subjects showed altered responses (compared to controls) after an unexpected increase in reinforcement when adults. These animals had a protracted cSPC (seven days).

On the other hand, in trials 11 and 13 (the last two positive contrast trials showing significant differences), the consummatory response of animals in the IE group exceeded that of the GE group. These results may not be accounted for by alternative explanations like hyperphagia or an overall increase in the solution consumption, as no differences were observed in the GTT of the control groups among animals with and without AI. Likewise, these data do not result from differences in locomotor activity among subjects with or with no history of AI. Although animals with AI displayed greater locomotor activity on the EPM (see Experiment 1), a rise in the nonspecific activity not only would interfere with the observation of an increased cSPC but would also hamper it, as consumption and locomotor activity are incompatible responses. In contrast, search behaviors and an increase in locomotion are observed in reinforcement devaluation or loss situations (e.g., Flaherty, Powell, & Hamilton, 1979; Kamenetzky, Mustaca, Pedrón, Cuenya, & Papini, 2009; Pecoraro, Timberlake, & Tinsley, 1999; Pellegrini & Mustaca, 2000).

Like in Experiment 1, the subjects in both conditions did not differ in their responses to reinforcement devaluation, when the solution was reduced from 32% to 4%, or to reinforcement omission, during the cE phase when the subjects were exposed to an empty sipper tube.

GENERAL DISCUSSION

The above experiments were focused on studying how exposure to chronic stress induced by social isolation during adolescence can have a long-lasting effect on responses to reinforcement shifts in adulthood.

It was demonstrated that AI (isolation from PND 21 to 36) increased locotomor activity and response to an unexpected increase in reinforcement during adulthood, both when going from 0% to a 32% sucrose solution (Experiment 1), and from 4% to 32% (Experiment 2). At the same time, it was noted that AI did not alter either anxiety levels in adulthood or responses to incentive omission or devaluation.

In this study all animals were isolated for 3 hr daily during training to ensure a homogeneous level of food deprivation, a critical condition in incentive contrast studies (Flaherty, 1996). It could be argued that this procedure of adult isolation may have contributed to the expression of the reported effects. Nevertheless, previous lab data show that the alteration pattern in subjects full isolated from adulthood (from PND 60) is substantially different; no alterations are detected either following a reinforcement increase and devaluation, or in the cPRIDE (Cuenya et al., 2011; Cuenya et al., 2012). Overall, and based on these data, it may be proposed that the long-lasting alterations found due to AI (i.e., an increased response following a reinforcement increase, with no differences in the case of reinforcement omission or devaluation) are typical of isolation during this ontogenetic stage.

Although some studies have documented that isolation from weaning generates greater sucrose consumption (Brenes & Fornaguera, 2008) and hyperphagia during adulthood (Morgan & Einon, 1975), in both experiments comparing subjects with or with no history of AI, the control animals showed a similar consummatory response when repeatedly exposed to a 32% solution, which rules out the assumption that differences are due to a higher overall consumption of sucrose solutions. Probably, the difference between previous studies and these experiments is that consuming tests in the former ones were performed during 48 hr (Brenes & Fornaguera, 2008), whereas in this work each test lasted 5 min. Hence, in this protocol AI did not modify the absolute value that the reinforcements used to have for the animals, but it did alter their relative value, specifically in situations of an unexpected increase in reinforcement. Hall et al. (1997) also discovered that rats isolated from weaning did not differ in their sucrose consumption compared to socially reared subjects, both in deprivation and ad libitum feeding situations. In the same work, the authors found that consumption among subjects

isolated from adolescence increased in positive contrast conditions but it did not in negative contrast conditions. Experiment 2 validates the data obtained by Hall et al. (1997); but unlike their study, it helps determine that the effect observed has a central origin and is specifically due to isolation during adolescence.

One of the characteristics of the AI model in rodents is the decline of certain inhibitory mechanisms and of sensorimotor gating evidenced in adulthood. Several studies have revealed that this treatment produces a deficit in prepulse inhibition of acoustic startle and impairment in the acquisition of latent inhibition (see Fone & Porkess, 2008). This kind of findings have promoted AI as an animal model of schizophrenia-like behavior, as a deterioration of both phenomena is also found in this type of patients (e.g., Bakshi, Swerdlow, Braff, & Geyer, 1998; Gray, Pilowsky, Gray, & Kerwin, 1995). It might be said that the positive contrast effect detected in animals with AI was due to a reduced inhibition of consummatory behavior vis-à-vis animals with no history of AI, which would show higher levels of impulsivity. If consummatory suppression following incentive devaluation and cE are seen as examples of inhibition of an already learned behavior, reduced consummatory suppression and greater persistence of cE might also have been expected. Lombardi and Flaherty (1978), discovered that cSNC was attenuated when the devaluation trial presented a novel stimulus (tone), thus demonstrating that the rate of consumption is sensitive to Pavlovian disinhibition and supporting the existence of inhibitory mechanisms in cSNC. Our experiments did not show any differences between conditions in the case of incentive omission and devaluation. However, the responses displayed in reinforcement omission and devaluation situations cannot be understood merely as behavioral inhibition, as the animals concurrently showed the activation of alternative behaviors like increased horizontal and vertical activity (Flaherty et al., 1979; Kamenetzky et al., 2009; Pellegrini & Mustaca, 2000).

Literature data show that subjects with AI develop a different sensitivity to natural or artificial reinforcers, so that AI becomes a particularly interesting model for studying the mechanisms of certain psychiatric disorders. Subjects with AI have been found to exhibit greater ethanol preference and increased motor response induced by the administration of psychostimulants like cocaine, amphetamine, or apomorphine (see Fone & Porkess, 2008). Probably, subjects with AI evidence increased incentive motivation and greater sensitivity to appetitive reinforcement properties, as well as greater sensitivity to reinforcement predictive stimuli. This interpretation finds its neurochemical correlate in numerous studies that document dopaminergic hyperactivity in the mesolimbic pathway, a brain circuit involved in the reinforcement system. For instance, Fabricius, Steiniger-Brach, Helboe, Fink-Jensen, and Wörtwein (2011) found that animals with or without history of AI, measured by microdialysis, did not show different basal dopamine levels in the NAcc, while a significantly higher release of this neurotransmitter was observed in subjects with AI when receiving amphetamine. These data would indicate an imbalance in dopaminergic function displayed when presenting an appetitive reinforcer. When the animal is in a consummatory positive and negative contrast situation, the dopamine release in the NAcc exhibits a bidirectional response; the negative contrast elicits attenuated release compared with a control group, while the positive contrast evidences increased release (see Phillips, Vacca, & Ahn, 2008).

The dopaminergic response is involved in prediction error or unpredictability of reinforcements (see Schultz, 2002), so that the dopaminergic imbalance caused by AI might affect the reaction to an unexpected shift of reinforcement. Nevertheless, why does not AI affect behavior in any given reinforcement shift situation but only specifically when the animal gets a bigger reward than the anticipated one? Bayer and Glimcher (2005); in experiments with monkeys, found that midbrain dopamine neurons encode a quantitative reward prediction error signal. Interestingly, an increment of the firing rates of the dopamine neurons was observed when the value of the current reward was greater than the weighted average of previous rewards, but this pattern was not observed when the value of the current reward was significantly lower than the weighted average of previous rewards. Diverse methodological aspects are different between the Bayer and Glimcher's experiments and those reported by Phillips et al. (2008). In any case, it might be that AI affects specific dopaminergic functioning related to the prediction error signal that could be contributing to an enhancement of the response when the animal gets a bigger than anticipated reward. Further experiments are required to investigate what specific neurochemical circuits are affected by AI to elucidate a plausible biological substrate of the long-lasting behavioral effects here reported.

NOTES

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