Effect of Neonatal Clomipramine Treatment on consummatory Successive Negative Contrast

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Neonatal administration of clomipramine (CLI) produces physiological, neuroendocrinal and behavioral abnormalities in rats when they reach adulthood, which are similar to those observed in animal models of depression. In consummatory successive negative contrast (cSNC), rats that have had experience drinking 32% sucrose solution drink significantly less 4% sucrose solution than rats that have drunk only 4% solution. It triggers an aversive-emotional reaction similar to fear or anxiety. We studied whether neonatal treatment with CLI alters the cSNC's response in adult rats. The findings of the present work suggest that the neonatal treatment with an antidepressive could generate an increase tolerance to frustration in adult animals. CLI rats showed a faster recovery from the cSNC than control animals, which may be explained by an alteration of the Hypothalamic-Pituitary-Adrenal axis (HPA), a serotonergic system deficit, a low expectative formation during pre-shift phase, or a combination of all these factors.

Administration of clomipramine (CLI), a serotonin and norepinephrine reuptake inhibitor, is commonly used for depression treatment. However, newborn animals treated with CLI exhibit behavioral and neurobiological abnormalities resembling endogenous depression in adult life (e.g., Vogel & Vogel, 1982; Vogel, Neill, Hagler & Kors, 1990a). Newborn rats treated with CLI show a decrease in their weight (e.g., de Boer, Mirmiran, van Haaren, Louwerse & van de Poll, 1989; Hansen & Mikkelsen, 1998; Maciag et al., 2006; Mirmiran et al., 1983), decreases pleasure-seeking behavior (Vogel, Neill, Hagler & Kors, 1990b), they were significantly less aggressive (e.g., Martínez-González, Prospero-García, Mihailescu & Drucker-Colín, 2002) and they increase immobility in a forced swim test, one of the most widely used procedures for the assessment of depressive behaviors (Baghya, Srikumar, Taju & Shankaranarayana Rao, 2008; Bonilla-Jaime, Retana-Márquez, Vázquez-Palacios & Velázquez-Moctezuma, 2003; Porsolt, Le Pichon & Jalfre, 1977; Vázquez-Palacios, Bonilla-Jaime & Velázquez-Moctezuma, 2005). Adding, they consume less of a 1% sucrose solution than controls in a preference test that was performed during two hours with animals deprived of food and water for 18 hours, and that had received previous training of 48 hours of exposure to water and a 1% sucrose solution (Baghya et al., 2008).

CLI rats have shortens the onset latency of REM sleep and increases REM sleep periods (Frank & Heller, 1997; Mirmiran, van de Poll, Corner, de Boer & van Oyen, 1980; Mirmiran, van de Poll, Corner, van Oyen & Bour 1981; Mirmiran et al., 1983; Vogel, Neill, Kors & Hagler, 1990) and produces permanent alteration of plasma corticosterone levels (e.g., Prathiba, Kumar & Karanth, 1998). CLI animals showed a lower increase of plasma corticosterone levels during the exposure to the stressor, and faster returned to basal levels. Similar results were found by others (Bonilla-Jaime et al., 2003). Besides CLI rats have an increase in their cholinergic activity (Mavanji & Datta, 2002; Prathiba, Kumar & Karanth, 2000) and Ogawa et al. (1994) showed that neonatal CLI treatment induced a lower response of the Hypothalamic-Pituitary-Adrenal axis (HPA) under a stressful situation. Otherwise, the neonatal CLI treatment produces a reduction in the serotonergic activity (Hansel & Mikkelsen, 1988). These results suggest that neonatal CLI treatment modifies the reactions to aversive situations. Furthermore, administration of antidepressants on adults rats treated with CLI during neonatal period reversed these changes (e.g., Nikiforuk & Popik, 2009, see Justel, Bentosela, Mustaca & Ruetti, 2011).

A phenomenon that was not studied in this model is the animal response to incentive downshift or frustration. One of the used procedures to evaluate the behavior of the animals to the suddenly changes in the

reward's value is the consummatory Successive Negative Contrast (cSNC), in which rats receive daily access to a 32% sucrose solution (pre-shift phase) and then they are shifted to a 4% sucrose solution (post-shift phase). Compared to unshifted animals that always receive access to the 4% solution, downshifted rats exhibit a sharp suppression of consummatory behavior, followed by a recovery during the following 2-4 trials (e.g., Flaherty, 1996). This phenomenon causes changes similar to those triggered by stress or fear response (e.g., Gray, 1987). There is an increase of corticosterone after reward devaluation (Mitchell & Flaherty, 1998), among others alterations (Mustaca et al., 2007, 2009).

While there is extensive evidence to justify that cSNC is a valid stress and "psychological pain" model (Papini, Wood, Daniel & Norris, 2006), little is known about the interactions between depression and cSNC. The data about the effects of antidepressives on cSNC are controversial. In a few studies in which antidepressants were administrated before the reward's devaluation in cSNC, authors did not found any change in the response of those animals (e.g., Flaherty, Grigson & Demetrikopoulos, 1987; Flaherty et al., 1990). However, Becker (1986) reported that nonspecific serotonin antagonists, like cyproheptadine and cinanserine, produced potent contrast-reducing action on the second post-shift trial, and Grigson and Flaherty (1991) showed that cyproheptadine reduced cSNC also during the first post-shift trial. More recently, Nikiforuk and Popik (2009) training rats in an operant task under progressive ratio schedule of reinforcement with the break point (BP, the value of the last completed response ratio) as a behavioral endpoint. In the main experiment, a 32% sucrose solution was initially used as the reinforcer. Once the stable responding was achieved, for the following 5 days animals were treated once daily with the experimental drugs, and they were offered a 4% sucrose solution instead. In vehicle-treated controls, the reduction of sucrose concentration resulted in a decrease in responding from a BP of about 40 (totaling 166 responses) to a BP of about 9 (totaling 22 responses). Fluoxetine and citalopram markedly inhibited this response decrement, while fluoxetine (6 mg/kg) augmented it. Neither desipramine nor morphine affected responding under the reduced sucrose concentration condition. The authors suggest that the antidepressants selectively inhibiting serotonin reuptake and a benzodiazepine anxiolytic and protect animals from the effects of a reinforcer downshift.

The goal of the present study was to assess whether neonatal CLI treatment alters cSNC in adulthood (Experiment 2). Previously, a forced swim test was conducted to validate the neonatal CLI treatment procedure in our laboratory (Experiment 1).This work will offer the first evidence

about the relationship between an animal model of endogenous depression and a frustration model. The results obtained with CLI rats suggest that they will have an attenuated cSNC effect.

EXPERIMENT 1: FORCED SWIM TEST

METHOD

Participants. Wistar male rats were experimentally naive and bred at the vivarium of our institution. Study procedures were approved by the Animal Care Committee and conformed to all the standards described in the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Fifteen naive rats were used, CLI group (n = 7) and vehicle (VEH) group (n = 8). Animals had free access to food and water. The body weight was (means ± SE) 324±28 g in CLI-treated rats, and 374 ± 23 g in VEH-treated rats. An ANOVA (CLI vs VEH) showed significant differences between CLI and VEH body weights, $F(1,14) = 16.31$, $p< .001$ indicating that neonatal CLI treatment induced a long-term negative effect in body weight gain.

Neonatal treatment. From postnatal day 8 to 21, rats received subcutaneously administration of CLI (Novartis, Buenos Aires, Argentina, 15mg/kg twice a day, dissolved in 0.9% saline solution, at 8.00 AM and 8.00 PM) or saline solution (VEH). When animals were 90 days old, they were housed individually and behavioural testing begun.

Apparatus and procedure. Rats were placed in a rectangular dark container (50 cm height, 30 cm wide) filled 30 cm high with water (24°C±3°C) for 5 min and the animal behavior was recorded using a Sony camera. Immobility (time spent still, making only small movements to hold the head above water; Porsolt et al., 1977; Velázquez-Moctezuma & Díaz-Ruiz, 1992) was analyzed by a blind scorer.

The forced swim test is one of the most widely used procedures for the assessment of depressive behaviors (Baghya et al., 2008; Bonilla-Jaime et al., 2003; Porsolt et al., 1977; Vázquez-Palacios et al., 2005). Because it was the first time that in our laboratory was used this neonatal treatment it was necessary to assess its validity.

Statistical analysis. Quantitative data were expressed as mean ± standard error mean (SEM) and ANOVA test were realized. Each goal tracking time (s) data for each animal was related to its body weight (g). An alpha level of statistical significance was set at 0.05.

RESULTS

Neonatal CLI rats remained motionless longer than those from VEH group, $F(1,14) = 11.15$; $p < .005$(data not shown) indicating the efficacy of CLI treatment according to previous results (Baghya et al., 2008; Bonilla-Jaime et al., 2003; Porsolt et al., 1977, 1978; Vázquez-Palacios et al., 2005; Velázquez-Moctezuma & Ruiz-Díaz, 1992).

EXPERIMENT 2: cSNC

Participants. Twenty-eight naïve rats, CLI (n = 13) and VEH (n = 15) were used. The body weight was 372±25 g in CLI, and 431±30 g in VEH-treated rats. ANOVA(CLI vs VEH) showed again differences in body weights, $F(1,26) = 23.89$, $p< .0001$. During the experiment, animals were maintained at 85% of their ad libitum weight, with free access to water.

Apparatus and procedure. Four identical conditioning boxes (MED Associates, Vermont, USA) were used. Each box measured 24.1x 29.2 x 21 cm (length x width x height). In the lateral wall there was a 5-cm hole, with a sipper tube. Goal-tracking time (measured in 0.01-s units) was automatically recorded by a computer that measured the cumulative amount of time that the photocell was activated during the trial. Goal-tracking time correlates with fluid intake for the two sucrose concentrations used in this experiment (Mustaca, Freidin & Papini, 2002).

Animals from treatments CLI and VEH were matched by body weight and were randomly assigned to one of the following groups: CLI 32 (n = 7); VEH 32 (n = 8); CLI 4 (n = 6), and VEH 4 (n = 7), depending on the neonatal treatment (CLI or VEH) and the reward magnitude received during pre-shift phase (Trials 1-10, 32% or 4% sucrose solutions). During the post-shift phase (Trials 11-15) all animals received access to the 4% sucrose solution. Each trial was 5 min duration.

RESULTS

Figure 1 shows the goal tracking time/body weight for each pair of groups during all training.
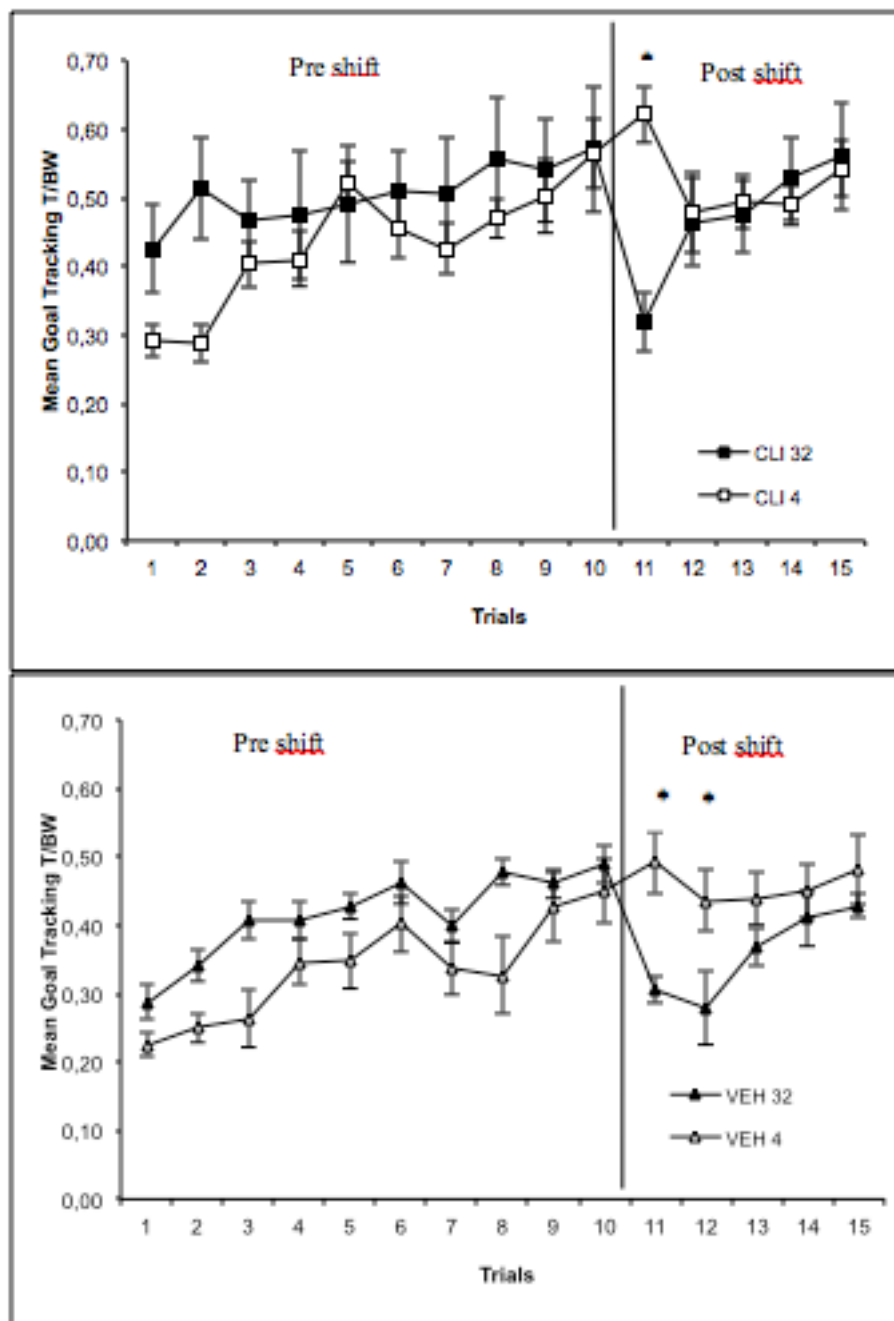
Figure 1. *Top panel*: **Pre-shift and post-shift phases of the cSNC in neonatal clomipramine (CLI)-treated groups. Animals were injected neonatally from postnatal day 8 to 21 with clomipramine.** *Bottom panel*: **Pre-shift and post-shift phases of the cSNC in neonatal vehicle (VEH)-treated groups. Animals were injected neonatally from postnatal day 8 to 21 with the vehicle solution. Results are expressed as mean ± SEM. * p<.05.   T/BW: Time (s) /Body Weight (g).**

Pre-shift phase. Goal-tracking time increased gradually during pre-shift phase. A Contrast (32%-4%) x Treatment (CLI-VEH) x Trial (1–10 trials) ANOVA indicated significant effects of Treatment, $F(1, 24) = 4,78$, $p< .04$; Trial, $F(9,216) = 22,79$, $p< .0004$, and a Contrast x Trial interaction, $F(9,216) = 2.52$, $p< .009$. Separate ANOVAs of each trial and its correspondent LSD comparisons test on trials 1 to 3 showed significant differences between CLI 32 vs. CLI 4, VEH 32 and VEH 4 (Trials 1, 2 and 3) and VEH 4 vs. CLI 4 and CLI 32 (Trial 4). ANOVAs of trials 4 to 10 did not show significant differences in any of the factors. In sum, during the first trials of the training the CLI 32 group consumed more sucrose solution than the other groups; nonetheless this difference was lost in the next trials.

Post-shift phase. A Contrast x Treatment x Trial (11-15) ANOVA showed significant differences of Treatment, $F(1, 24) = 4.34$, $p< .04$; Trial, $F(4,96) = 7.73$, $p< .0001$; a Contrast x Trial interaction, $F(4,96) = 18.23$, $p< .0001$, and a Contrast x Treatment x Trial interaction, $F(4,96) = 3.82$, $p< .006$. Separate ANOVAs of each trial and its correspondent LSD comparisons test showed that CLI 32 vs CLI 4 groups differed on trial 11 ($p< .0003$), VEH 32 vs VEH 4 groups on trials 11 and 12 ($p< .04$), and CLI 32vs. VEH 32 on trial 11 ($p< 0,003$). Despite that the Figure 1 shows a decrease in the consume of the CLI 4 group, and this decrease would be the cause of the absence of the contrast effect in the CLI 32 group, the comparison between CLI 4 vs. VEH 4 groups in the trial 11 didn't show significant differences ($p> 0.05$). To corroborate this result, we realize an ANOVA between CLI 4 vs VEH 4 groups on the post-shift phase that only shows significant differences in Trial factor, $F(4,44) = 9,82$, $p< 0,0001$. The others factors didn't show significant differences. In summary, the animals that receive CLI neonatally presented a faster recovery of cSNC than animals without the neonatal treatment.

DISCUSSION

This is the first evidence that links an animal model of frustration with another of endogenous depression. The main results of this study indicated that CLI rats showed a cSNC effect only during the first downshift trial, while VEH animals showed this effect on two post-shift trials, which indicates a faster recovery of CLI animals.

This result does not seem to be due to differences in sensitivity to sucrose solutions in CLI rats, because during the pre-shift phase all groups

had the same acquisition curve, even with increased intake of CLI 32 at the beginning of the training.

The results are consistent with previous research that showed that neonatal administration of CLI may play an important role on the regulation of the stress responsiveness in adulthood.

A similar result with CLI rats was found with the administration of a neonatal stress treatment on cSNC effect in adult rats (Ruetti, Justel, Mustaca, Torrecilla & González Jatuff, 2010). Suggestively that chronic random neonatal stress, just like that the CLI rats, produce a response' reduction of the Hypothalamic-Pituitary-Adrenal and same response of corticosterone in situation on stress (Ruetti et al., 2010). Seems like the response to appetitive reward devaluation of both neonatal treatments are associated with the same neurophysiological mechanisms.

On the other hand, Amsel' theory (1992) suggests that the initial rejection of the 4% solution is triggered by a state of primary frustration induced by the discrepancy between the expectation of a 32% reward acquired during the pre-shift trials and the actual 4% reward encountered on the first post-shift trial. This initial emotional reaction (primary frustration) is hedonically aversive, and it induces rejection with minimal or no conflict, serving as an unconditioned stimulus for the acquisition of secondary frustration through Pavlovian conditioning (i.e., pairings of stimuli present at the time of reward downshift with the internal state of primary frustration (Amsel, 1992). Once acquired, secondary frustration plays its role as the avoidance component of the conflict induced at full strength during the second postshift trial. Also, the Amsel' theory predicts that the cSNC intensity depends on a magnitude discrepancy between the expectancy provoked during de pre-shift phase and the one obtained during the post-shift phase, especially on secondary frustration. Although depressive subjects can evaluate correctly positive and negative rewards (e.g. McChargue, Spring, Cook & Neumann, 2004), their expectative formation might be altered due to amygdala dysfunctions (Abler, Erk, Herwig & Walter, 2007). Studies with humans show evidence of deficit in the evaluation of positive reinforcement and the formation of expectations in depressive patients (e.g., Layne, Merry, Christian & Ginn, 1982; Lewinsohn, Larson & Muñoz, 1982). On the basis of Amsel' theory (1992) the results suggest that the CLI and VEH rats expressed a same response pattern when they face to the downshift solution (unconditioned response), but the secondary frustration was attenuated may be because a diminished expectative in the CLI rats.

In summary, neonatal CLI treatment, which is a model of endogenous depression, induces a faster recovery from cSNC in adulthood (an animal model of frustration). The result may be explained by a low expectative formation during pre-shift phase, for an alteration of the HPA axis, a serotonergic system deficit, or a combination of all these factors. Further research is needed to elucidate these issues.

RESUMEN

Efecto del tratamiento neonatal con clomipramina sobre el Contraste Sucesivo Negativo consumatorio. La administración neonatal de clomipramina (CLI) produce alteraciones fisiológicas, neuroendocrinas y comportamentales en las ratas adultas, que son similares a las observadas en los modelos animales de depresión. En el Contraste Sucesivo Negativo consumatorio (CSNc), las ratas que recibieron una solución de sacarosa al 32%, consumen menos de una solución de sacarosa al 4%, que los animales que siempre recibieron la solución 4%. Este modelo de devaluación del incentivo produce en los animales una reacción emocional similar al miedo y la ansiedad. En el presente trabajo, estudiamos si el tratamiento neonatal con CLI altera la respuesta de CSNc en ratas adultas. Los hallazgos del presente trabajo sugieren que el tratamiento neonatal con un antidepresivo podría generar un aumento en la tolerancia a la frustración en animales adultos. Los animales a los que se les administró neonatalmente CLI presentaron una recuperación más rápida en el CSNc, que los animales controles sin tratamiento neonatal. Este resultado puede explicarse por una alteración del eje Hipotálamo-Pituitario-Adrenal (HPA), por un deterioro del sistema serotoninérgico, por la formación de una baja expectativa durante la fase de pre-cambio, o por una combinación de estos factores.

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