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Domperidone Interferes with Conditioned Disgust Reactions but Not Taste Avoidance Evoked by a LiCl-Paired Taste in Infant Rats

ABSTRACT: *Rats exhibit taste avoidance and conditioned disgust reactions when stimulated with a tastant paired with lithium chloride (LiCl). Lithium-mediated activation of chemoreceptor nuclei at the brainstem appears to determine the acquisition of conditioned taste aversion (CTA) in adult rodents. Domperidone (DOM), an anti-emetic drug that does not cross the blood–brain barrier, was employed to analyze mechanisms underlying LiCl-mediated CTA in infant rats. On postnatal day 13 animals were given DOM followed by a pairing between intraoral saccharin and LiCl. Saccharin consumption at testing was lower in lithium-treated pups than in controls. DOM did not interfere with this LiCl-mediated taste avoidance but significantly decreased LiCl-mediated disgust reactions (head-shaking and wall climbing). Activation of the emetic system of the brainstem does not seem necessary for the acquisition of LiCl-mediated conditioned taste avoidance. Yet, these centers seem to be involved in the palatability shift resulting from taste-LiCl pairings. These results indicate an early dissociation between conditioned disgust reactions and conditioned taste avoidance. © 2008 Wiley Periodicals, Inc.* Dev Psychobiol 50: 343–352, 2008.

Keywords: *lithium chloride; aversion; disgust reactions; anti-emetic; infant rat*

INTRODUCTION

Several species of mammals (i.e., ferrets, house musk shrew) suppress consumption of a distinctive taste after this conditioned stimulus (CS) is paired with the postabsorptive effects of lithium chloride (LiCl, unconditioned stimulus, US) (Rabin & Hunt, 1992; Smith, Friedman, & Andrews, 2001) and other drugs (e.g., cisplatin; McCarthy & Borison, 1984, and cyclophosphamide; Fetting, Grochow, Folstein, Ettinger, & Colvin, 1982) that exert emetic effects. Although rats are incapable of vomiting, they readily avoid a LiCl-paired taste (Palmerino, Rusiniak, & Garcia, 1980; Parker, 1995, 2003) and also display a distinctive set of behavioral reactions when intraorally stimulated with the CS. These reactions include head shaking, increased locomotion, wall-climbing and decreased mouthing (Parker, 1984, 1995). Activation of the chemoreceptor centers at the midbrain and brainstem [area postrema (AP) and nuclei of the solitary tract (NST), Miller & Leslie, 1994] appear to represent necessary factors for the establishment of conditioned taste aversion (CTA, Garcia, 1989). The AP is situated on the dorsal surface of the medulla oblongata on the floor of the fourth ventricle (Miller & Leslie, 1994). Yet, it does not exhibit a blood–brain barrier. The latter

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feature allows AP, which has reciprocal connectivity with the NST, to quickly detect chemicals in the blood or cerebrospinal fluid (Chambers, 1990). In other words, AP serves as an interface conveying information to higher brain structures involved in emesis and/or nausea.

Paradoxically, rats also suppress consumption of taste CSs previously associated with drugs known to exert central appetitive effects, including amphetamine and cocaine (Parker, 1991, 1993, 1995). These drugs induce appetitive learning when employing conditioned place preference procedures (Tzschentke, 1998). Originally, it was proposed that a side-effect of these drugs was to activate the emetic centers at the brainstem, an effect that would explain their capability to mediate CTA acquisition. Yet, conditioned disgust reactions are absent when animals are stimulated with a tastant previously paired with reinforcing drugs, even after titrating drug dosage so as to induce similar taste avoidance as observed with LiCl (Parker, 1995). This suggested that different mechanisms may underlie avoidance of the taste CS and the behavioral expression of disgust reactions. According to Parker (2003), rats will suppress consumption of flavors paired with different changes in their physiological state, including emesis like effects, general malaise as well as rewarding effects. Conditioned disgust reactions, on the other hand, will be exhibited only when the US has the capability to activate the chemoreceptor nuclei at the brainstem.

Anti-emetic treatments have been useful in assessing the role of nausea in CTA. It has been observed that anti-emetic agents like scopolamine interfere with expression of LiCl-mediated taste avoidance (Coil, Hankins, Jenden, & Garcia, 1978). Yet, further attempts to replicate these results were not successful (Parker & McLeod, 2003). On the other hand, several experiments have provided converging evidence indicating that antinausea agents do not affect acquisition of LiCl-mediated taste avoidance. Limebeer and Parker (2000) observed no effect of ondansetron pretreatment (a selective serotonin antagonist, highly effective for treating nausea in human; Ye, Ponnudurai, & Schaefer, 2001) on either LiCl-induced taste avoidance or unconditioned disgust reactions induced by intraoral infusion of quinine. Yet, Ondansetron selectively inhibited conditioned rejection reactions elicited by a LiCl-paired flavor. Patterns of extinction of LiCl-mediated taste avoidance and disgust reactions also differed. Rats suppressed CS consumption even when disgust reactions were absent (Cántora, López, Aguado, Rana, & Parker, 2006). Dissociation between lithium-induced taste avoidance and disgust reactions was also found after reducing serotonin availability by performing lesions of the raphe nuclei (Limebeer & Parker, 2006).

All the above studies (e.g., Limebeer & Parker, 2000; Parker, 1995, 2003) assessed the nature of the relationship between LiCl-mediated taste avoidance and conditioned disgust reactions in adult rats. Previous studies suggest that ontogenetic differences are likely to be expected for these and related phenomena. Hoffmann, Hunt, and Spear (1991) found that 15- but not 5-day-old rats displayed specific conditioned disgust reactions when stimulated with a LiCl-paired taste. Specifically, 15-day-old pups were given pairings of a taste CS and emetic (LiCl) or nonemetic USs (footshock and intraoral citric acid). Magnitude of taste avoidance assessed through an intake test was similar across USs. Nevertheless, conditioned disgust reactions elicited by the LiCl-paired taste were qualitatively different from those mediated by nonemetic agents. LiCl-treated pups exhibited conditioned increments in wall-climbing and paw-treading as well as decreased mouthing. On the other hand, nonemetic USs induced only a significant reduction in mouthing.

The main aim of the present study was to analyze mechanisms underlying LiCl-mediated CTA during early ontogeny of the rat. Specifically, 13-day-old rats were pretreated with a peripheral-acting anti-emetic agent (Domperidone, DOM; Barone, 1999) and then given a single pairing between a novel taste CS (saccharin) and postabsorptive effects of LiCl. Saccharin intake and emission of disgust reactions were assessed 24 and 48 hr after training. Repeated measurement of responsiveness towards the taste CS was meant to allow analysis of the patterns of extinction of both lithium-induced taste avoidance and taste reactivity. Although comparison between patterns of extinction of taste avoidance learning and conditioned disgust reaction has been assessed in adult rats (Cántora et al., 2006), the expression of such phenomenon in infants has yet to be investigated. Cántora et al. (2006) found that rats continued to avoid a lithium-paired flavor, even when conditioned disgust reactions were completely extinguished.

MATERIALS AND METHODS

Subjects

Thirty-nine 13-day-old Sprague-Dawley pups (21 females and 18 males), representative of 10 litters were employed. Animals were born and reared at the vivarium of the Center for Developmental Psychobiology (Binghamton University, NY) under conditions of constant room temperature ($22 \pm 1.0^{\circ}$C), on a 12-hr light 12-hr dark cycle. Births were examined daily and the day of parturition was considered as postnatal day 0 (PD0). All litters were culled to 10 pups (5 females and 5 males, whenever possible) within 48 hr after birth. All procedures were in accordance with the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996) and the guidelines indicated by the Binghamton University animal handling review committee.

Procedures

Conditioning (PD13). Pups were separated from their mothers and randomly assigned to one of four conditions defined by the following independent variables: Conditioning treatment (LiCl or saline) and Domperidone treatment (DOM or vehicle). Only one pup from a given litter was assigned to a specific group. Number of pups in each group was as follows: LiCl–DOM, $n = 10$; LiCl–Vehicle, $n = 10$; Saline–DOM, $n = 9$; and Saline–Vehicle, $n = 10$.

Pups were placed in couples in holding cages (45 cm × 20 cm × 20 cm) lined with clean wood shavings and maintained at 32–33°C by means of a heating pad. An intraoral cannula (PE 10 polyethylene tubing, length: 5 cm, Clay Adams, Parsippany, NJ) was implanted in the right cheek of each pup. The cannulation procedure has been extensively described in prior studies (Arias & Chotro, 2006; Chotro & Arias, 2003; Hoffmann, Hunt, & Spear, 1990; Pautassi, Godoy, Spear, & Molina, 2002). This procedure requires no more than 10 s per subject and induces minimal stress to the infant rat (Spear, Specht, Kirstein, & Kuhn, 1989).

Ninety minutes after cannulation, body weights were recorded (±.01 g) and pups were intraperitoneally (i.p.) injected with either Domperidone (1 mg/kg, Sigma–Aldrich, St. Louis, MO) or vehicle (1 ml/kg dimethyl sulfoxide, Sigma–Aldrich). The DOM dose here utilized (1 mg/kg) is effective in terms of suppressing apomorphine-induced retching and emesis in ferrets (Lau, Ngan, Rudd, & Yew, 2005). DOM is a peripheral, dopamine competitive receptor antagonist, that exerts anti-emetic effects in various species (Osinski et al., 2005). DOM does not readily cross the blood–brain barrier and is known to cause less central side-effects than alternative anti-emetic treatments (i.e., metoclopramide; Jolliet et al., 2007). Specifically, extremely large doses of DOM are required to obtain central effects (Wauquier, Niemegeers, & Janssen, 1981). It has been shown that DOM does not bind with dopamine receptors in the rat's striatum and also fails to cause significant increases in dopamine metabolites (i.e., homovanillic acid) as is usually observed with central dopamine antagonists, such as neuroleptic drugs (Laduron & Leysen, 1979). More recent studies have also confirmed that DOM exerts its activity in areas not protected by the blood–brain barrier (Schinkel, Wagenaar, Mol, & Van Deemter, 1996). Its peripheral site of action (Barone, 1999) makes DOM particularly suitable for the purposes of the present study. Alternative, more recently developed antinausea treatments are also available (i.e., ondansetron, Limebeer & Parker, 2006). However, most of these drugs cross the blood–brain barrier, raising the possibility of effects upon CTA that can be unrelated with anti-emetic effects but rather related with centrally-mediated motivational consequences such as anti-anxiety effects (Ye et al., 2001). Employment of the peripheral-acting DOM minimizes this potential confounding factor.

Following DOM or vehicle administration, animals were returned to their holding chambers for 30 min. Hence, animals were food and fluid deprived for 120 min prior to commencement of conditioning. Previous studies suggest that this time interval provides adequate parameters for acquisition of conditioned taste aversion in preweanling pups (Hunt, Spear, & Spear, 1991). Employment of this relatively short deprivation was aimed at reducing stress associated with lengthier deprivation and maternal separation treatments, which can exert aversive unconditional effects in itself (Smith, Kucharski, & Spear, 1985) and therefore are likely to interact with the learning processes under analysis. Previous work found that 180 min of maternal deprivation increased stress-mediated pituitary–adrenal axis responsiveness in rat pups (Huot, Thrivikraman, Meaney, & Plotsky, 2001). Notably, activation of this hormonal system affects LiCl-mediated taste aversion (Gorzalka, Hanson, Harrington, Killam, & Campbell-Meiklejohn, 2003).

Pup's bladders were then voided by gentle stimulation of the anogenital area with a cotton swab. Following this procedure body weights were recorded again and subjects were placed in individual Plexiglas chambers (10 cm × 10 cm × 12 cm). Pups were given two minutes of habituation in these chambers. After completion of the habituation phase, pups were intraorally stimulated with the taste CS (.05% (w/v), Saccharin, Sigma–Aldrich; vehicle: distilled water) during 7.5 min. The overall amount of fluid intraorally delivered was equivalent to 2.75% of the subject's preinfusion weight. Saccharin was delivered at a constant rate by means of a 10-syringe infusion pump (KD Scientific, Holliston, MA) connected to the oral cannula of each pup by a polyethylene catheter (Clay Adams, PE 50 Parsippany, NJ). When employing these infusion parameters, pups are capable of consuming or rejecting the liquid CS (Arias & Chotro, 2006; Dominguez, Lopez, & Molina, 1998). Infusion procedures at conditioning and testing were conducted under room temperature (22°C). Several previous studies (Hunt et al., 1991; Pautassi et al., 2002) observed reliable acquisition and expression of conditioned taste aversion under these thermal conditions. After the infusion subjects were weighed to estimate saccharin consumption scores in terms of percentage body weight gain (% BWG). This index was calculated as follows: 100 × [(post-(postinfusion weight − preinfusion weight)/preinfusion weight]. Infants were subsequently given an intraperitoneal injection of LiCl (.5% of the body weight of a .3 M solution, Sigma–Aldrich) or an equivalent volume of physiological saline. Pups were then returned to their holding cages, where they remained undisturbed for 3 hr until being reunited with their mothers.

Test (PDs 14–15). Intraoral infusion with the taste CS was conducted on postnatal days 14 and 15. The rationale for conducting two test trials was to be able to detect potential differences in the course of an eventual extinction of the aversive memories under analysis. Pups were cannulated immediately after being separated from the homecage every testing day. Then, they were placed in couples during ninety minutes in a heated holding cage (32–33°C). A saccharin intake test was later conducted. The saccharin infusion procedure was the same as the one utilized during conditioning. Briefly, a 2-min habituation phase was followed by intraoral infusion of the saccharin CS (intake phase, duration: 7.5 min).

Saccharin consumption as well as behavioral reactivity were measured during the habituation and intake phases of the test. Based on previous taste reactivity studies conducted with infant (Arias & Chotro, 2006; Chotro, Kraebel, McKinzie, Molina, & Spear, 1996; Hall & Bryan, 1981; Vigorito & Sclafani, 1988) and adult rats (Grill & Norgren, 1978; Parker, 1988, 1995) the

following behavioral measures were selected: general activity, wall climbing and head shaking. These variables are considered aversive indices in the hedonic assessment of tastants. General activity was automatically monitored using a photocell system composed by six infrared photo emitters and six infrared photoreceptors. The photo beams crossed the Plexiglas chamber generating a matrix of cells. Each chamber was in turn connected to a computer. Custom-made software served to analyze the number of beams crossed by each subject every 10th of a second. Wall climbing duration was registered when pups stood on their rear limbs with the forepaws placed on the walls of the chamber. Finally, frequency of head shaking was registered when observing rapid side-to-side movements of the head. Hence, general activity (number of beams break), wall climbing duration and number of head shakes were registered throughout the habituation (2 min) and intake (7.5-min) phases of the test. Researchers blind to the experimental conditions measured these behaviors.

Immediately after the end of every testing day, cannulae were removed and pups were reunited with their mother.

Data Analysis

A two-way ANOVA [conditioning (LiCl or saline) × drug treatment (DOM or vehicle)] served to analyze intake scores (% BWG) during conditioning. Intake at testing was analyzed with a three-way mixed ANOVA defined by the following factors: conditioning treatment (LiCl or saline), Domperidone (DOM or vehicle) and day of testing (1 or 2).

General activity, wall climbing and head shaking registered during the 2-min habituation phase were analyzed using a mixed ANOVA that included the following factors: conditioning treatment (LiCl or saline) × domperidone (DOM or vehicle) × day of testing (1 or 2). A similar ANOVA was employed to analyze scores of these variables obtained during the 7.5 min intake phase of the test.

Finally, a general index of behavioral reactivity at testing was calculated. This multivariate aversion index (further referred as ''overall aversion index'') comprised head shaking, wall climbing and general activity scores. Specifically, scores corresponding to these dependent variables were standardized (z-scores, relative to the entire sample of subjects) and then added up to obtain a single score for each testing session. In other words, general aversive reaction scores were obtained by adding standardized values corresponding to head shaking, wall climbing and general activity. This index was analyzed with separate mixed ANOVAs (conditioning treatment × domperidone × day) for each of the testing phases (habituation and intake). Similar multivariate aversive indexes have been previously employed to successfully reveal expression of conditioned disgust reactions (Parker, 1995).

The loci of significant main effects or interactions were further examined through follow-up ANOVAs and post hoc comparisons (Fisher's LSD, type I error set at .05).

RESULTS

Baseline body weights increased significantly as a function of the age of the animals [$F(2, 70) = 802.25$, $p < .001$] and were not affected by conditioning or domperidone treatments. Body weights across sessions were as follows: PD13: $30.01 \pm .35$ g; PD14: $31.91 \pm .36$ g; PD15: $34.59 \pm .35$ g; values represent mean ± SEM.

No significant main effects or significant interactions were detected when analyzing saccharin intake (% BWG) during the conditioning trial (PD 13). Mean and standard error across groups were as follows: LiCl–DOM = $1.23 \pm .17$; LiCl–Vehicle = $1.05 \pm .16$; Saline–DOM = $1.11 \pm .18$; and Saline–Vehicle = $1.25 \pm .16$.

Intake scores at testing are depicted in Figure 1a. The ANOVA yielded a borderline significant effect of conditioning as well as a significant effect of day of testing [$F(1, 35) = 3.31$, $p = .07$; $F(1, 35) = 12.18$, $p < .005$; respectively]. The interaction between these factors also achieved significance, $F(1, 35) = 4.77$, $p < .05$. Domperidone treatment was not observed to exert a main significant effect or to significantly interact with any of the remaining variables. The locus of the conditioning × day of testing interaction was further analyzed by means of one-way ANOVAs for each testing day. In this analysis conditioning treatment (LiCl or saline) served as the independent factor. A significant conditioning treatment effect was only observed during the second testing day, $F(1, 35) = 5.56$, $p < .05$. Pups treated with LiCl showed lower saccharin intake on PD 15 than those treated with Saline. Also, intake across days of testing was analyzed within each conditioning treatment (LiCl or Sal) by means of repeated-measures ANOVAs. Intake scores for LiCl-treated pups did not significantly change across testing sessions. Interestingly, this was not the case for Saline-treated pups, which showed a progressive increase in their acceptance of the tastant CS, $F(1, 18) = 12.52$, $p < .005$. That is, results derived from both between- and within-measures analyses indicated expression of LiCl-mediated conditioned taste avoidance, a phenomenon that was not modulated by domperidone administration (1 mg/kg) prior to conditioning. In other words, administration of a peripheral, dopaminergic-mediated anti-emetic treatment failed to affect LiCl-induced conditioned taste avoidance in preweanling pups.

No significant main effects or significant interactions were found when analyzing individual behavioral scores (general activity, wall climbing and head shakes; Figure 1, sections b, c, and d, respectively) during the 2-min habituation phase. However, the ANOVA for the overall aversion index during habituation yielded a significant main effect of conditioning, $F(1, 35) = 7.30$, $p < .05$. As depicted in Figure 1e, LiCl-treated pups showed significantly higher scores than control counterparts during habituation, a result likely to be interpreted as a conditioned response evoked by the contextual cues

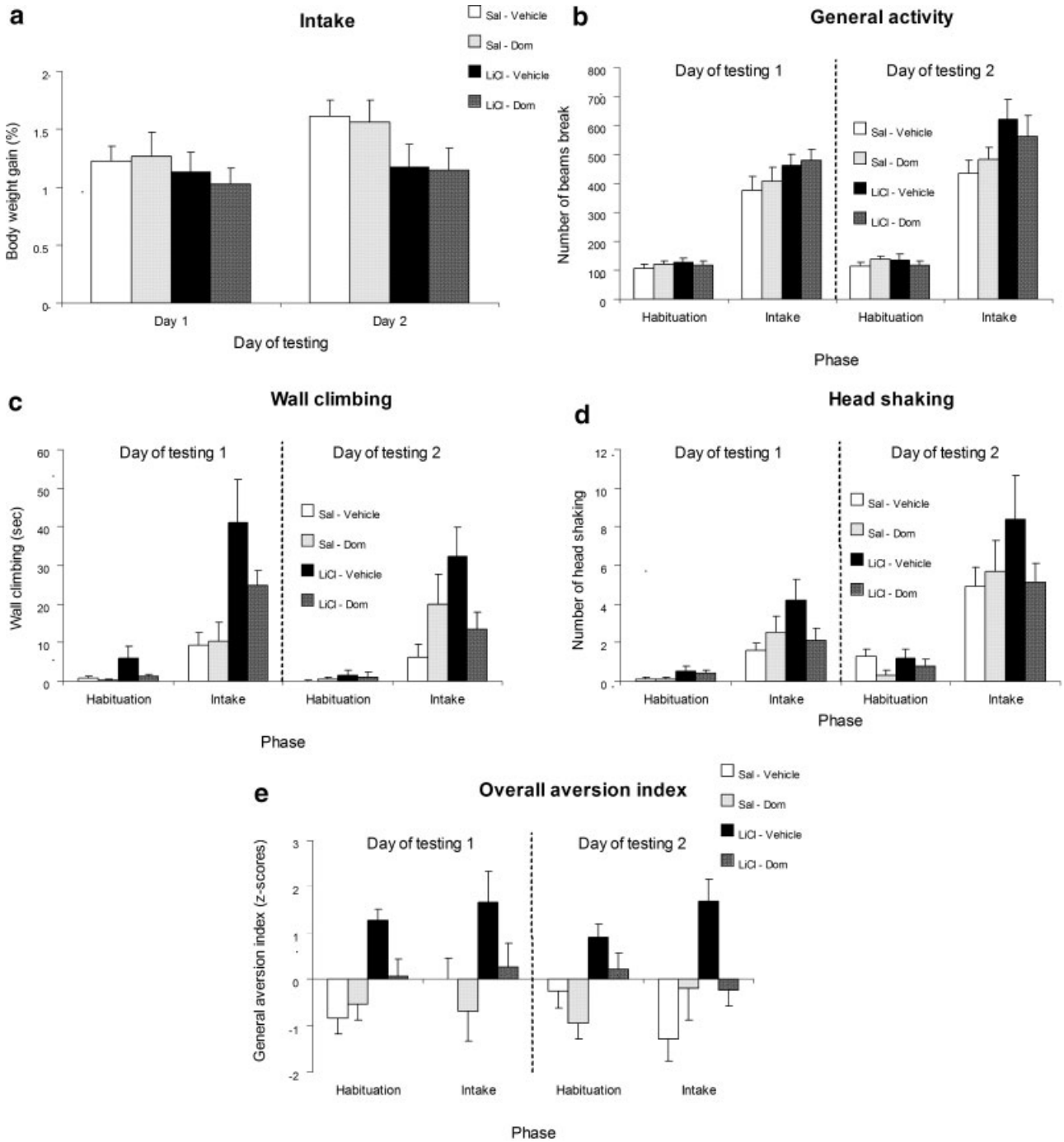

FIGURE 1 Saccharin consumption and behavioral reactions elicited by intraoral infusion of the tastant in rat pups given Domperidone (.0 or 1.0 mg/kg, i.p.) followed by a pairing between intraoral saccharin and Lithium Chloride (.5% of the body weight, .3 M). Conditioning was performed at postnatal day (PD) 13; testing took place at PD 14 and 15 (Days 1 and 2, respectively). The test involved a 2-min habituation phase followed by a 10-min intake phase. Vertical lines represent standard errors of the means. (a) Mean saccharin intake (% body weight increases) during conditioning and testing sessions as a function of conditioning treatment (LiCl or Saline) and domperidone administration (.0 or 1.0 mg/kg, i.p.). (b) General activity (number of beam breaks) at testing as a function of test phase (habituation or intake), conditioning treatment (LiCl or Saline) and domperidone administration (.0 or 1.0 mg/kg, i.p.). (c–e) Wall Climbing (s), frequency of head-shaking and overall behavioral reactivity (general activity + wall climbing + head-shaking) registered at testing, respectively, as a function of conditioning treatment (LiCl or Saline), domperidone administration (.0 or 1.0 mg/kg, i.p.) and test phase (habituation or intake).

present at test (for a similar result in adult rats, see Limebeer, Hall, & Parker, 2006).

During the intake phase of testing defined by saccharin intraoral delivery, animals treated with LiCl showed higher activity scores (number of beams broken) than saline-treated controls (Fig. 1b). The ANOVA for activity scores registered during the intake phase yielded significant main effects of conditioning treatment and day of testing [$F(1, 35) = 5.56$, $p < .05$; $F(1, 35) = 12.77$, $p < .05$; respectively]. LiCl-treated pups were more active than saline control pups during the intake phase. Activity scores were significantly higher on PD15 than on PD14. The analysis for general activity indicated a lack of main effect of domperidone treatment. Also, the latter factor was found not to significantly interact with any of the remaining variables in terms of modulating general activity.

Figure 1c and d depicts total time of wall climbing and number of head-shaking movements at test. Similar treatment effects were observed with both response measures. Across day of testing, LiCl–vehicle pups engaged in more wall climbing and head-shaking movements than Sal–vehicle animals. This difference was significantly inhibited by DOM treatment. The ANOVA for wall climbing scores confirmed these impressions. The analysis found significant main effects of conditioning treatment $F(1, 35) = 11.13$, $p < .005$; as well as a conditioning treatment × domperidone interaction $F(1, 35) = 6.47$, $p < .05$. Post hoc tests indicated that pups in the LiCl–Vehicle group showed more wall climbing than pups in groups LiCl–Domperidone or Saline–Vehicle. No significant main effect nor significant interaction comprising day of testing was revealed by the ANOVA, hence indicating that the pattern of wall-climbing emission was not affected by repeated testing.

The ANOVA for head-shaking scores indicated significant effects of day of testing [$F(1, 35) = 16.84$, $p < .001$] as well as a borderline significant interaction between conditioning treatment and domperidone, $F(1, 35) = 3.92$, $p = .055$. Head-shaking was more frequently observed on the second testing day than in the first one. Post hoc tests indicated that pups that received LiCl preceded by vehicle displayed more head-shaking than pups injected with DOM prior to LiCl. Head-shaking was also significantly higher in the LiCl–Vehicle group when compared with the Saline–Vehicle group.

The inferential analysis related with the overall aversion index during the intake phase of the test revealed a significant effect of conditioning treatment, $F(1, 35) = 14.36$, $p < .001$. The interaction comprising conditioning treatment and domperidone was also significant, $F(1, 35) = 7.80$, $p < .01$. LSDs analyses indicated that pups treated with LiCl and saline exhibited higher scores in this index of aversiveness than those treated with LiCl and DOM or with saline and vehicle. In other words, pups given a single pairing of LiCl and saccharin displayed a behavioral pattern indicative of conditioned aversion when reexposed to the taste CS. DOM treatment prior to conditioning inhibited the expression of disgust reactions (see Fig. 1e). The inferential analysis indicated no significant main effect nor significant interaction involving day of testing. We found no significant correlation between saccharin consumption levels at test and overall aversive scores. Pearson product-moment correlations between these variables were as follows: PD 14: $r = -.06$, PD 15: $r = -.21$, both p's $> .05$. Similar absence of significant correlations between saccharin intake and overall aversive scores was found when this relationship was analyzed separately for each group (LiCl–vehicle, LiCl–DOM, SAL–vehicle, SAL–DOM).

DISCUSSION

Under conditions of relatively short maternal separation and food/fluid deprivation, preweanling rats given a single pairing of intraoral saccharin and LiCl on postnatal day 13 subsequently exhibited less consumption of the tastant CS when compared to pups that originally were exposed to this CS paired with saline administration. This effect achieved statistical significance during the second testing day at PD 15. Saline-treated pups also exhibited a progressive increase in terms of intake of the CS, an effect likely to be mediated by nonassociative processes (familiarization and/or neophobia reduction). This effect was not observed in saccharin-LiCl pups. In other words, LiCl-treated pups showed an overall reduction in intake relative to saline-treated counterparts and did not show the day-to-day increase in saccharin intake seen in the saline controls. Both effects indicate that preweanlings acquired a significant, albeit modest, LiCl-mediated conditioned taste avoidance. More rapid and robust LiCl-induced CTA has been previously reported in infant rats (Arias & Chotro, 2006; Hoffmann et al., 1990; Hoffmann, Molina, Kucharski, & Spear, 1987). Yet, these studies employed not only higher LiCl dosage but also lengthier intervals of maternal separation prior to conditioning (i.e., up to 6 hr). The latter factor is known to exert facilitating effects upon LiCl-mediated CTA in preweanlings (Hoffmann et al., 1987). Employment of a relatively short deprivation schedule in the present work was aimed at reducing aversive unconditional effects associated with isolation (Smith et al., 1985).

LiCl-treated rats not only ingested less of the tastant CS than control counterparts but also exhibited heightened disgust reactions at test. When stimulated with saccharin, lithium-treated pups showed greater locomotion, head-shaking and wall climbing than did controls. Pretreatment

with the peripheral anti-emetic domperidone at conditioning markedly attenuated LiCl-mediated disgust reactions. Pups given the pairing between LiCl and intraoral saccharin after DOM administration showed similar levels of head shaking and wall climbing when compared with controls (group Sal–Vehicle). LiCl–DOM pups also displayed fewer disgust reactions than counterparts assigned to the LiCl–Vehicle group. Apparently, DOM treatment interfered with the shift in the palatability of the tastant CS mediated by LiCl. Yet, saccharin consumption at test was not affected by the anti-emetic treatment, indicating that DOM did not affect LiCl-mediated avoidance of the tastant. It is interesting to note that enhanced wall climbing in 15-day-old pups—viewed as a disgust reaction—has been observed in response to a tastant paired with LiCl but not with alternative aversive agents, such as footshock and citric acid (Hoffmann et al., 1991). Our results suggest that lithium-mediated activation of the chemoreceptors at the brainstem is responsible for this increase in wall-climbing.

Previous research conducted in adult rodents (Limebeer & Parker, 2003) found that intake of a tastant paired with lithium chloride and conditioned disgust reactions evoked by this CS are differentially affected by pretreatment with ondansetron, a 5-HT3 antagonist with potent antinausea effects. Results obtained in the present study agree with Limebeer and Parker (2003), showing dissociation between avoidance patterns and disgust reaction evoked by a LiCl-related CS. These results were obtained with the use of Domperidone, a peripheral dopaminergic antagonist that exerts its pharmacological effects outside of the area protected by the blood–brain barrier (Laduron & Leysen, 1979; Jolliet et al., 2007). This suggests that dopamine antagonism at the brainstem level (i.e., area postrema and/or nuclei of the solitary tract) is the mechanism underlying the phenomena reported here as well as in previous studies employing alternative antinausea treatments (i.e., Limebeer & Parker, 2003). As already stated, AP and NST do not exhibit an effective blood brain barrier (Chambers, 1990). The divergence between avoidance and disgust reactions seems to be present early in life during a developmental stage in which independent feeding is emerging in the rat (Hall, 1985).

DOM successfully suppressed conditioned head-shaking and wall-climbing mediated by systemic administration of LiCl. However, pretreatment with the peripheral anti-emetic agent failed to affect the enhanced general activity (i.e., locomotion) exhibited by lithium-treated pups. An increase in general activity has been commonly considered an index of aversive reactivity in studies assessing either aversive conditioning or acute response to aversive stimuli (Arias & Chotro, 2006; Brining, Belecky, & Smith, 1991). Nevertheless, this variable does not seem to be as sensitive (Arias & Chotro 2005, 2006) or as specific as wall climbing (Hoffmann et al., 1991). For instance, Pautassi, Nizhnikov, Molina, & Spear (2007) observed enhanced locomotion in animals that had experienced pairings of an odor CS and citric acid infusion. However, a follow-up experiment failed to replicate this result, indicating that the apparent locomotor conditioned response effect was not very reliable. Also, Arias and Chotro (2006) recently found enhanced wall-climbing but absence of changes in locomotion as a function of a treatment likely to induce aversive reactions.

During the habituation phase of testing LiCl-treated pups showed significantly higher overall aversion scores than saline-treated counterparts. This effect may indicate a conditioned response elicited by the testing context which had been previously paired with lithium-induced malaise (Limebeer et al., 2006). It might seem paradoxical that separate analyses of the component variables during the habituation stage revealed no significant main effects or interactions but the overall aversion index was affected by LiCl treatment. It needs to be noted that the overall aversion index is a composite, multivariate score derived from a linear addition of individual measures of taste reactivity. Hence, it is likely to be more sensitive for detection of aversive conditioning than each isolated dependent variable.

Suppressed consumption and rejection reactions were originally considered as homologous behavioral components in the expression of conditioned taste aversions (Garcia, Hankins, & Rusiniak, 1974). Under this theoretical framework, a shift in the palatability of a tastant is expected after pairing it with a nausea-inducing treatment. Taste avoidance induced by psychoactive drugs (e.g., amphetamine and cocaine) is not necessarily associated with the expression of conditioned disgust reactions (Parker, 1995) and antinausea treatments selectively alter disgust reactions but not taste avoidance in adult rodents (Limebeer & Parker, 2003). This has led Parker (2003) to postulate a two-factor process model. In the rat, reduction in the intake of a taste CS is likely to occur when the CS predicts a change in physiological state, even if that state is rewarding. According to Parker (2006), rats developed this highly sensitive defense system because they are incapable of vomiting. On the other hand, conditioned disgust reactions will be exhibited only if the US exerts emetic effects. Parker (2003) agrees with Balleine, Gerner, and Dickinson (1995) in that two processes are involved in CTA. Taste avoidance involves a signal-learning process in which the taste becomes a predictor of danger (i.e., homeostatic change), whereas conditioned rejections result from an incentive process in which the hedonic value of the taste shifts from neutral to aversive.

Overall, the results of the present study support Parker's hypothesis (2006); that is, emesis like effects are related with the establishment of conditioned disgust reactions but play no role in the acquisition of conditioned taste avoidance. We observed that an antinausea treatment inhibited disgust reactions while not affecting LiCl-mediated taste avoidance. The claim that taste avoidance and disgust reactions reflect different processes is further supported by the lack of association observed between intake values at testing and overall aversion scores.

It is of interest to discuss the findings of the present work within the frame of the study by Cántora et al. (2006), the first study that systematically assessed extinction of lithium-mediated taste avoidance and taste reactivity in adult rats. These authors found that extinction of conditioned disgust reactions preceded extinction of taste avoidance. Our results agree with Cántora et al. (2006) in that taste avoidance and conditioned disgust reactions yielded different patterns of extinction. However, in the present study LiCl-treated infants showed enhanced conditioned disgust reactions across days of assessment. On the other hand, taste avoidance was better expressed during the second day of assessment. Further research is needed to establish whether this reflects differences in the sensitivity of these indices or an specific behavioral characteristic of the infant rat. The apparent discrepancy could also relate to procedural differences underlying methods to assess CTA in infants and adult rats (i.e., intraoral infusion of the taste CS vs. two-bottle test).

These results also show that dissociation between taste avoidance and conditioned disgust reactions can be found in 2-week-old pups, a stage in development in which aversive interoceptive and exteroceptive USs differ in the specific patterns of conditioned disgust reactions they elicit (Hoffmann et al., 1990). Future research is needed to assess whether similar relations between LiCl-mediated taste avoidance and conditioned disgust reactions are observed at those younger ages at which similar patterns of CTA are found across a variety of USs, regardless of their capability to activate the chemoreceptor nuclei located at the midbrain and brainstem (Hoffmann et al., 1990).

NOTES

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