Peculiar Modulation of Taste Aversion Learning by the Time of Day in Developing Rats

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ABSTRACT:

The ontogeny of the temporal context modulation of conditioned taste aversion was studied in male Wistar rats using a palatable 1% NaCl solution. A procedure that included two saline preexposures, a single pairing saline-lithium chloride (0.15 M; 1% b.w.) either at the same or a different time of day of preexposures and a one-bottle test at the same time than preexposure was applied. Four age groups (PN32, PN48, PN64, and PN100) covering the complete range from adolescence to the adult period were tested. The results showed no effect of a temporal context shift in PN32. A peculiar enhancement of temporal context specific saline aversions was exhibited by PN48 and PN64 rats, while the adult typical temporal context specificity of latent inhibition was only evident in PN100 rats. The results are discussed in terms of the peculiar brain functional organization during a protracted adolescence period.

Keywords: taste aversion; temporal context; adolescence; rats; latent inhibition; post-weaning; neophobia.

INTRODUCTION

Conditioned taste aversions (CTA) are readily acquired in one-trial by adult rats if intake of a taste solution is followed by visceral distress induced by lithium chloride (Bures, Bermúdez-Rattoni, & Yamamoto, 1998). The learned response consists of a shift of the taste hedonic value, thus becoming unpalatable and being avoided in later encounters (Grill & Norgren, 1978). Among other factors, such as palatability, taste novelty is a potent modulator of CTA. Previous exposure to the taste without consequences retards CTA acquisition, a well known phenomenon called latent inhibition (Lubow, 1989).

Context may also modulate CTA. In addition to context aversions that may be induced by lithium chloride if several conditioning trials are applied (Boakes, Westbrook, & Barnes, 1992; Rodríguez, López, Symonds, & Hall, 2000; Symonds&Hall, 1997), both latent inhibition (LI) of CTA and CTA itself may exhibit context-dependency under certain circumstances. First, a context change between preexposure and conditioning may attenuate the latent inhibition effect (Hall & Channell, 1986; Rudy, Rosenberg, & Sandell, 1977). Second, a context change between conditioning and testing may interfere with learned taste aversions retrieval (Boakes, Westbrook, Elliot, & Swinbourne, 1997; Bonardi, Honey, & Hall, 1990; Loy, Álvarez, Rey,&López, 1993; Puente, Cannon, Best, & Carell, 1988).

We have previously reported that a time of day shift may also act as a context, thus modulating CTA in the absence of other environmental changes (Manrique et al., 2004; Morón et al., 2002). Using a behavioral procedure that included changing the temporal context between preexposure and testing and also between conditioning and testing, we have demonstrated the temporal context dependency of both latent inhibition and conditioned taste aversion. Evidencing the former or the latter phenomenon depends on subtle modifications of the same basic behavioral procedure. Thus, if the procedure includes a long habituation period to drink twice a day and nonrestricted intake during conditioning, those groups preexposed and conditioned at a different time of day exhibit stronger aversions than those preexposed and conditioned at the same time of day. Therefore, a time of day shift between preexposure and conditioning interferes with LI (Manrique et al., 2004). However, the opposite pattern is seen by applying only 2 days of previous habituation to drink water twice a day and restricted drinking during the conditioning session. The group conditioned and tested at the same time of day shows stronger aversions than that conditioned and tested at a different time of day (Morón et al., 2002). This indicates that the same time of day of conditioning facilitates retrieval of the aversive taste memory during testing (Manrique et al., 2004).

Ontogenetically, CTAis a primitive and early developing type of associative learning. The ability to associate flavor cues with subsequent lithium-induced visceral distress and to exhibit conditioned flavor aversions in later encounters has been reported in rat foetuses (Abate, Pepino, Domínguez, Spear, & Molina, 2000; Smotherman, 2002a,b; Smotherman&Robinson, 1985). Neonatal rats are able to learn odor and taste aversions. Lemon-quinine pairings 3–5 hr after birth result in odor aversions that reduce both attachment to a surrogate nipple and milk intake in the odor presence (Nizhnikov, Petrov,&Spear, 2002).Rudy and Cheatle (1977) reported aversions in 8-day-old rat pups exposed to an odor-lithium chloride pairing at the age of 2 days. Although the nursing situationmay interferewith the acquisition of learned taste aversions (Alberts&Gubernick, 1984;Martin & Alberts, 1979), aversions to sweet and salty solutions followed by lithium chloride injections are evidente in 5-day-old rat pups when tested 5 or 16 days later (Kehoe& Blass, 1986). Several other studies have confirmed that conditioned taste aversions can be established in preweanling rats (Chotro & Alonso, 1999, 2001, 2003; Hoffmann, Molina, Kucharski, & Spear, 1987; Kraemer, Hoffmann, & Spear, 1988).

As it has been described in other types of learning, new CTA capabilities emerge at older ages, supporting both longer retention intervals (Gregg, Kittrell, Domjan, & Amsel, 1978; Guanowsky, Misanin, & Riccio, 1983; Schweitzer & Green, 1982) and the appearance of more complex associative phenomena, such as latent inhibition or second-order conditioning (Ader&Peck, 1977; Cheatle & Rudy, 1979). With respect to the ontogeny of LI using taste aversion tasks, there are different results depending probably on procedural variations. On one hand, Kraemer et al. (1988) reported LI in 12-day-old but not 6-day-old rat pups using an intraoral infusion procedure and a short interval between preexposure and conditioning, provided that testing took place 20 hr after conditioning but not 10 days later. However, since the effect appeared also in a control group preexposed to a different nonconditioned flavor, it cannot be considered a proper ‘‘adult like’’ LI. Other studies (Franchina,Domato, Patsiokas,& Griesemer, 1980) have demonstrated LI of CTA at postnatal Day 20 (PN20), and it has been reported even greater preexposure effects at about this age than in adults (Franchina & Horowitz, 1982; Misanin, Blatt, & Hinderlitter, 1985; Misanin, Guanowsky,&Riccio, 1983). All of these studies shared the use of a choice-test applied 24 hr after conditioning. On the other hand, there are studies reporting deficits in latent inhibition of CTA before 20–25 days of

age (Klein, Mikulka, Domato, & Hallstead, 1977; Nicolle, Barry, Veronesi, & Stanton, 1989). Nicolle et al. (1989) performed a well controlled study which included 18-, 25-, and 32-day-old rats in order to assess LI of CTA to coffee and saccharin solutions. By applying four preexposures to the solutions via intraoral cannulae along 2 days and lithium injection after a fifth solution infusion the next day, they did not found evidence of LI of CTA in rats younger than 32 days of age, the aversion being tested in a one-bottle test performed 4 days later. Therefore, the ability of taste preexposure to retard the acquisition of later conditioned taste aversions emerges early and perhaps prior to weaning age in rats, but evolves during the post-weaning age before becoming an adult-like LI. This is consistentwith the effect of conditioned stimulus (CS) preexposure in young rats using other learning tasks, such as conditioned emotional response (CER) (Kraemer & Randall, 1992; Zuckerman, Rimmermann, & Weiner, 2003), eyeblink conditioning (Stanton, 2000), odor aversive conditioning (Hoffman & Spear, 1988) and odor-shock potentiated startle (Richardson, Fan, & Parnas, 2003). While there is an early emergence of LI with different developmental courses depending on the specific task, theCS-preexposure effect is present in 35-day-old rats but exhibits peculiar features not found in adults. Accordingly, Zuckerman et al. (2003) have reported LI of CER in 35-day-old rats which is not affected by amphetamine, by decreasing the number of preexposures, increasing the number of conditioning trials nor by a context shift between preexposure and conditioning.

To the best of our knowledge no data are available on the ontogeny of the LI contextual specificity using CTA. Moreover, studies on conditioned emotional response (CER) provide conflicting results. Rudy (1994) reported context-specific LI in 23-day-old rats, while Zuckerman, Rimmerman, and Weiner (2003) showed that 35-day-old rats exhibited LI ‘‘resistant’’ to a context shift between preexposure and conditioning. Using odor-aversive conditioning Yap and Richardson (2005) have reported a late emergence of the context effects on LI, since 25-day-old, but not 18-day-old rats exhibit context-specific LI.

The present study aimed to assess LI of CTAin order to explore the ontogeny of its contextual specificity. Since we have previously reported context-specific LI of conditioned saline aversions in adult rats (Manrique et al., 2004) by using the time of day as context,we applied the same behavioral procedure that included 5 days of habituation to drink twice a day and nonrestricted intake during conditioning. In order to examine the emergence of the temporal context dependency of LI during the adolescence period, which has been widely defined as covering a wide age range from PN28 to around PN60 (Spear, 2000), the performance of three adolescent groups (PN32, PN48, PN64) were compared with adult 3-monthold groups (PN100). It can be hypothesized that all the groups should show latent inhibition of CTA and a late onset during adolescent of the contextual specificity of latent inhibition using a conditioned taste aversion procedure. Thus, the context specificity of LI should not appear in the younger PN32 group but it could be evidente either from PN48 or PN64.

METHOD

Subjects

One hundred and forty one male Wistar rats obtained from 31 litters were used. The female pregnant rats were checked daily for new births being the first postnatal day (PN0) the morning in which the new litters were observed. Three days after birth each litter was culled to ten pups (being the males always preserved) and housed with their dams in standard clear polyethylene hanging cages.Weaning took place on postnatal day 19 (PN19). The litters were individually housed in an isolated room with constant temperature (22–24□C) and a 12:12 hr. light-dark cycle

(lights on at 8:00 am and off at 8:00 pm). Food was available *ad libitum*, but water availability depended on the behavioral procedure. After weaning, the rats were housed in groups of three to four subjects until the training procedure required individual housing.

Pups were randomly assigned to one of 16 groups as dictated by the 4x2x2 (AgeXPreexposureXGroup) design (see Tab. 1). The behavioral procedures were approved by the University of Granada Ethics Committee for Animal Research, and were in accordance with both the NIH of the United States guidelines for the ethical treatment of animals, and the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Apparatus

During the behavioral training the rats remained in individual home cages, which consisted of one chamber (30x15x30 cm) made of four walls: two opposing walls were made of opaque polyethylene; the front and back walls were made of clear polyethylene. The front wall had two holes of 1.6 cm of diameter, placed to equal distance from the center and 10 cm from the bottom of the cage, thus allowing us to introduce the graduated burettes containing the taste solution. The luminance provided by the lights located on the ceiling of the room provided 40 nit.A ventilation fan that was located on the side wall of the room produced a low-level background noise of 66 dB.

Procedure

The animals were subjected to the long-habituation procedure described by Manrique et al. (2004). Throughout the behavioral procedure they had two daily 15 min drinking sessions. Morning (9:00 am) and evening (7:00 pm) drinking sessions were used to maximally differentiate the temporal contexts. The procedure consisted in five phases: Habituation, Taste Preexposure,Conditioning, Recovery and Testing (see Tab. 1).

After being habituated for 5 days to the water deprivation procedure, the rats belonging to the preexposed groups (Pre) were allowed to drink saline solution (NaCl, 1% diluted in distilled water) for 15 min during the evening session for the following 2 days while those belonging to the non-preexposed groups (Ctrl) were allowed to drink water. The next day conditioning took place during the evening session. All the rats were allowed to drink a sodium chloride solution (1%) for 15 min. Fifteen minutes later they received an intraperitoneal (i.p.) injection of lithium chloride (LiCl 0.15 M; 2% body weight) and were returned to the home cage. After two recovery days with water available during the drinking sessions, a one-bottle test was applied during the evening drinking session. The amount of fluid ingested was recorded to the nearest 0.1 ml.

In order to test LI and its contextual specificity, each age group was further divided in four groups according to the presence or absence both of a time of day shift during conditioning and of saline preexposures. Same groups were conditioned during the evening drinking session while Diff groups were conditioned during the morning drinking session, that is, at a different time of preexposure and testing. In order to facilitate comparisons between different age groups a test intake rate was calculated (test/CTA□100), representing 100 no aversion, since the animals would have drunk the same amount during testing than during conditioning and 0 maximum aversion.

RESULTS

Table 2 shows the mean (±SEM) body weights of the different groups at the behavioral procedure onset. A 4x2x2 (AgexPreexposurexGroup) ANOVA análisis evidenced a significant main effect of Age ($F(3,125)=847.86$; $p<.01$), showing an expected weight increase related with increasing age. Thus, post hoc LSD comparisons showed differences between all the age groups ($p<.01$). No other effects were significant.

A 4x2x2 (AgexPreexposurexGroup) ANOVA analysis of the water consumption during the baseline showed a significant main effect of Age ($F(3,127)=12.96$; $p<.01$), but no other main effects nor interactions were significant. Post-hoc LSD comparisons indicated that PN32 group consumed less water than the rest of the groups ($p<.01$). In spite of the age-related weight increase, no differences were seen among the rest of the groups in the water intake.

In order to explore the neophobic response and its potential habituation a 4x4 (AgexDays) mixed ANOVA analysis of the amount of water and saline solution drunk during the baseline, preexposures and conditioning sessions in those groups conditioned at the same time of preexposures (Pre-Same) was performed. There was a significant effect of Age ($F(3,32)=11.29$; $p<.01$), Days ($F(3,96)=24.90$; $p<.01$) and the interaction AgexDays ($F(9,96)=2.11$; $p<.05$). Repeated measures ANOVA analyses of the amount drunk by each age group including the baseline, preexposures and conditioning sessions revealed a significant main effect of Days in each group, PN32 ($F(3,21)=7.84$; $p<.01$), PN48 ($F(3,24)=3.47$; $p<.05$), PN64 ($F(3,24)=11.50$; $p<.01$) and PN100 ($F(3,27)= 15.96$; $p<.01$).

Post-hoc LSD comparisons showed increase of saline intake during the first preexposure compared with the previous baseline water drinking session in the each of the age groups ($p<.05$). An increase of saline consumption during the second preexposure compared with the first preexposure was evident only in PN32 ($p<.01$) and PN100 groups ($p<.01$). However, PN32 rats reduced again the saline intake during the third preexposure with no significant differences in comparison with the first preexposure, while adult rats maintained a high saline intake with no differences between the second and third preexposures, thus confirming attenuation of neophobia only in adult rats. Unexpectedly, PN64 rats that did not show attenuation of neophobia in the second preexposure, exhibited a later increase in saline solution intake during the third preexposure, that is, the conditioning sesión compared with the previous preexposure session ($p<.01$) which did not appear in the younger groups.

Regarding the ontogeny of taste neophobia, the data support not only the presence of a neophobic response to a highly accepted lowsaline concentration that requires two exposures to habituate in PN100 group, but also a saline neophobic response at 64 days of age that required three exposures to habituate. No evidence of neophobic responses were found in the younger groups.

Figure 1 shows the mean (±SEM) test intake rates of the different groups. A 4x2x2 (AgexPreexposurexGroup) ANOVA analysis showed a significant effect of the interaction AgexPreexposurexGroup ($F(3,125)=6.12$; $p<.01$). Therefore, 2x2 (PreexposurexGroup) two way ANOVA analyses were performed in each age group. In PN32 there was a significant effect of Preexposure ($F(1,28)=16.14$; $p<.01$), evidencing the non-preexposed groups stronger aversions (mean: 60.26±7.17) than the preexposed groups (mean: 24.30±4.9), but no effect of time of day shift ($F(1,28)=.21$; $p>.65$), nor interaction ($F(1,28)=.04$; $p>.84$). Thus, the results revealed the presence of latent inhibition and no effect of the temporal context shift on LI of CTA at this age.

In PN48 there was a significant main effect of Preexposure ($F(1,32)=33.77$; $p<.01$), Group ($F(1,32)=5.25$; $p<.05$) and the interaction PreexposurexGroup ($F(1,32)=4.76$; $p<.05$). Post-hoc LSD

comparisons revealed that the effect could be attributed to the differences between the preexposed groups, since no differences were seen between the Ctrl non-preexposed groups ($p>.94$) which exhibited a low test intake rate, that is, strong saline aversions. LI was evident in both preexposed groups, since both Pre-Same ($p<.01$) and Pre-Diff ($p<.05$) showed higher intake rates than their respective control non-preexposed groups. The Pre-Diff group also showed a weaker aversion than Pre-Same group ($p<.01$). This pattern of results supported the presence of context specific saline aversions in the preexposed animals and absence of context specific LI.

Unexpectedly, similar results were obtained in PN64 rats. Again, there was a significant main effect of Preexposure ($F(1,31)=39.01$; $p<.01$), Group ($F(1,31)= 6.71$ $p<.01$) and the interaction PreexposurexGroup ($F(1,31)=10.74$; $p<.01$). Post-hoc LSD comparisons confirmed that the effect could be attributed to the differences between the preexposed groups, since no differences were seen between the Ctrl non-preexposed groups ($p>.64$), which exhibited a low test intake rate, that is, strong saline aversions. LI was evident in both preexposed groups, since both Pre-Same ($p<.05$) and Pre-Diff ($p<.01$) showed higher intake rates than their respective control non-preexposed groups. Again, the Prediff group showed also a weaker aversion than Pre-Same group ($p<.01$).

The results obtained with the adult group confirmed previously reported results showing that a time of day shift during conditioning disrupt LI (Manrique et al., 2004). There was a significant main effect of Preexposure ($F(1,34)=15.39$; $p<.01$), Group ($F(1,34)=4.06$; $p<.05$) and the interaction Preexposure□Group ($F(1,34)=6.19$; $p<.05$). Post-hoc LSD comparisons revealed that the effect could be attributed to the differences between the preexposed groups, since no differences were seen between the Ctrl non-preexposed groups ($p>.75$) which exhibited a low test intake rate, that is, strong saline aversions. Interestingly, the pattern of results shown by the preexposed groups was opposite to that found in the younger groups. LI was evident only in the Pre-Same group ($p<.01$), but not in the Pre-Diff group ($p>.32$). In fact, the Pre-diff group showed also a stronger aversion than Pre-Same group ($p<.01$).

Taken together the results indicated a late emergence of the adult time-of-day specific LI (Manrique et al., 2004), which is only observed in the adult (PN100) group. Consistent with a context-independent LI, the deleterious effect of saline preexposures on later CTAwas resistant to a time of day shift in PN32. However, the time of day shift induced a peculiar strong effect both in PN48 and PN64. Those preexposed groups conditioned at a different time of day of preexposure and testing exhibited weaker saline aversions than those conditioned at the same time of day of preexposure and testing. This is an effect previously reported by using a short-habituation procedure in adult rats (Morón et al., 2002), but never obtained with the present long-habituation protocol in adult rats (Manrique et al., 2004).

Regarding the ability to learn a saline aversion, a oneway ANOVA analysis of the test intake rates showed by the Ctrl non-preexposed groups at the different ages showed a significant main effect of Age ($F(3,59)=16.38$; $p<.01$), evidencing weaker aversions in the younger PN32 group than in the rest of the groups ($p<.01$). No differences were seen among the rest of the groups. There was not significant effects of Group ($F(1,59)=.24$; $p>.63$) nor an AgexGroup interaction ($F(3,59)=.39$; $p>.76$). This result evidenced that the learning ability improved with age, having reached the adult level by 48 days of age.

DISCUSSION

Several findings regarding the ontogeny of taste neophobic responses, the ability to learn taste aversions, the latent inhibition phenomenon and the effects of context modulation on CTA

are reported using a low concentration NaCl solution as the taste stimulus in rats. First, regarding the ontogeny of the neophobic response to a 1% sodium chloride (NaCl) solution, the results showed increased intake of the novel salty solution during the first preexposure session in all the age groups. We have previously reported similar results in adult and aged Wistar rats (Morón & Gallo, 2007). These data confirm the high palatability of this low saline concentration solution for rats. Accordingly, previous results have shown that this solution is preferable to water (Bare, 1949; Kare, Fregly, & Bernard, 1980; Kiefer & Grijalva, 1980; Pfaffmann, 1960; Weiner & Stellar, 1951).

Although taste neophobia is defined as the reluctance to consume novel-taste solutions (Bures et al., 1998), the demonstration of such reluctance requires an increase in taste consumption induced by the successive presentations without negative consequences (neophobia attenuation), regardless of previous water consumption. This is especially true if a palatable solution is used, as in our case. Therefore, we determined the absence of the neophobic response to a highly palatable NaCl solution not only by comparisons between saline consumption and previous-day water consumption, but also by further comparisons with saline intake on a second presentation as proposed by Reilly and Bornovalova (2005), and even on a third presentation.

The results of the water baseline and saline intake during the two preexposure sessions confirmed the absence of saline neophobia both in PN48 and PN64, since no further saline consumption increase was seen in the second preexposure session. The transitory increase of saline consumption observed in PN32 group during the second exposition does not seem to reflect attenuation of neophobia, since it disappeared during the following presentation in which the volume ingested decreased to the first preexposure level. However, in adult rats an increase in saline ingestion during the second presentation, which was maintained on the following presentation, evidenced the presence of neophobia.

Thus, the data concerning previous water intake and two saline presentations could be initially interpreted in terms of absence of neophobia until the age of 64 days. However, the analysis of the saline intake in the third presentation, that is, the saline intake during the conditioning session in those groups preexposed and conditioned at the same time of day, revealed the presence of neophobia not only in the 100-day-old group but also in the 64-day-old group, since an increase of saline intake was seen during the conditioning session. No such increase occurred in the younger groups confirming the absence of neophobia. The possibility that determining the presence of neophobic responses to highly palatable solutions may require a minimum of three taste presentations instead of two as previously proposed (Reilly & Bornovalova, 2005) should be considered.

There are three different explanations for the absence of neofobia to a NaCl solution in the younger groups aged from 32 to 48 days. First, the absence of neophobia could be attributed to immaturity of the gustatory system. Important changes in the sensitivity of the gustatory system to NaCl take place during development as shown by neurophysiological data. It has been reported that functional responses of the nucleus of the solitary tract, which is the first relay level of the gustatory system in rats, are not mature until after 35 days of age (Hill, Bradley, & Mistretta, 1983). Thus responsiveness to salts changes dramatically during a prolonged developmental period. Compared with adult rats, younger 25-day-old rats drink higher amounts of high concentration NaCl solution that is aversive to mature rats (Midkiff & Bernstein, 1983). This could be interpreted as the younger rats failing to perceive qualitative or quantitative features of the stimulus. However, in the present experiment all the age groups demonstrated

the ability to detect the saline solution, because learned aversions were evident. While a reduced sensitivity to the low saline concentration used in the present experiment cannot be fully discarded in 32-day-old rats that showed weaker saline aversions tan adult rats, this explanation is not supported in the 48-dayold group exhibiting robust saline aversions similar to that of older age groups.

In the second place, the absence of saline neophobia in the younger groups could be due to an unspecific effect of early handling and training after weaning. It has been reported decreased neophobia induced by early handling (Weinberg, Smotherman, & Levine, 1978). However, all the groups were similarly treated concerning weaning and early handling.

Finally, the most feasible explanation for the absence of neophobia, at least in PN32 and PN48 groups, is that based in the increased novelty seeking associated to adolescence (Spear, 2000). Reduced neophobic responses have been reported in adolescent rats (Darmani, Shaddy, & Gerdes, 1996; Spear, 2000; Spear, Shalaby, & Brick, 1980). Although a reduced salt sensitivity cannot be discarded in the younger 32-day-old group, the absence of neophobia in 48-day-old rats is more likely due to the peculiar features of the adolescent behavior leading to novelty seeking. If this were so, the results support that the performance of 64-day-old rats may reflect the transition to the mature behavioral pattern regarding responses to taste novelty.

With respect to the ontogeny of CTA, the ability to learn aversions to a low saline concentration was present in 32-day-old rats which exhibited weaker aversion tan the rest of the groups that did not differ between them. The results confirm previous reports showing lithium-induced aversions to salty solutions at early ages (Kehoe & Blass, 1986), but they indicate a developmental course of CTA capability throughout the preadolescent and early adolescent period. As mentioned above, the protracted developmental course of the brain mechanisms involved in NaCl perception which may have led to a potential reduced sensitivity to the taste solution, as well as the delayed maturation of the associative and memory processes required (Vogt & Rudy, 1984), can provide a feasible explanation for the improvement in long-delay CTA ability from 32 to 48 days.

Third, regarding the ontogeny of the latent inhibition phenomenon, the results confirmed the presence of LI from 32 days of age, using a one-bottle test applied 3 days after conditioning. Although LI of CTA has been demonstrated in younger rats (Franchina et al., 1980; Franchina & Horowitz, 1982; Klein et al., 1977; Misanin et al., 1983, 1985), our testing procedure and conditioning- testing interval better resembles that applied by Nicolle et al. (1989). Nicolle et al. (1989) found evidence of latent inhibition of CTA using four intraoral taste preexposures applied along 2 days at 32 days of age but not in younger 18- or 25-day-old rats. The authors attributed the delayed onset of latent inhibition to immaturity of the hippocampal system, since fórnix transections performed in preweanling rats disrupted the emergence of latent inhibition at 32 days of age. However, at present, there is no evidence for a critical hipocampal involvement in latent inhibition of CTA (for reviews see Buhusi, Gray, & Schmajuk, 1998; Gallo, Ballesteros, Molero, & Moro´n, 1999). Lesion studies in adult rats showed no effect (Gallo & Ca´ndido, 1995) or enhancement of latent inhibition (Purves, Bonardi, & Hall, 1995; Reilly, Harley, & Revusky, 1993). Moreover, fórnix transection has been shown not to disrupt LI in adult rats (Weiner, Feldon, Tarrasch, Hairston, & Joel, 1998). It can be envisaged that fornix transection during the early development can have widespread effects on the neural networks organization hindering a simple explanation.

Finally, the most outstanding finding in the present study is the modification of the temporal context effect on CTA throughout the adolescence period. The range of developmental ages chosen, from PN32 to PN64, covered the complete adolescence period in rats, according to the most wide behavioral criteria (Spear, 2000). According to the hypotheses, the results show a late emergence of the temporal context modulation of CTA, which is not evidente in 32-day-old rats. Moreover, unexpectedly the temporal context modulation of CTA in PN48 and PN64 dramatically differs of that seen in the PN100 group. In fact, a time of day shift during the conditioning session induced a similar pattern of results at 48 and 64 days that was opposite to that seen at 100 days of age. In adult rats the temporal context change disrupted LI of CTA, thus showing a similar aversion to that exhibited by the nonpreexposed groups. Therefore, the group conditioned at a different time of preexposure (Diff) showed a stronger aversion than the group preexposed and conditioned at the same time of day (Same). This temporal context specificity of LI has been previously reported in adult rats using an identical procedure (Manrique et al., 2004).

However, the latent inhibition of CTA was not disrupted by conditioning the saline aversion at a different time of day than the preexposure sessions both in PN48 and PN64 groups. The Pre-Same and Pre-Diff groups both had weaker aversions than their respective non-preexposed control groups. Context non-specific LI has also been previously reported (Hall&Channell, 1986; Kurz & Levitsky, 1983; Rudy, Rosenberg, & Sandell, 1977). Moreover, in these age groups the time of day shift induced an opposite pattern of differences, with stronger aversions in the Pre-Same groups than in the Pre-Diff groups. We have previously reported a similar pattern of differences between Same and Diff preexposed groups in adult rats (Morón et al., 2002) applying a behavioral protocol that included only two habituation days and restricted drinking during conditioning. A weaker aversion in those groups conditioned and tested at a different time of day than in those conditioned and tested at the same time of day may be the result of a temporal context specific aversion in the preexposed groups. The absence of differences between the Ctrl Pre and Ctrl Diff non-preexposed groups confirms previous results using one-trial CTA and one-bottle tests (Bonardi, Honey, & Hall, 1990; Rosas & Bouton, 1997) and it is consistent with previous findings showing that taste familiarity increases the contextual control of the aversion (Boakes et al., 1997; Puente et al., 1988).

It can be proposed that the contextual specificity of LI and the contextual specificity of CTAare developmentally dissociable phenomena, showing the former a later emergence than the later. This is consistent with data showing thatNMDA lesions of the dorsal hippocampus in adult rats disrupted the context specificity of LI but they did not impair the context specificity of CTA (Molero et al., 2005). A bulk of results has pointed to a delayed emergence during development of learning and memory functions requiring a mature hippocampus (Bachevalier & Vargha-Khadem, 2005; Stanton, 2000). In fact, context dependent learning effects show a late emergence during development in other aversive learning tasks, such as fear conditioning (Rudy, 1994; Rudy & Morledge, 1994; Yap & Richardson, 2007). Furthermore, it has also been suggested that different functions of context cues in learning and memory (Holland & Bouton, 1999) may show different developmental courses (Carew & Rudy, 1991; Rudy, 1993).

The contextual modulation of CTA exhibited by the adolescent groups in our study may involve associations between the time of day and the taste CS. However, adult rats would have represented the saline taste separately from the time of day it was experienced, since the opposite pattern of results was evident. Thus, the time of day specificity of LI could depend on the ability for segregating elements of an experience into separate internal representations, a

function which has been attributed to hippocampus using spatial cues (Kubik & Fenton, 2005). Consistently, previous research has indicated that taste and the time of day are more likely to be represented as a compound stimulus (saline-AM or saline-PM) and associated with malaise after hipocampal lesions in aged rats, thus leading to a similar pattern to that shown by adolescents in the present study (Manrique et al., 2008). This could support the idea that a late emerging function of hippocampus and related cortical networks is to facilitate the segregation of stimulus representations even when the context does not include spatial information. Therefore, the behavioral procedure that evidenced the temporal context specificity of LI in adult rats may have disclosed the temporal context specificity of CTA in developing rats, leading to the opposite pattern of differences seen in adolescent rats.

Additionally, the peculiar organization of the learning and memory systems during this developmental period could have facilitated the formation of a compound representation of saline and time-of-day. Brain development involves not only progressive but also critical regressive processes, taking place at different developmental stages in different brain areas. Thus, the developing brain systems involved in learning and memory exhibit peculiar patterns of organization that are not seen in adults and that may promote unique types of learning at early developmental stages. Although the evidence is limited to earlier ages than those used in the present study, peculiar features of learning have been demonstrated in developing rats, such as the enhancement of the ability to establish associations between stimuli not found in adults (Bordner & Spear, 2006; Campbell & Spear, 1972; Hoffmann & Spear, 1988; Molina, Hoffmann, Serwatka, & Spear, 1991), the potentiation of the contextual conditioning by CS conditioning (Brasser & Spear, 2004) and facilitation of sensory preconditioning (Barr, Marrott, & Rovee-Collier, 2003). Our results add evidence for a peculiar organization of the learning and memory systems which is still evident at 64 days of age. This shows a protracted time frame of adolescence which is consistent with the extended course of brain maturation both in rats (Bockhorst et al., 2008) and humans (Durston & Casey, 2006).

In summary, the results reported in the present study show a dissociated developmental emergence of the phenomena studied. Latent inhibition appears at 32 days of age. Neophobic responses to the palatable saline solution showed a later emergence being evident after the attenuation of neophobia at the third exposure in PN64 while at the second exposure in PN100 rats. No effect of a temporal context shift was seen in PN32. Moreover, a peculiar enhancement of a temporal context specific CTA was exhibited only by PN48 and PN64 rats, while the adult typical temporal context-dependency of LI was evident in PN100 rats.

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Table 1. Behavioral Procedure

	Habituation (5 days)	Preexposure (2 sessions)	Conditioning (1 session)	Recovery (2 days)	Testing (1 session)
Pre-Same	am and pm water	am: water pm: Sal	am: water pm: Sal ± LiCl	am and pm water	am: water pm: Sal
Pre-Diff	am and pm water	am: water pm: Sal	am: Sal ± LiCl pm: water	am and pm water	am: water pm: Sal
Ctrl-Same	am and pm water	am and pm water	am: water pm: Sal ± LiCl	am and pm water	am: water m: Sal
Ctrl-Diff	am and pm water	am and pm water	am: Sal ± LiCl pm: water	am and pm water	am: water pm: Sal

am/pm, temporal contexts morning/evening; Sal, Isotonic saline, 1%; LiCl, Lithium Chloride. Pre, Preexposed group; Ctrl, Control group; Same, groups with preexposure, conditioning and testing during evening session; Diff, groups with conditioning during morning session.

Table 2. Experimental Design Including Number of Subjects in Each Group and Mean ± SEM of Weight by Age

	PN32	PN48	PN64	Adult
Preexposed				
Same	$n = 8$, 83.47 gr ± 5.35	$n = 9$, 141.22 gr ± 6.28	$n = 9$, 171.13 gr ± 6.69	$n = 10$, 255.84 gr ± 4.6
Diff	$n = 8$, 85.03 gr ± 3.35	$n = 10$, 154.16 gr ± 6.15	$n = 10$, 155.93 gr ± 5.06	$n = 10$, 257.82 gr ± 4.31
Control				
Same	$n = 8$, 86.7gr ± 4.48	$n = 9$, 139.98 gr ± 4.03	$n = 8$, 161.02 gr ± 4.71	$n = 9$, 257.71 gr ± 3.04
Diff	$n = 8$, 84.32 gr ± 3.66	$n = 8$, 145.15 gr ± 3.85	$n = 8$, 156.16 gr ± 5.53	$n = 9$, 253.89 gr ± 4.18

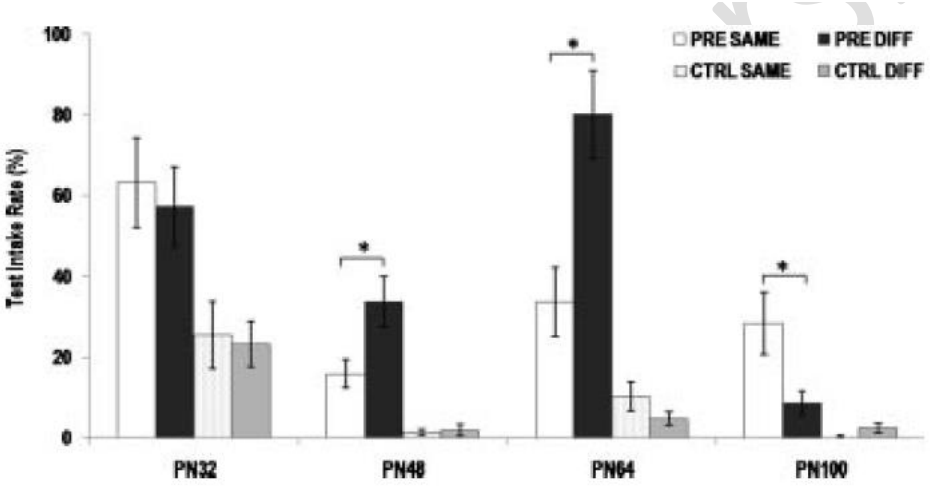

FIGURE 1 Saline solution test intake rates (Test/CTA × 100) of the different groups receiving a taste-lithium pairing at postnatal Day 32 (PN32), postnatal Day 48 (PN48), postnatal Day 64 (PN64) and postnatal Day 100 (Adult). (Pre, preexposed groups; Ctrl, non-preexposed groups; Same, groups receiving preexposures, conditioning and testing at the same time of day; Diff, groups conditioned at a different time of day of preexposure and testing.)