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Ontogenetic Differences in Sensitivity to LiCl- and Amphetamine-Induced Taste Avoidance in Preweanling Rats

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Abstract

When amphetamine is associated with a tastant conditioned stimulus, rats learn to avoid the taste even when employing doses that promote conditioned place preference. One hypothesis raised to account for this effect proposes that taste avoidance induced by amphetamine may be motivated by fear. A sensitive period has been identified in the rat (until postnatal day 10) in which infants learn conditioned appetitive effects to stimuli to which aversions are conditioned after this period. Exogenous administration of corticosterone within this period reverses this effect, generating aversive conditioning. In the present study, we tested conditioning of aversions to amphetamine or LiCl, within and after the sensitive period (Experiments 1 and 2). A third experiment evaluated unconditioned rejection of an aversive quinine solution within the sensitive period. Finally, we tested whether corticosterone administration before conditioning modulates amphetamine-induced taste avoidance. After the sensitive period, infant rats rejected the solution paired with amphetamine or LiCl after 2 conditioning trials, but within the sensitive period, aversions were conditioned only by LiCl and after 4 conditioning trials. Amphetamine-induced taste avoidance was not observed even when corticosterone was administered before conditioning. Additionally, during the sensitive period, a low LiCl dose promoted conditioned taste preference. According to Experiment 3, parameters employed in this study were suitable to yield rejection of aversive solutions within the sensitive period. These results suggest that during the sensitive period, there is a notable resistance to the acquisition of taste avoidance induced by amphetamine. The present experimental framework may represent a useful tool for studying mechanisms underlying taste avoidance and aversion effects.

Key words: amphetamine, infant, lithium chloride, rat, taste avoidance

Introduction

In the conditioned taste aversion paradigm, a gustatory conditioned stimulus (CS) is associated with an aversive unconditioned stimulus (US). This training results in avoidance of the taste signaling the US (Garcia et al. 1985; Parker 1995). This response is also accompanied by a distinctive set of behavioral reactions when subjects are intraorally stimulated with this CS (Parker 1995, 2003), which can be measured by means of the taste reactivity test (TRT) (Parker 1995, 2003; Berridge 2000), and are thought to reflect the palatability of the gustatory CS. Following taste aversion training, the typical behavioral repertoire in terms of taste reactivity includes gaping, chin rubbing, paw treading, head shaking, increased locomotion, facial wiping, and wall climbing (Parker 1995, 2003; Berridge 2000). Mouthing and tongue protrusions are common reactions of the positive hedonic component of the TRT. A reduction of these responses after conditioned taste aversion training is considered a reduction in palatability (Parker 1995, 2003).

When a tastant CS is associated with drugs of abuse (such as amphetamine, morphine, or cocaine), rats also avoid the CS even when employing doses of these drugs that induce conditioned place preference (Parker 1995, 2003). This paradoxical effect, referred as conditioned taste avoidance, is considered to be a different process than conditioned taste aversion induced by emetic drugs, such as LiCl (Parker 1995, 2003). Taste aversion, but not taste avoidance, seems to be specifically mediated by conditioned nausea (Parker 1995, 2003, 2006). This hypothesis is supported by the fact

that taste aversion, but not taste avoidance, is accompanied by changes in the palatability of the gustatory CS (Parker 1995, 2003). Additionally, taste aversion is reduced by antiemetic drugs that do not affect taste avoidance process (Parker 1995, 2003, 2006; Limebeer and Parker 2000).

Although during the last years researchers have accumulated a considerable amount of empirical evidence regarding the neural mechanisms of taste avoidance, the psychological mechanism underlying this process is still under discussion (Grigson 1997; Huang and Hsiao 2008). Grigson (1997) explained taste avoidance in terms of the anticipatory contrast effect. According to this hypothesis, rats avoid the gustatory CS paired with the drug in anticipation of receiving the psychoactive drug, a more rewarding stimulus. According to Grigson (1997), the value of the CS pales in comparison with the value of the rewarding drug and the reduction in the CS intake after conditioning represent another form of anticipatory contrast. An alternative hypothesis proposed that conditioned taste avoidance induced by rewarding drugs is a phenomenon observable in the rat because this species cannot vomit (Parker 1995, 2003, 2006). A gustatory CS paired with a change in physiological state may indicate danger to the rat (Hunt and Amit 1987; Parker 1995, 2003, 2006) resulting in avoidance of the CS. This hypothesis is supported by the fact that the extent to which rewarding drugs induce taste avoidance but not taste aversion depends on whether the animal tested can vomit or not (Parker 2006). Parker hypothesized that taste avoidance in rats is mediated by fear conditioning (Parker 2003). In this regard, Rana and Parker (2007) reported that amphetamine-paired flavor, but not LiCl-paired flavor, potentiated an acoustic startle reaction.

Conditioned taste aversion has been well studied from an ontogenetic approach. Early in development (Hoffmann et al. 1987; Hoffmann and Spear 1988), and even during the gestational period (Stickrod et al. 1982), rats can learn taste aversion induced by LiCl. However, the ontogenetic emergence of taste avoidance learning induced by rewarding drugs such as amphetamine has not been well explored. The ontogenetic analysis of taste avoidance learning may represent an experimental framework for study of neural mechanisms that underlie this process, by establishing the relationship between the maturity of specific neural systems and emergence of this learning process.

There are important reasons to expect changes in taste avoidance learning through the first 2 postnatal weeks of life. As mentioned above, some authors hypothesize that taste avoidance learning in rats is mediated by fear (Parker 1995, 2003). Sullivan et al. (2000) have defined an ontogenetic period, which ends by postnatal day 10, in which infant rats learn conditioned preferences even for aversive stimuli that induce fear conditioning after this sensitive period (Sullivan et al. 2000). The acquisition of conditioned preferences induced by aversive stimuli during this sensitive period seems to be associated with a variety of neurochemical mechanisms that predispose infants to appetitive learning. For example, this period partially coincides with the so-called stress hyporesponsive period (Moriceau and Sullivan 2004; Moriceau et al. 2006). The low corticosterone levels induced by mild aversive stimuli are insufficient for activation of the basolateral amygdala (BLA) during conditioning. Corticosterone-induced activation of BLA seems to be critical for modulation of fear conditioning after the sensitive period (Moriceau and Sullivan 2004, 2006; Moriceau et al. 2006).

If conditioned taste avoidance induced by rewarding drugs is mediated by fear, we may expect that during the sensitive period described by Sullivan and collaborators, rewarding drugs will induce conditioned preference instead of avoidance. Previous data from our laboratory help to support this hypothesis. During the sensitive period, ethanol was found to induce conditioned preference at doses that generate aversive learning after postnatal day 10 (Arias and Chotro 2006a; Chotro and Arias 2007; Chotro et al. 2009). However, ethanol is a complex drug and can support taste avoidance in infant rats even in quite low doses (0.4 g/kg) (Hunt et al. 1990), whereas relatively high doses (2.5 g/kg) induce disgust reactions similar to LiCl (Arias, Pautassi, et al. 2010). In adults it has been shown that high ethanol doses induce c-fos activity in brain stem nuclei (such as area postrema) that modulate nausea (Thiele et al. 1996). Although amphetamine-induced learning has not been systematically studied in preweanling rats, Pedersen et al. (1982) reported some results suggesting that this drug may act as an appetitive US before PD10. These authors found appetitive odor conditioning induced by amphetamine in newborns, although they were utilizing a very different paradigm, measuring nipple attachment behavior.

The specific goal of the present study is to analyze conditioned taste avoidance induced by a rewarding drug, amphetamine, in preweanling rats. In Experiment 1, we tested taste avoidance induced by moderate-to-high amphetamine doses (1 or 3 mg/kg) during and after the sensitive period described by Sullivan et al. (2000). In Experiment 2, we compared taste avoidance induced by amphetamine and taste aversion induced by LiCl in infant rats within and after the sensitive period. For this purpose, we employed LiCl doses which promote after the sensitive period, a level of conditioned taste avoidance similar to that induced by the amphetamine doses in this experiment. In Experiment 3, we tested the rat pup's capability of rejecting an aversive solution (quinine) within the sensitive period; the purpose was to assess the suitability of the parameters employed in the present study for detecting taste avoidance. Finally, in Experiment 4, we pharmacologically manipulated corticosterone levels before conditioning within and after the sensitive period. In the younger rats, we administered corticosterone before conditioning (Experiment 4a), and in the older subjects, we blocked corticosterone synthesis by giving metyrapone

before conditioning (Experiment 4b). The purpose of these experiments was to analyze the role of corticosterone in amphetamine-induced taste avoidance.

The working hypothesis guiding this study is that sensitivity to amphetamine-induced taste avoidance will differ for infants within and after the sensitive period. More specifically, if taste avoidance induced by rewarding drugs is mediated by fear (Rana and Parker 2007), we expect that during the sensitive period, amphetamine will generate conditioned taste preference (similar to that induced by footshock (Sullivan et al. 2000) or ethanol (Arias and Chotro 2006a; Chotro and Arias 2007; Chotro et al. 2009)). We also expect that ontogenetic differences in taste avoidance learning induced by amphetamine are unrelated to those in taste aversion learning induced by LiCl. Finally, we also hypothesize that exogenous corticosterone administration during the sensitive period will enhance acquisition of amphetamine-induced taste avoidance. In contrast, a reduction in corticosterone activity (by means of metyrapone) after this developmental period is expected to attenuate this avoidance learning.

Materials and methods

Subjects

Forty-seven (23 females and 24 males) 14-day-old and 33 (15 females and 18 males) 7-day-old Wistar WKAH/HOK rats, representative of 14 litters, were utilized for Experiment 1. In Experiment 2a, we employed 35 (19 males and 16 females) 14-day-old and 58 (30 males and 28 females) 7-day-old Wistar WKAH/HOK rats representative of 14 litters; in Experiment 2b, we used 39 (21 males and 18 females) 7-day-old Wistar WKAH/HOK rats derived from 6 litters, whereas in Experiments 2c and 2d, we employed 21 (14 males and 7 females) and 19 (8 males and 11 females) Wistar WKAH/HOK rats derived from 5 litters. Twenty-seven (15 males and 12 females) 7-day-old rats derived from 5 litters were used in Experiment 3. Finally, in Experiments 4a and 4b, we used 69 (38 males and 31 females) and 45 (25 males and 20 females) Wistar WKAH/HOK rats derived from 14 and 7 litters, respectively. Animals were born and reared at the vivarium of the Instituto de Investigacion Medica Mercedes y Martin Ferreyra (Cordoba, Argentina) under conditions of constant room temperature (22 ± 1.0 °C), on a 12 h light:dark cycle. The day of parturition was considered postnatal day 0 (PD0). All procedures were in accordance with the guidelines for animal care and use established by the Institute of Laboratory Animal Resources (1996).

Procedures

Experiment 1

Conditioning phase. In Experiment 1, amphetamine-induced conditioned taste avoidance was tested in infant rats within or after the sensitive period described by Sullivan et al. (2000) (before or after PD10, respectively). Two consecutive conditioning trials (one per day) were performed in the same way for each age group starting on PD7 and PD14. On the first conditioning day, pups from a given litter were assigned to one experimental group defined by each combination of age (PD7 or PD14) and drug treatment (vehicle, 1 or 3 mg/kg). In all following experiments, no more than one male and one female from a given litter was assigned to the same treatment condition to avoid overrepresentation of a given litter in any particular treatment (Holson and Pearce 1992). Male and female subjects were balanced across experimental groups. Immediately after assignment to their experimental condition, pups were separated from the mother and an intraoral cannula (PE 10 polyethylene tubing, length: 5 cm, Clay Adams) was implanted in the right cheek of each pup, as previously described (Chotro and Alonso 2003; Arias and Chotro 2006b). Briefly, a flanged end of the cannula was shaped by exposure to a heat source (external diameter: 1.2 mm). A dental needle (30-gauge Monoject, Sherwood Medical) was attached to the non-flanged end of the cannula and positioned in the middle portion of the intraoral mucosa. The needle was inserted through the cheek and the cannula was pulled through the tissue until the flange end rested on the mouth's mucosa. This cannulation procedure requires no more than 20 s per subject and does not induce major stress in infant rats (Spear et al. 1989). Ninety minutes after cannulation, pup's bladders were voided by gentle brushing of the anogenital area. Following this procedure, body weights were recorded and subjects were placed into individual Plexiglas chambers (10 × 10 × 12 cm). Then pups received an intraoral infusion of saccharin (CS, 0.05% w/v, duration: 15 min). Total administration volume was equivalent to 5% of the subject's preinfusion body weight. Saccharin was delivered at a constant rate by means of an infusion pump (KD Scientific) connected to the oral cannula of each pup by a polyethylene catheter (Clay Adams, PE 50 Parsippany). Considering the normal variation in body weight between litters, in the present study, the infusion rate for the older pups varied between 0.085 and 0.1 mL/min (from P14 to P16). For the younger pups (from P7 to P10), the infusion rates varied between 0.047 and 0.059. With similar infusion parameters, pups are capable of either consuming or rejecting the infused solution (Chotro and Alonso 2003; Arias and Chotro 2005; Diaz-Cenzano and Chotro 2010). After the infusion procedure, subjects were weighed to estimate saccharin consumption scores. Percentage body weight gain (% BWG) was calculated as follows: 100 × [(postinfusion weight – preinfusion weight)/preinfusion weight]. This dependent variable has been previously employed to estimate saccharin consumption in infant rats (Arias, Molina, and Spear 2010). Immediately following CS exposure, pups received the corresponding amphetamine administration (1 or 3 mg/kg, intraperitoneally). The type of amphetamine used in all the experiments from the present study was D-amphetamine

sulfate (Parafarm). Amphetamine (1 or 3 mg/kg) was administered in a volume equivalent to 0.01 mL/g (1%) of body weight. Amphetamine was dissolved in NaCl (0.9%) in a concentration equivalent to 1 mg/10 mL for the lower dose and 3 mg/10 mL for the highest one. Control pups received an equivalent volume of vehicle (NaCl 0.9%). After drug treatment, pups were reunited with their mother. The second conditioning trial was conducted the following day (PD 8 for the younger or PD15 for the older subjects) applying the exact same procedures described for the first conditioning trial.

Testing phase. On PD9 or PD16, pups were separated from their mothers, intraorally cannulated, and placed in pairs for 90 min in a heated holding cage. Then, pups were tested in terms of saccharin intake for 15 min. The apparatus and parameters were the same as those described for conditioning.

Experiment 2

Experiment 2a. The goal of this experiment was to assess whether the lack of taste avoidance induced by amphetamine in 7-day-old rats in Experiment 1 is associated with the sensitivity in acquisition of aversive learning induced by LiCl. For this purpose, in Experiment 2a, we compared 7- and 14-day-old rats in their conditioning of taste avoidance induced by amphetamine (3 mg/kg) or LiCl. Doses of LiCl (Sigma Aldrich; 0.5% or 1.0% of body weight of a 0.3 M LiCl solution) selected were known from previous studies to generate levels of conditioned taste avoidance in 14-day-olds similar to levels produced by the amphetamine dose employed (Arias et al. 2009; Arias, Pautassi, et al. 2010). The volume injected for the highest LiCl dose was equivalent to those corresponding to amphetamine injections (1 or 3 mg/kg) to avoid possible differential effects of the volume on learning induced by the drugs. Experiment 2 included only the highest amphetamine dose (3 mg/kg) because in Experiment 1, 1 and 3 mg/kg induced similar magnitudes of CS rejection. After 2 conditioning trials, pups were tested for their acceptance of the tastant CS. Procedures and parameters employed in Experiment 2a were similar to those in Experiment 1.

Experiment 2b. In Experiment 2a, neither amphetamine nor LiCl induced conditioned taste rejection during the sensitive period after 2 conditioning trials, so Experiment 2b replicated 2a but included 4 conditioning trials. The first conditioning trial was on PD6 (instead of PD7) to avoid having the last conditioning trial after the sensitive period (thought to end on PD10). Only the highest doses of amphetamine (3 mg/kg) and LiCl (1% body weight) were employed. All other procedures and parameters were identical to those used in Experiment 1.

Experiments 2c and 2d. In Experiments 1, 2a, and 2b, the control group was represented by a CS-only condition. This control group was selected because an unpaired control condition requires longer maternal deprivation time, which is highly stressful for infants (Smith et al. 1985) and may interact with the effects of LiCl or amphetamine. However, the CS-only control group limits our conclusions because we cannot rule out that the rejection of the CS is due to the mere US administration. For this reason, in additional experiments, we analyzed the effect of the US-only (amphetamine or LiCl) administration upon saccharin intake within (Experiment 2c) and after (Experiment 2d) the sensitive period. In these experiments, pups were separated from the mother and assigned to the US condition (vehicle, 3 mg/kg amphetamine or 1% LiCl). During the treatment days, pups were given the corresponding US treatment following the procedures described for Experiment 2a. On test day, pups were evaluated in terms of saccharin acceptance. In Experiment 2c, we included 4 treatment days (beginning on PD6) because LiCl-induced taste aversion was only observed after 4 conditioning trials in Experiment 2b. In Experiment 2d, 2 treatment days were employed because in Experiment 1 and 2a LiCl or amphetamine-induced rejection of the CS were clearly observed after 2 conditioning trials.

Experiment 3

According to Experiment 2, infants evaluated during the sensitive period required 4 conditioning trials to show taste aversion induced by a relatively high LiCl dose, whereas after the sensitive period, only 2 conditioning trials were needed. However, we cannot discard the possibility that parameters employed in this study are not suitable to detect differences in taste rejection in the youngest rats. For this reason, we conducted an additional experiment to provide additional evidence on the relative resistance of infant rats to taste aversion induced by LiCl within and after the sensitive period. With this aim, in Experiment 3, we tested whether 7-day-old rats were able to reject an unconditioned aversive tastant (quinine, 0.006% or 0.025% w/v) within the range of parameters applied throughout the present study. The 2 selected quinine concentrations were found in preliminary tests to yield a level of taste avoidance similar to that for amphetamine and LiCl in Experiment 2a (in the older pups; data not shown). In Experiment 3, quinine consumption was compared with that of saccharin during the sensitive period. Procedures and parameters employed in this experiment coincide with those employed in Experiment 1.

Experiment 4

In Experiment 4, we manipulated corticosterone levels before conditioning trials employing amphetamine. Because the sensitive period defined by Sullivan and coworkers coincides with the stress hyporesponsive period (Sapolsky and Meaney 1986; Levine 2001) in which low levels of this hormone are produced in response to stressful stimuli, the low corticosterone levels induced by aversive stimuli during this

period may help to explain why infants during this period are relatively resistant to taste avoidance learning induced by amphetamine. According to Parker (2003), taste avoidance induced by rewarding drugs such as amphetamine may be mediated by fear conditioning, and corticosterone has been associated with taste avoidance induced by rewarding drugs such as morphine (Gomez et al. 2000). Rats given exogenous administration of corticosterone before conditioning during this period of infancy acquire fear responses and avoidance while controls learn conditioned preferences (Moriceau et al. 2004; Moriceau and Sullivan 2004). In contrast, removing corticosterone after the sensitive period attenuates fear conditioning (Moriceau and Sullivan 2004; Moriceau et al. 2004). In Experiment 4a, pups during the sensitive period (PDs 7 and 8) were given corticosterone (Sigma Aldrich; 0 or 3 mg/kg) 30 minutes before taste conditioning with amphetamine (0, 1, or 3 mg/kg). The corticosterone dose was selected because in previous studies it has been shown that 3 mg/kg is enough to activate BLA during this ontogenetic period (Moriceau and Sullivan 2004; Moriceau et al. 2004). In Experiment 4b, metyrapone (Sigma Aldrich), a corticosterone synthesis blocker, was administered 30 min before conditioning with amphetamine (0 or 1 mg/kg), after the sensitive period (PDs 14 and 15). Metyrapone dosage (0 or 50 mg/kg) was selected from prior studies with preweanling rats (Upton and Sullivan 2010). Corticosterone and metyrapone were dissolved in a solution of saline and 0.25% acetic acid (volume administered was 10 mL/kg). The day after the last conditioning trial, subjects from Experiments 4a and 4b were tested following the procedures described in Experiment 1.

Data analysis

No significant effect of sex or interaction with the remaining factors was found in any of the dependent variables considered in any experiment in this report. Hence, for the inferential analysis and descriptive representation of the results, data were collapsed across sex. Intake was analyzed by means of 2 indexes: The % BWG and a difference score calculated through the subtraction of saccharin intake during the testing from saccharin intake on the first conditioning trial. Because the total volume of CS infused depended on body weight and subjects from both age groups obviously differ in body weight, we calculated change in intake (% BWG) to better compare intake across age.

In Experiments 1, 2a, 2b, 3, 4a, and 4b, the % BWG was analyzed by means of mixed analyses of variance (ANOVAs) including the following between-subject variables: Experiment 1: age (PD7 or PD14) and drug treatment (vehicle, 1 or 3 mg/kg amphetamine). Experiment 2a: age and drug treatment (vehicle, 3 mg/kg amphetamine, 0.5% LiCl, 1% LiCl). Experiment 2b: drug treatment (vehicle, 3 mg/kg amphetamine or 1% LiCl). Experiment 3: intake solution (saccharin, quinine 0.006% or 0.025%). Experiment 4a: drug (vehicle, 1 or 3 mg/kg amphetamine) and corticosterone (vehicle or 3 mg/kg) treatments, and Experiment 4b: drug treatment (vehicle or 1 mg/kg amphetamine) and metyrapone (vehicle or 10 mg/kg). In all these analyses, day was considered as a within-factor variable. The % BWG of experiments 2c and 2d was analyzed by means of one-way between-factor ANOVA, in which drug treatment (vehicle, 3 mg/kg amphetamine or 1% LiCl) was treated as the between-factor variable.

The difference scores were analyzed by means of between-factor analyses with the following between-subject variables: Experiment 1: age (PD7 or PD14) and drug treatment (vehicle, 1 or 3 mg/kg amphetamine). Experiment 2a: age and drug treatment (vehicle, 3 mg/kg amphetamine, 0.5% LiCl, 1% LiCl). Experiment 2b: drug treatment (vehicle, 3 mg/kg amphetamine or 1% LiCl). Experiment 3: intake solution (saccharin, quinine 0.006% or 0.025%). Experiment 4a: drug (vehicle, 1 or 3 mg/kg amphetamine) and corticosterone (vehicle or 3 mg/kg) treatments, and Experiment 4b: drug treatment (vehicle or 1 mg/kg amphetamine) and metyrapone (vehicle or 10 mg/kg).

Significant main effects and/or interactions were further analyzed by means of follow-up ANOVAs and post hoc analysis (Newman–Keuls). All inferential analyses conducted in the present study employed an α level equal to 0.05. When appropriate we reported the effect size (Cohen's f).

Results

Experiment 1

Figure 1 (left side) represents % BWG during conditioning and testing as a function of age and drug treatment. In the insert on the right is shown the difference score (test intake subtracted from intake during the first day of conditioning). In the older group, acceptance of the tastant CS was significantly affected by both amphetamine doses. However, amphetamine did not modify saccharin intake in the younger rats. For this analysis, we used a 2 (age: PD7 or PD14) by 3 (drug treatment: vehicle, 1 or 3 mg/kg amphetamine) by 3 (day) mixed ANOVA. The ANOVA conducted with the absolute scores revealed significant main effects of age and drug treatment ($F_{1,74}$ = 64.31, $P < 0.05$ and $F_{2,74}$ = 5.57, $P < 0.05$, respectively) and the following significant interactions: age by drug treatment ($F_{2,74} = 5.71$, $P < 0.05$), age by day ($F_{2,148}$ = 13.79, $P < 0.05$), drug treatment by day ($F_{4,148}$ = 3.06, $P < 0.05$), and the triple interaction of age by drug treatment by day ($F_{4,148} = 2.70$, $P < 0.05$). To determine the loci of this significant interaction, follow-up one-way ANOVAs were performed considering drug treatment as the only between-subject variable. We analyzed intake scores from each day independently in each age group. The analyses performed with the younger rats did not reveal any significant effect. The ANOVAs conducted with the older subjects indicated only a significant effect of drug treatment on PD16 ($F_{2,44}$ = 39.56, $P < 0.05$; Cohen's f = 1.34).

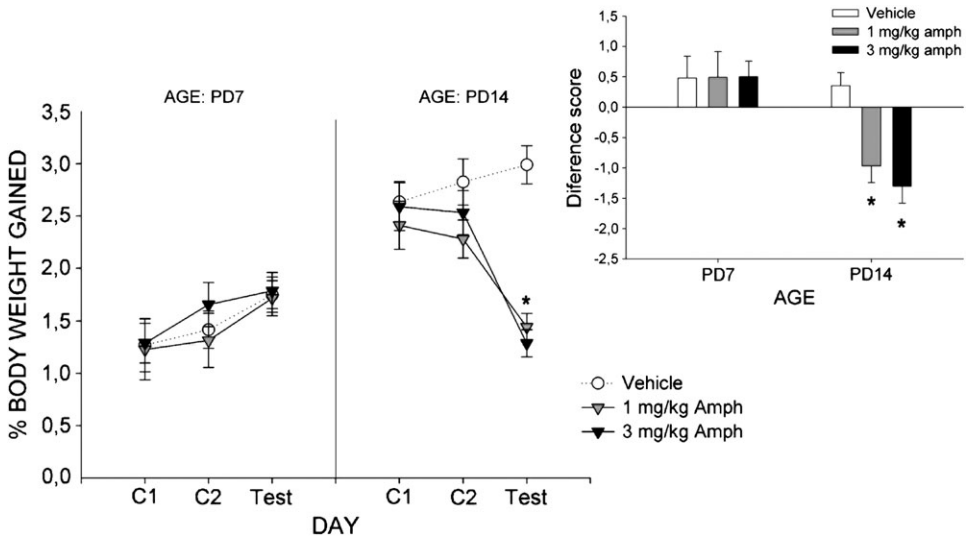

Figure 1 Left side: saccharin consumption (% BWG) as a function of age and amphetamine treatment (0, 1, or 3 mg/kg) across conditioning (C1 and C2) and testing days. Right side: difference score (test intake subtracted from intake during the first day of conditioning) as a function of amphetamine and age. Vertical lines illustrate standard errors of the means. $P < 0.05$ versus vehicle-control.

According to post hoc analyses, vehicle-treated controls ingested more saccharin than subjects treated with amphetamine regardless of dose.

The difference score was analyzed by means of a 2 (age: PD7 or PD14) by 3 (drug treatment: vehicle, 1 or 3 mg/kg amphetamine) between-factor ANOVA. This analysis also confirmed our prior observation (Figure 1, right insert). The ANOVA revealed significant effects of the main factors age ($F_{1,74} = 21.13$, $P < 0.05$) and drug treatment ($F_{2,74} = 4.15$, $P < 0.05$), as well the interaction between them ($F_{2,74} = 4.34$, $P < 0.05$; Cohen's $f = 0.49$). Post hoc analyses indicated that the increment in saccharin acceptance for the younger rats given amphetamine (1 or 3 mg/kg) was significantly higher than that for the older subjects. The difference score from subjects treated with vehicle did not differ across age. Additionally, older pups given 1 or 3 mg/kg amphetamine showed lower difference scores than the corresponding control group, whereas younger rats did not differ in this dependent variable regardless of drug treatment.

Experiment 2

Experiment 2a

Intake data from Experiment 2a are depicted in Figure 2a. These data were analyzed by a 2 (age: PD7 or PD14) by 3 (drug treatment: vehicle, 3 mg/kg amphetamine, 0.5% LiCl, 1% LiCl) by 3 (day) mixed ANOVA. The ANOVA revealed significant main effects of age ($F_{1,85} = 14.59$, $P < 0.05$), drug treatment ($F_{3,85} = 3.70$, $P < 0.05$), and day ($F_{2,170} = 8.74$, $P < 0.05$), and the following interactions: age by drug treatment ($F_{3,85} = 3.63$, $P < 0.05$), age by day ($F_{2,170} = 19.75$, $P < 0.05$), drug treatment by day ($F_{6,170} = 2.23$, $P < 0.05$), and the 3-way interaction of age by drug treatment by day ($F_{6,170} = 2.55$, $P < 0.05$). To determine the loci of this interaction, we utilized follow-up one-way ANOVAs, with drug treatment as the only between-factor variable, and intake data from each day for each age group as the dependent variable. In the younger age group, the ANOVA revealed a significant effect of drug treatment on PD9 ($F_{3,54} = 5.79$, $P < 0.05$; Cohen's $f = 0.56$). Post hoc analyses indicated that pups given the lower LiCl (0.5 g/kg) dose consumed more saccharin than the remaining experimental conditions, including pups injected with only vehicle during conditioning. Saccharin acceptance in subjects given amphetamine, the highest LiCl dose or vehicle did not differ. In the older rats, the ANOVA also revealed a significant effect of drug treatment on PD 16 ($F_{3,31} = 13.53$, $P < 0.05$; Cohen's $f = 1.14$), but for very different reasons implicating conventional effects of conditioned taste aversion. According to the post hoc analyses, pups given amphetamine or LiCl consumed significantly less than those given vehicle. Intake of subjects treated with amphetamine or LiCl did not differ.

The difference score was analyzed by a between-factor ANOVA in which age and drug treatment represented the between-subject variables. This analysis revealed significant main effects of age ($F_{1,85} = 46.89$, $P < 0.05$) and drug treatment ($F_{3,85} = 4.56$, $P < 0.05$) and the interaction between these variables ($F_{3,85} = 6.60$, $P < 0.05$; Cohen's $f = 0.62$). Post hoc analyses indicated that control pups from both ages did not differ, whereas increments in saccharin consumption were significantly greater in younger rats given amphetamine or LiCl than in the corresponding older subjects (see Figure 2a, right insert). Additionally, intake scores from older pups given amphetamine or LiCl were significantly lower than the corresponding control group. In the young rats, only pups

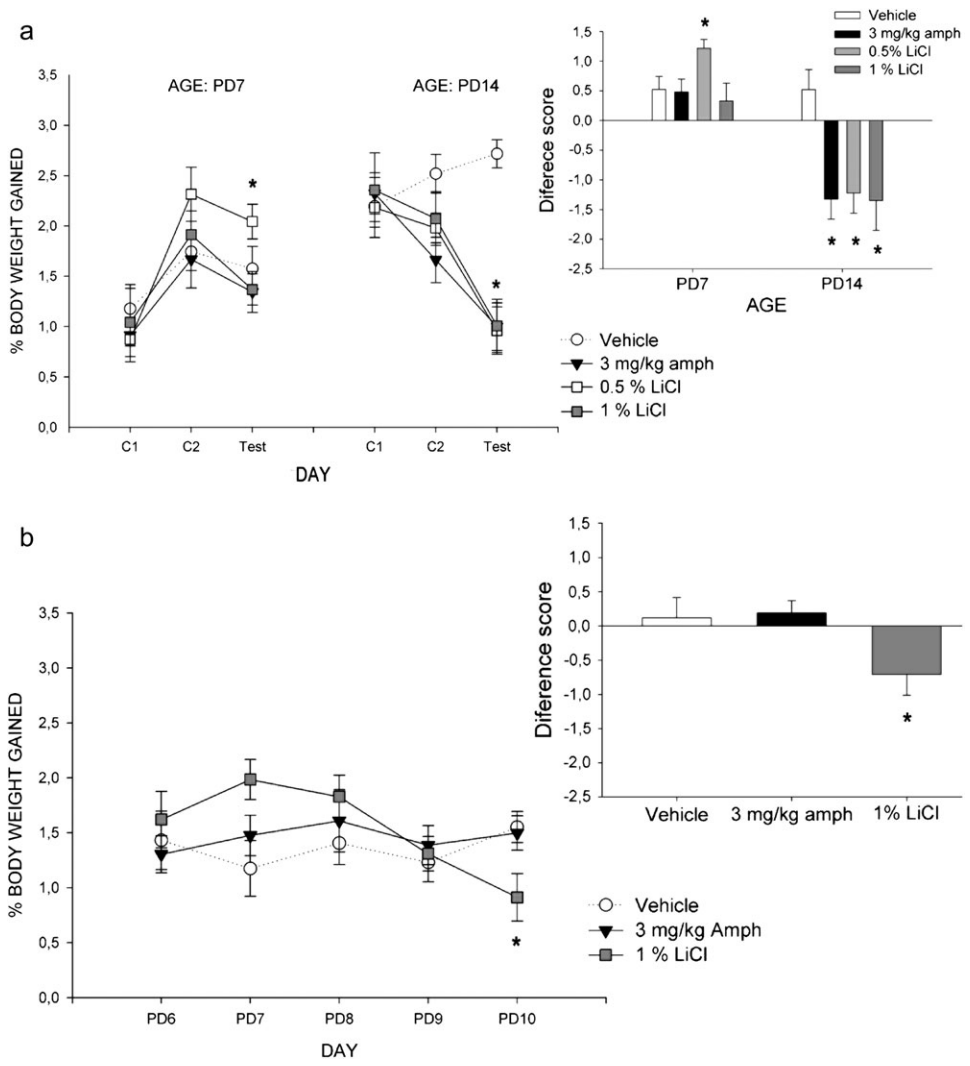

Figure 2 **(a)** Left side: saccharin consumption (% BWG) as a function of age and drug treatment (vehicle, 3 mg/kg amphetamine, 0.5% LiCl or 1% LiCl) across conditioning (C1 and C2) and testing days. Right side: difference score (test intake subtracted from intake during the first day of conditioning) as a function of drug treatment and age. Vertical lines illustrate standard errors of the means. *$P < 0.05$ versus vehicle-control. **(b)** Left side: saccharin consumption (% BWG) as a function of drug treatment (vehicle, 3 mg/kg amphetamine or 1% LiCl) across conditioning (C1, C2, C3 and C4) and testing days. Right side: difference score (test intake subtracted from intake during the first day of conditioning) as a function of drug treatment. Vertical lines illustrate standard errors of the means. *$P < 0.05$ versus vehicle-control.

given 0.5% LiCl showed significantly higher scores than the remaining groups.

Experiment 2b

Figure 2b represents intake of saccharin during the sensitive period (PDs 6 to 10) as a function of drug treatment (vehicle, 1% LiCl, 3 mg/kg amphetamine). The % BWG was analyzed by a 3 (drug treatment: vehicle, 3 mg/kg amphetamine or 1% LiCl) by 5 (day) mixed ANOVA. This analysis indicated a significant interaction between drug treatment and day ($F_{8,144} = 2{,}19$, $P < 0.05$). Follow-up one-way ANOVAs were performed with saccharin intake scores from each day, including drug treatment as the only between-factor variable. These analyses revealed a significant effect of drug treatment on PD7 and 10 ($F_{2,36} = 3.97$, $P < 0.05$, Cohen's $f = 0.48$, and $F_{2,36} = 4.09$, $P < 0.05$, Cohen's $f = 0.48$, respectively). Post hoc analyses revealed that LiCl-treated pups, when compared with the remaining conditions, consumed more saccharin on PD7 but less saccharin on PD10. Saccharin intake in amphetamine and vehicle-treated subjects did not differ across days (Figure 2b, left side).

The difference scores were analyzed by a one-way ANOVA with drug treatment (vehicle, 1% LiCl, 3 mg/kg amphetamine) was considered the only between-subject variable. This analyses provided a similar result, indicating a significant main effect of drug treatment, $F_{2,36} = 3.49$, $P < 0.05$, Cohen's $f = 0.43$. According to post hoc analyses, the increase in saccharin intake

was significantly lower in the LiCl-treated group when compared with the remaining conditions (Figure 2b, right insert).

Experiment 2c

In this control experiment, we analyzed saccharin intake on PD10 as a function of the US-only treatment (vehicle, amphetamine 3 mg/kg or LiCl 1% body weight) administered from PD6 to PD9. The one-way ANOVA failed to find significant differences between groups (data not shown). Means and standard errors of the means obtained in each condition were vehicle: 1.90 ± 0.16 (n = 7); 3 mg/kg amphetamine: 1.55 ± 0.24 (n = 6); 1% LiCl: 1.76 ± 0.32 (n = 6).

Experiment 2d

Experiment 2d, the control experiment for the older age group, tested saccharin consumption on PD16 as a function of the US-only treatment (vehicle, amphetamine 3 mg/kg or LiCl 1% body weight) administered on PDs 14 and 15. The one-way ANOVA did not revealed significant differences across US conditions (data not shown). Means and standard errors of the means from each experimental condition were vehicle: 3.10 ± 0.31 (n = 7); 3 mg/kg amphetamine: 3.14 ± 0.39 (n = 7); 1% LiCl: 3.74 ± 0.35 (n = 7).

Experiment 3

Figure 3 shows saccharin and quinine consumption in infant rats during the sensitive period (from PD 7 to 9). The percentage of body weight was analyzed by a 3 (intake solution: saccharin, quinine 0.006% or 0.025%) by 3 (day) mixed ANOVA. The ANOVA revealed a significant effect of solution ($F_{2,24}$ = 4.54, $P < 0.05$, Cohen's f = 0.50) and day ($F_{2,48}$ = 3.60, $P <$ 0.05, Cohen's f = 0.33). Post hoc analyses revealed that subjects given saccharin consumed more than those given quinine (0.025%). Intake of the 0.006% quinine solution did not differ from the remaining solutions. Additionally, subjects consumed significantly more on PD9 than on PD7.

Although pups given saccharin had greater increase in intake across days than those given quinine (see Figure 3, right insert), differences between groups did not reach statistic significance. The one-way ANOVA conducted with the difference scores did not find a significant effect of solution.

Experiment 4a

Figure 4a represents saccharin intake during the sensitive period (PDs 7, 8, and 9) as a function of drug (vehicle, 1 or 3 mg/kg amphetamine) and corticosterone (vehicle or 3 mg/kg) treatments administered during the conditioning days (PDs 7 and 8). The analysis of the % BWG (Figure 4a, left side) was performed by a 3 (drug treatment: vehicle, 1 or 3 mg/kg amphetamine) by 2 (corticosterone treatment: vehicle or 3 mg/kg) by 3 (day) mixed ANOVA. This analysis revealed only a significant effect of day ($F_{2,126}$ = 8.05, $P < 0.05$). According to the post hoc analyses, subjects consumed less saccharin the first day than the remaining ones. The difference scores were analyzed by a 3 (drug treatment) by 2 (corticosterone treatment) between-factor ANVOA. This analysis did not reveal any significant effects (Figure 4a, right insert).

Experiment 4b

Figure 4b represents saccharin intake after the sensitive period (PDs 14, 15, and 16) as a function of drug (vehicle or 1 mg/kg amphetamine) or metyrapone (vehicle or 10 mg/kg) treatments. These data were analyzed by a 2 (drug

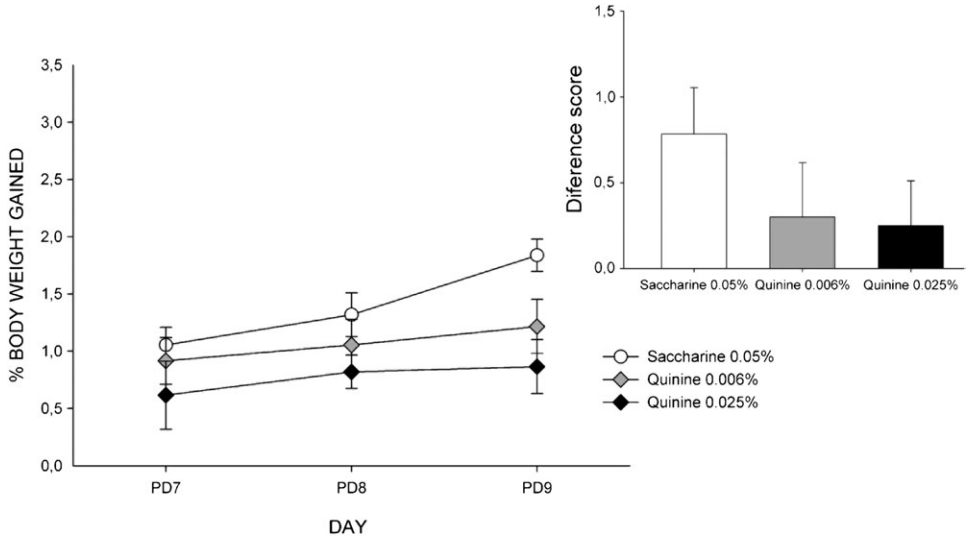

Figure 3 Left side: quinine intake (% BWG) as a function of solution (saccharin, quinine 0.006% or 0.025%) across postnatal days 7, 8, and 9. Right side: difference score (test intake subtracted from intake during the first day of conditioning) as a function the solution (saccharin, quinine 0.006% or 0.025%). Vertical lines illustrate standard errors of the means. Rats ingested significantly more saccharine than quinine solutions.

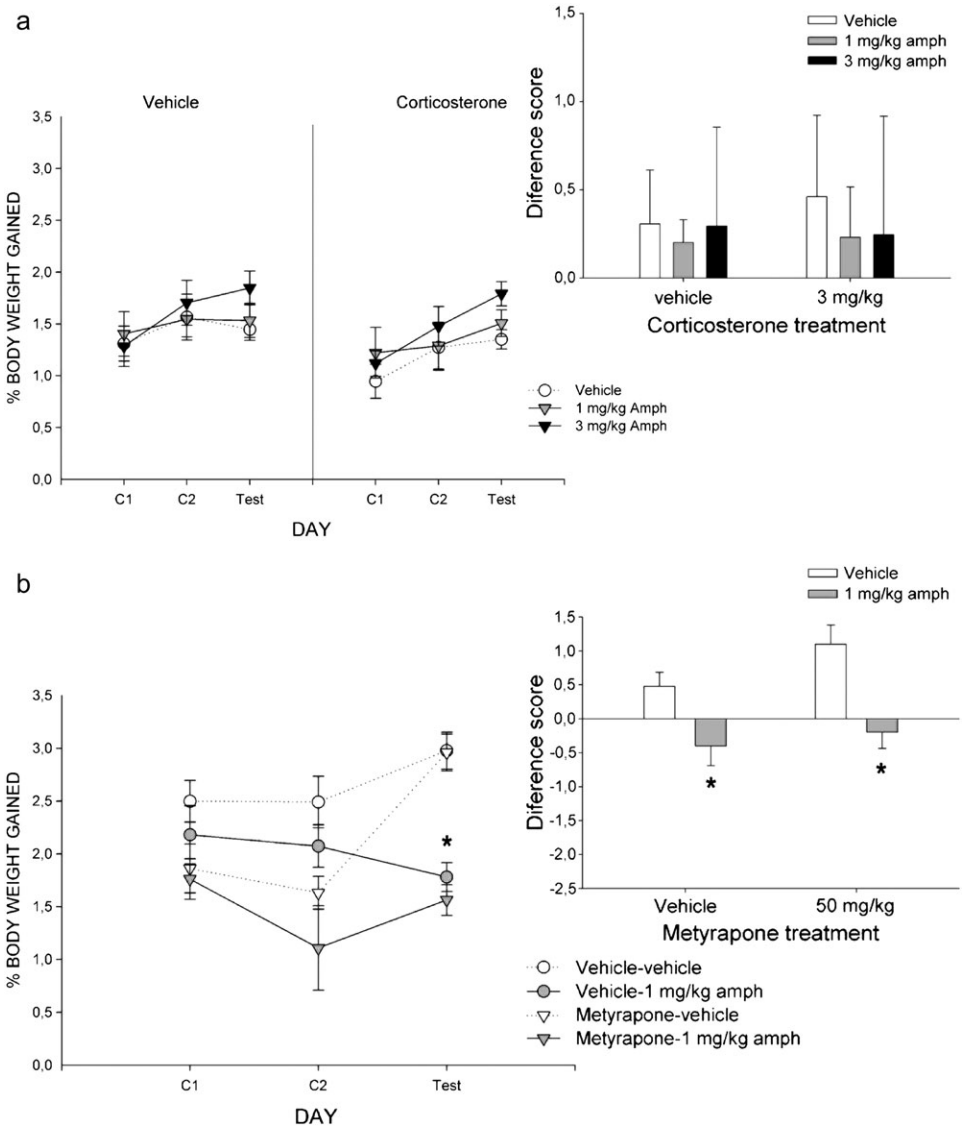

Figure 4 **(a)** Left side: saccharin consumption (% BWG) as a function of corticosterone (0 or 3 mg/kg) and amphetamine treatment (vehicle, 1 or 3 mg/kg) across conditioning (C1 and C2) and testing days. Right side: difference score (test intake subtracted from intake during the first day of conditioning) as a function of corticosterone and amphetamine treatments. Vertical lines illustrate standard errors of the means. **(b)** Left side: saccharin consumption (% BWG) as a function of metyrapone (0 or 10 mg/kg) and amphetamine treatment (vehicle or 1 mg/kg) across conditioning (C1 and C2) and testing days. Right side: difference score (test intake subtracted from intake during the first day of conditioning) as a function of metyrapone and amphetamine treatments. Vertical lines illustrate standard errors of the means. *$P < 0.05$ versus vehicle-control.

treatment: vehicle or 1 mg/kg amphetamine) by 2 (metyrapone treatment: vehicle or 10 mg/kg) by 3 (day) mixed ANOVA. This analysis revealed significant main effects of drug and metyrapone treatments ($F_{1,40} = 11.20$, $P < 0.05$; $F_{1,40} = 18.02$, $P < 0.05$, respectively) and of day ($F_{1,80} = 6.37$, $P < 0.05$) and the interactions, drug dose by day and drug treatment by day ($F_{2,80} = 4.13$, $P < 0.05$; $F_{2,80} = 8.42$, $P < 0.05$, respectively). The loci of these interactions were explored by means of follow-up one-way between-factor ANOVAs with the intake data for each day. In these analyses, drug treatment was treated as the only between-factor variable. According to these analyses, acute administration of metyrapone during conditioning significantly reduced saccharin intake when the drug was administered during the conditioning days (PDs 14 and 15, $F_{1,42} = 6.03$ $P < 0.05$; $F_{1,42} = 11.72$, $P < 0.05$, respectively), but there was no effect on the test day (PD16) (when metyrapone was not administered). Additionally, amphetamine administered during conditioning suppressed saccharin acceptance on the test day ($F_{1,42} = 66.81$, $P < 0.05$, Cohen's $f = 0.90$).

The difference scores were analyzed by a 2 by 2 between-factor ANOVA, in which drug treatment and metyrapone were the between-subject variables. This analysis revealed a significant effect of drug treatment ($F_{1,40} = 18.41$,

$P < 0.05$, Cohen's $f = 0.64$, see Figure 4b, right insert), indicating that increments in saccharin consumption were significantly greater in rats given vehicle than in those treated with amphetamine.

Discussion

The present study was designed to analyze ontogenetic differences in sensitivity to conditioning of taste avoidance induced by amphetamine in preweanling rats. Results from these experiments indicate that rats younger than PD10 (within the "sensitive period") are highly resistant to amphetamine-induced taste avoidance learning. No evidence of taste avoidance learning was found at this age even with 4 conditioning trials during the sensitive period (Experiments 1, 2a, 2b, and 4a), whereas rats only a few days older, at the end of the second postnatal week, rapidly acquired this learning (Experiments 1, 2a, and 4b). Sensitivity to LiCl-induced aversive learning also varied across age. Under the present experimental conditions, within the sensitive period, infants required 4 conditioning trials (Experiment 2b) to condition avoidance of a taste paired with LiCl (although no conditioning occurred if amphetamine rather than LiCl was the US), whereas after this period, only 2 conditioning sessions were needed to induce conditioned avoidance with either LiCl or amphetamine as US (Experiment 2a). Finally, we found that resistance to acquisition of amphetamine-induced taste avoidance within the sensitive period is not related to the low corticosterone response characterizing this ontogenetic period. Pharmacological manipulations of this hormone did not modified amphetamine-induced conditioning within or after the sensitive period (Experiments 4a and 4b).

Resistance to the acquisition of taste avoidance in the youngest rats cannot be explained by the insensitivity of the procedures employed in the present study because in Experiment 3 infant rats evaluated within the sensitive period rejected quinine solutions. Additionally, infants during this period acquired LiCl-induced taste aversion, although this learning took more conditioning trials than after the sensitive period (Experiment 2b). Hence, ontogenetic changes in taste avoidance learning mediated by amphetamine cannot be attributed to age-related differences in sensitivity for conditioned taste avoidance generally. This result also supports the hypothesis that taste avoidance learning generated by rewarding drugs (such as amphetamine) is governed by different mechanisms than taste aversion induced by emetic agents (such as LiCl). As we mentioned above, mechanisms regulating taste aversion induced by LiCl are functional early in development (Hoffmann et al. 1987; Hoffmann and Spear 1988; Hoffmann et al. 1990), even prenatally (Stickrod et al. 1982). Our results suggest, however, that mechanisms underlying taste avoidance induced by amphetamine may not be functional before postnatal day 10. The highest amphetamine dose employed (3 mg/kg) is considered in the literature as a moderate dose (Parker 1995). Moreover, previous research has shown that even younger infant rats are sensitive to other amphetamine effects. For example, Raskin and Campbell (1981) reported that amphetamine doses still lower than those in the present study induced locomotor stimulation in 5-day-old rats. Hence, the lack of sensitivity in acquiring amphetamine-induced taste avoidance during the sensitive period prior to P10 is not associated with insensitivity to other effects of this drug.

From our results, we can also conclude that during the sensitive period, rats are resistant to LiCl-induced taste aversion relative to older rats. Before PD10, rats required 4 conditioning trials (Experiment 2b), whereas by the end of the second postnatal week of life, they only needed 2 trials to learn the aversion. This result is congruent with previous empirical observations showing ontogenetic differences in taste aversion learning during this developmental stage (Hoffmann et al. 1987). Surprisingly LiCl generated conditioned taste preference in the youngest rats after one conditioning trial (Experiment 2a and 2b). A similar result was previously reported by Kehoe (1988) employing similar procedures. This paradoxical effect may be related to the same effect observed during the same ontogenetic period in response to other aversive stimuli, such a mild footshock (Sullivan et al. 2000), morphine (Kehoe 1988), or relatively high ethanol doses (Arias and Chotro 2006a). It is interesting that the same footshock, morphine, LiCl, or ethanol treatment that generated conditioned preference during this "sensitive" ontogenetic period, promoted aversive learning after the sensitive period (Kehoe 1988; Sullivan et al. 2000; Arias and Chotro 2006a). In our study, however, amphetamine failed to generate preference for the associated taste during this sensitive period.

Is it plausible that footshock, morphine, LiCl, and ethanol may share a common mechanism responsible of their paradoxical effects during this period? It has been reported that opioid antagonists such as Naloxone or Naltrexone reversed conditioned preferences produced by these aversive US agents during the sensitive period (Kehoe 1988; Sullivan et al. 2000; Chotro and Arias 2007). Hence, it is likely that the paradoxical responses to LiCl, footshock or ethanol during this period are associated with the capability of such stimuli to induce opioid activity, in view also of the critical role this neurochemical system seems to play in learning during this developmental stage (Roth and Sullivan 2006).

One mechanism supporting the paradoxical learning induced by footshock during the sensitive period is associated with the low corticosterone response that characterizes this period. Exogenous corticosterone administration before odor–shock pairings during the sensitive period has been found to suppress appetitive learning and allow emergence of aversive learning (Moriceau and Sullivan 2004). In contrast, preweanling rats after the sensitive period learned preferences when corticosterone activity was suppressed before conditioning (Moriceau and Sullivan 2004). Parker and collaborators have suggested that taste avoidance induced by

rewarding drugs is a learning process mediated by fear (Parker 2003). In our study, amphetamine failed to generate taste preference, a result which contrasts with the capability of other aversive stimuli to induce preferences during this period. Additionally, corticosterone manipulations did not alter learning induced by this drug, suggesting that sensitivity to amphetamine-induced taste learning is not associated with the corticosterone response during this ontogenetic period.

In our study, amphetamine was peripherally administered. Although there is much evidence showing that structures of the central nervous system modulate taste learning induced by rewarding (Grupp et al. 1976; Carr and White 1986; Lovaglio et al. 2010) or aversive (Grupp et al. 1976; St Andre et al. 2007) drugs, we cannot ignore their effects on the peripheral nervous system. Some authors have suggested that the activity of the sympathetic-adrenal system may contribute to the expression of the aversive response elicited by a taste previously associated with an aversive stimulus (Amaro et al. 1996; Kassil et al. 1998). Interestingly, between the first and second postnatal week of life in the rat, there are critical changes in the functional development of the sympathetic nervous system. For example, the heart rate response to a Beta-adrenergic agonist (isoprenaline) is lower in PDs 4, 7, and 11 than in PD 16. At PD16, the response is similar to the one observed in adults. This ontogenetic profile is correlated with the one that we observed in terms of taste learning. The aversive effect of psychostimulants such as amphetamine or cocaine is also mediated by the action of these drugs on the catecholaminergic systems, an effect that seems to be more related with the dopaminergic than the noradrenergic system (Freeman et al. 2008). In fact, Beta-adrenergic antagonists, such as propanolol, enhanced the aversive effects of cocaine instead of suppressing this effect (Freeman et al. 2008). Considering these antecedents, the question remains open whether the functional maturation of the sympathetic nervous system may contribute to the ontogenetic differences observed in response to different aversive stimuli during the first 2 postnatal weeks of life, after which this system starts functioning in a adult-like way.

As mentioned, several hypotheses have been raised to explain taste avoidance induced by rewarding drugs. Parker (2003) proposed that taste avoidance in rats is mediated by fear conditioning (Parker 2003). An alternative hypothesis explained taste avoidance in terms of the anticipatory contrast effect (Grigson 1997). Our results do not exclude any of these hypotheses. Fear induced by amphetamine or footshock may be mediated by different mechanisms that may also have a different temporal pattern of development. And, to our knowledge, there is no evidence of anticipatory contrast effect in this stage of development. So we cannot rule out the possibility that the neural substrates that mediate this learning process are not functionally developed before PD10.

In sum, during the sensitive period prior to P10, rats seem to be resistant to the acquisition of taste avoidance induced by amphetamine. This experimental setting can aid in understanding the nature of taste avoidance induced by rewarding drugs and the neural mechanisms underlying this controversial and paradoxical effect. This strategy has been effective for obtaining important information about mechanisms underlying a variety of behavioral phenomena, such as fear conditioning (Sullivan et al. 2000), freezing (Takahashi 1996), acute or chronic response to psychostimulants (Duke et al. 1997; Sibole et al. 2003; McDougall et al. 2005), or the affinity and response to drugs of abuse such as ethanol (Arias and Chotro 2006a; Chotro and Arias 2007; Sanders and Spear 2007). Additionally, the present data also provide useful information for research focused on affinity for drugs of abuse, such as ethanol, early in development (Spear and Molina 2005; Chotro et al. 2007).

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References

Amaro S, Monda M, De Luca B. 1996. EEG arousal, sympathetic activity, and brown adipose tissue thermogenesis after conditioned taste aversion. Physiol Behav. 60(1):71–75.

Arias C, Chotro MG. 2005. Increased palatability of ethanol after prenatal ethanol exposure is mediated by the opioid system. Pharmacol Biochem Behav. 82(3):434–442.

Arias C, Chotro MG. 2006a. Ethanol-induced preferences or aversions as a function of age in preweanling rats. Behav Neurosci. 120(3):710–718.

Arias C, Chotro MG. 2006b. Interactions between prenatal ethanol exposure and postnatal learning about ethanol in rat pups. Alcohol. 40(1):51–59.

Arias C, Molina JC, Spear NE. 2009. Ethanol-mediated aversive learning as a function of locomotor activity in a novel environment in infant Sprague-Dawley rats. Pharmacol Biochem Behav. 92(4):621–628.

Arias C, Molina JC, Spear NE. 2010. Differential role of micro, delta and kappa opioid receptors in ethanol-mediated locomotor activation and ethanol intake in preweanling rats. Physiol Behav. 99(3):348–354.

Arias C, Pautassi RM, Molina JC, Spear NE. 2010. A comparison between taste avoidance and conditioned disgust reactions induced by ethanol and lithium chloride in preweanling rats. 52(6):545–557.

Berridge KC. 2000. Measuring hedonic impact in animals and infants: microstructure of affective taste reactivity patterns. Neurosci Biobehav Rev. 24(2):173–198.

Carr GD, White NM. 1986. Anatomical disassociation of amphetamine's rewarding and aversive effects: an intracranial microinjection study. Psychopharmacology. 89(3):340–346.

Chotro MG, Alonso G. 2003. Stimulus preexposure reduces generalization of conditioned taste aversions between alcohol and non-alcohol flavors in infant rats. Behav Neurosci. 117(1):113–122.

Chotro MG, Arias C. 2007. Ontogenetic difference in ethanol reinforcing properties: the role of the opioid system. Behav Pharm. 18:661–666.

Chotro MG, Arias C, Laviola G. 2007. Increased ethanol intake after prenatal ethanol exposure: studies with animals. Neurosci Biobehav Rev. 31(2): 181–191.

Chotro MG, Arias C, Spear NE. 2009. Binge ethanol exposure in late gestation induces ethanol aversion in the dam but enhances ethanol intake in the offspring and affects their postnatal learning about ethanol. Alcohol. 43(6):453–463.

Diaz-Cenzano E, Chotro MG. 2010. The effect of taste familiarity on intake and taste reactivity in infant rats. Dev Psychobiol. 52(2):109–120.

Duke MA, O'Neal J, McDougall SA. 1997. Ontogeny of dopamine agonist-induced sensitization: role of NMDA receptors. Psychopharmacology (Berl). 129(2):153–160.

Freeman KB, Verendeev A, Riley AL. 2008. Noradrenergic antagonism enhances the conditioned aversive effects of cocaine. Pharmacol Biochem Behav. 88(4):523–532.

Garcia J, Lasiter PS, Bermudez-Rattoni F, Deems DA. 1985. A general theory of aversion learning. Ann N Y Acad Sci. 443:8–21.

Gomez F, Leo NA, Grigson PS. 2000. Morphine-induced suppression of saccharin intake is correlated with elevated corticosterone levels. Brain Res. 863(1–2):52–58.

Grigson PS. 1997. Conditioned taste aversions and drugs of abuse: a reinterpretation. Behav Neurosci. 111(1):129–136.

Grupp LA, Linseman MA, Cappell H. 1976. Effects of amygdala lesions on taste aversions produced by amphetamine and LiCl. Pharmacol Biochem Behav. 4(5):541–544.

Hoffmann H, Hunt P, Spear NE. 1990. Ontogenetic differences in the association of gustatory and tactile cues with lithium chloride and footshock. Behav Neural Biol. 53(3):441–450.

Hoffmann H, Molina JC, Kucharski D, Spear NE. 1987. Further examination of ontogenetic limitations on conditioned taste aversion. Dev Psychobiol. 20(4):455–463.

Hoffmann H, Spear NE. 1988. Ontogenetic differences in conditioning of an aversion to a gustatory CS with a peripheral US. Behav Neural Biol. 50(1):16–23.

Holson RR, Pearce B. 1992. Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. Neurotoxicol Teratol. 14(3): 221–228.

Huang AC, Hsiao S. 2008. Re-examination of amphetamine-induced conditioned suppression of tastant intake in rats: the task-dependent drug effects hypothesis. Behav Neurosci. 122(6):1207–1216.

Hunt PS, Molina JC, Spear LP, Spear NE. 1990. Ethanol-mediated taste aversions and state-dependency in preweanling (16-day-old) rats. Behav Neural Biol. 54(3):300–322.

Hunt T, Amit Z. 1987. Conditioned taste aversion induced by self-administered drugs: paradox revisited. Neurosci Biobehav Rev. 11(1):107–130.

Institute of Laboratory Animal Resources. 1996. National Research Council. Guide for the Care and Use of Laboratory Animals. Washington (DC): National Academic Press.

Kassil' VG, Yu Bondarenko M, Mikhailenko VA. 1998. Conditioned reflex responses of the sympathetico-adrenal system to an aversive taste stimulus. Neurosci Behav Physiol. 28(1):31–37.

Kehoe P. 1988. Opioids, behavior, and learning in mammalian development. In: Blass EM, editor. Developmental psychology and behavioral ecology. New York: Plenum Press. p. 309–340.

Levine S. 2001. Primary social relationships influence the development of the hypothalamic–pituitary–adrenal axis in the rat. Physiol Behav. 73(3): 255–260.

Limebeer CL, Parker LA. 2000. The antiemetic drug ondansetron interferes with lithium-induced conditioned rejection reactions, but not lithium-induced taste avoidance in rats. J Exp Psychol Anim Behav Process. 26(4):371–384.

Lovaglio J, Lin JY, Roman C, Reilly S. 2010. Basolateral amygdala and morphine-induced taste avoidance in the rat. Physiol Behav. 99(3): 419–423.

McDougall SA, Hernandez RM, Reichel CM, Farley CM. 2005. The partial D2-like dopamine receptor agonist terguride acts as a functional antagonist in states of high and low dopaminergic tone: evidence from preweanling rats. Psychopharmacology (Berl). 178(4):431–439.

Moriceau S, Roth TL, Okotoghaide T, Sullivan RM. 2004. Corticosterone controls the developmental emergence of fear and amygdala function to predator odors in infant rat pups. Int J Dev Neurosci. 22(5–6):415–422.

Moriceau S, Sullivan RM. 2004. Corticosterone influences on mammalian neonatal sensitive-period learning. Behav Neurosci. 118(2):274–281.

Moriceau S, Sullivan RM. 2006. Maternal presence serves as a switch between learning fear and attraction in infancy. Nat Neurosci. 9(8): 1004–1006.

Moriceau S, Wilson DA, Levine S, Sullivan RM. 2006. Dual circuitry for odor-shock conditioning during infancy: corticosterone switches between fear and attraction via amygdala. J Neurosci. 26(25):6737–6748.

Parker LA. 1995. Rewarding drugs produce taste avoidance, but not taste aversion. Neurosci Biobehav Rev. 19(1):143–157.

Parker LA. 2003. Taste avoidance and taste aversion: evidence for two different processes. Learn Behav. 31(2):165–172.

Parker LA. 2006. The role of nausea in taste avoidance learning in rats and shrews. Auton Neurosci. 125(1–2):34–41.

Pedersen PE, Williams CL, Blass EM. 1982. Activation and odor conditioning of suckling behavior in 3-day-old albino rats. J Exp Psychol Anim Behav Process. 8(4):329–341.

Rana SA, Parker LA. 2007. Effect of prior exposure to a lithium- and an amphetamine-paired flavor on the acoustic startle response in rats. J Exp Psychol Anim Behav Process. 33(2):172–184.

Raskin LA, Campbell BA. 1981. Ontogeny of amphetamine anorexia in rats: a behavioral analysis. J Comp Physiol Psychol. 95(3):425–435.

Roth TL, Sullivan RM. 2006. Examining the role of endogenous opioids in learned odor-stroke associations in infant rats. Dev Psychobiol. 48(1):71–78.

Sanders S, Spear NE. 2007. Ethanol acceptance is high during early infancy and becomes still higher after previous ethanol ingestion. Alcohol Clin Exp Res. 31(7):1148–1158.

Sapolsky RM, Meaney MJ. 1986. Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. Brain Res. 396(1):64–76.

Sibole JM, Matea PJ, Krall CM, McDougall SA. 2003. Effects of a partial dopamine D2-like agonist on the cocaine-induced behavioral sensitization of preweanling rats. Pharmacol Biochem Behav. 76(1):27–34.

Smith GJ, Kucharski D, Spear NE. 1985. Conditioning of an odor aversion in preweanlings with isolation from home nest as the unconditioned stimulus. Dev Psychobiol. 18(5):421–434.

Spear LP, Specht SM, Kirstein CL, Kuhn CM. 1989. Anterior and posterior, but not cheek, intraoral cannulation procedures elevate serum corticosterone levels in neonatal rat pups. Dev Psychobiol. 22(4): 401–411.

Spear NE, Molina JC. 2005. Fetal or infantile exposure to ethanol promotes ethanol ingestion in adolescence and adulthood: a theoretical review. Alcohol Clin Exp Res. 29(6):909–929.

St Andre J, Albanos K, Reilly S. 2007. C-fos expression in the rat brain following lithium chloride-induced illness. Brain Res. 1135(1):122–128.

Stickrod G, Kimble DP, Smotherman WP. 1982. In utero taste/odor aversion conditioning in the rat. Physiol Behav. 28(1):5–7.

Sullivan RM, Landers M, Yeaman B, Wilson DA. 2000. Good memories of bad events in infancy. Nature. 407(6800):38–39.

Takahashi LK. 1996. Glucocorticoids and the hippocampus. Developmental interactions facilitating the expression of behavioral inhibition. Mol Neurobiol. 13(3):213–226.

Thiele TE, Roitman MF, Bernstein IL. 1996. c-Fos induction in rat brainstem in response to ethanol- and lithium chloride-induced conditioned taste aversions. Alcohol Clin Exp Res. 20(6):1023–1028.

Upton KJ, Sullivan RM. 2010. Defining age limits of the sensitive period for attachment learning in rat pups. Dev Psychobiol. 52(5):453–464.