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Research report

Odor-avoidance or odor-preference induced by amphetamine in the infant rat depending on the dose and testing modality

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ABSTRACT

By the second postnatal week of life infant rats can acquire taste avoidance induced by amphetamine. Psychostimulant drugs supports appetitive and aversive learning in adult rats. Their appetitive effects are more likely to become associated with contextual cues, while the aversive ones have been consistently found in taste aversion learning. To explain this paradox, it has been proposed that rats would avoid a taste that predicts a change in their homeostasis because this species cannot vomit. In this study we assessed the motivational properties of amphetamine in preweanling rats by means of an odor conditioning preparation, which enables the analysis of the hedonic value of the memory by means of a consumption test or in terms of locomotor approach to the odor. Results indicate that regardless of the amphetamine dose (1 or 5 mg/kg), when animals were evaluated in the intake test, subjects avoided the odor. However, the outcome in the locomotor avoidance test varied as a function of the amphetamine dose. Rats trained with the low dose (1 mg/kg) showed odor preference, while the highest amphetamine dose (5 mg/kg) induced odor avoidance. When LiCl was employed as an unconditioned stimulus (US), rats showed avoidance in the intake and locomotor activity tests. These data indicate that amphetamine, like other drugs of abuse, supports appetitive conditioning in preweanling rats. Interestingly, infant rats expressed conditioned odor avoidance or preference depending on the dose and testing modality. Results were discussed considering current theories of avoidance learning induced by rewarding drugs.

Psychostimulant drugs, such as cocaine or amphetamine, can induce appetitive and avoidance learning in rodents [1]. Depending on their nature, some stimuli are easier to get associated than others with the different consequences of the drug [2]. For example, the appetitive effects produced by psychostimulant drugs are more likely to become associated with contextual or environmental cues, while avoidance has been consistently found in conditioned taste aversion preparations [3]. Rats show approach behavior to a context in which they have experienced the effects of amphetamine, the typical conditioned place preference effect [4]. This approach behavior is considered an index of the positive hedonic value or the appetitive properties of the drug [5]. However, rats also reject a taste that predicts the effects of psychostimulants [1,6–8]. The question of whether taste avoidance induced by drugs of abuse reflects their appetitive or aversive properties is still being debated [9,10]. For example, it has been proposed that taste avoidance induced by drugs of abuse is due to an anticipatory contrast effect [11]. According to this hypothesis, taste avoidance prompted by drugs such as cocaine or amphetamine would reflect their appetitive properties. In contrast, other authors have suggested that taste avoidance is caused by the aversive effects of these drugs [10]. It is important to point out that these aversive and appetitive effects are promoted by the same range of drug dose [1,7], and both effects can be generated simultaneously in the same subject within the same intoxication process [3].

There are important empirical evidences supporting the hypothesis that taste avoidance induced by drugs of abuse is a process that can be observed particularly in rats, because this species cannot vomit [1,7,12]. Consequently, rats will avoid any taste that predicts a change in their homeostasis [1,6,7,12]. This process is different from taste aversion induced by emetic drugs such as LiCl, which is mediated by conditioned nausea [1]. One critical finding that supports this hypothesis is that drugs of abuse, such as amphetamine or cocaine, induce conditioned taste preference in shrews, a rodent that can vomit. Emetic drugs, however, induce conditioned taste aversion in both rats and shrews [12].

There are important changes in the sensitivity to the reinforcement induced by drugs of abuse during the infancy of the rat. Before

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postnatal day (PD) 10, infant rats are resistant to the aversive effects of several drugs of abuse, such as ethanol [13,14], amphetamine [15], or morphine [16]. During this period some of these drugs can induce conditioned preferences with the same doses that promote conditioned aversions few days later [13,14,16]. However, by the second postnatal week of life infant rats are able to acquire taste avoidance induced by LiCl [15] and different drugs of abuse such as morphine [16], ethanol [17–19], or amphetamine [15], indicating that the mechanisms regulating this learning process are already functional. Infant rats are also able to acquire and express appetitive conditioning induced by a variety of rewarding drugs such as morphine [20], ethanol [21], or cocaine [22].

In the present study we explored whether amphetamine, similar to other drugs, can induce appetitive conditioning in preweanling rats. Although it has been shown appetitive conditioning mediated by cocaine in this period [22], recent data indicate that mechanisms underlying some amphetamine and cocaine effects mature at a different rate. For example, cocaine, but not amphetamine, generates sensitization in preweanling rats with a single exposure to the drug [23]. Additionally, it has been suggested that, during the preweanling period, cocaine may be more effective than other psychostimulants (including amphetamine) to generate conditioning to environmental cues [23].

We used an odor conditioning procedure, because it enables the analysis of the hedonic value of amphetamine by means of two different indexes: consumption and locomotor approach to the odor. Usually, studies that have employed odor conditioning to test the motivational properties of psychoactive drugs, evaluated the consequences of conditioning in terms of physical approach to the odor. The inclusion of the odor consumption test will allow us to evaluate additionally Parker's hypothesis about the behavioral mechanism underlying taste avoidance. If this later hypothesis is correct, when rats are tested in the consumption test, they should avoid the odor that predicted amphetamine, because they cannot vomit. However, a different result would be expected for rats evaluated in a test in which they are able to approach or to avoid an area containing the conditioned odor. In this case, no avoidance should be observed, because the defense barrier that protects them from ingesting potentially toxic substances would not be activated. Instead, it is likely that subjects trained with amphetamine will express odor preference, because in most of studies showing conditioned place preference in preweanling rats, the conditioned environment contains an explicit odor [20,22] that may facilitate context conditioning [24]. If this prediction is correct, with the same conditioned stimulus (CS) and with the same unconditioned stimulus (US), and after the same experience with both stimuli, we would be able to detect avoidance or preference, depending on the test employed. In the present study we compared odor learning induced by amphetamine (1 or 5 mg/kg) with learning induced by LiCl, an emetic drug classically employed in the taste aversion paradigm. LiCl is expected to induce conditioned avoidance in both the consumption and locomotor activity tests.

1. Experiment 1

The Please check the hierarchy of the section headings.goal of the first experiment was to compare odor avoidance induced by LiCl (1% of body weight of a 0.3 M LiCl) and a low amphetamine dose (1 mg/kg) by means of an intake test. In preweanling rats these doses of amphetamine and LiCl promoted an equivalent level of taste avoidance [15]. The CS in Experiment 1a was an almond odor solution. If taste avoidance induced by amphetamine is related to rats' inability to vomit, we expect that in this test subjects treated with amphetamine will avoid the almond odor, similar to what we previously observed when LiCl was used as a US [25]. In Experiment 1b we tested whether or not contingent exposure to almond odor and LiCl or amphetamine affects the intake of an alternative odor, vanilla.

1.1. Materials and methods

1.1.1. Subjects

Twenty-one Wistar rats representative of 8 litters, were utilized for Experiment 1a, while in Experiment 1b we employed 20 Wistar rats, derived from 9 litters. In the present study we used only female rats. In previous studies conducted with infant rats in different laboratories, no significant effect of sex was observed at this age in terms of the magnitude of the conditioned taste avoidance induced by a variety of drugs, such as LiCl [19], amphetamine [15] or ethanol [13,19]. In the present study each litter contributed only with one score to each experimental group. In those cases in which more than one subject from the same litter was assigned to the same experimental condition, scores from these subjects were averaged, and only one score was considered in the statistical analysis to avoid overrepresentation of a given litter in any particular treatment [26,27]. All animals employed in the present study 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 ($22 \pm 1.0\,^{\circ}C$), 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 National Department of Animal Care and Health (SENASA–ARGENTINA) and were in compliance with the National Institute of Health's general guidelines for the Care and Use of Laboratory Animals.

1.1.2. Procedures

Conditioning phase: two consecutive conditioning trials (one per day) were performed on postnatal days (PD) 15 and 16. On the first conditioning day, pups from a given litter were separated from the mother and placed in pairs for 15 min in a heated holding cage. During this period pups were weighed and marked, and assigned to one of the three experimental conditions defined by the drug treatment [vehicle (CS-only), amphetamine (1 mg/kg) or LiCl (0.3 M at 1% of body weight)]. After this procedure, pups were placed into individual Plexiglas chambers ($15 \times 7 \times 15$ cm) where they were exposed for 10 min to an almond odor (Esencias del boticario, Cordoba, Argentine). Odor concentrations employed both in this and in the subsequent experiments were selected in prior preliminary studies [25]. In this first experiment, the odor consisted of 1 ml of a 0.1% solution of an almond scent dissolved in distilled water, placed on a small piece of cotton located on the top of the Plexiglas chamber. Immediately after odor exposure, pups received an intraperitoneal injection of vehicle, 1 mg/kg amph (D-amphetamine sulfate, Parafarm, Buenos Aires, Argentina) or 1% body weight of 0.3 M LiCl. Amphetamine and LiCl were dissolved in NaCl (0.9%). Control pups received an equivalent volume of vehicle (NaCl 0.9%). After drug treatment, pups were placed in pairs for another 15 min in the holding cage before being returned to the home cage. The second conditioning trial was conducted the following day (PD 16), applying the exact same procedure described for the first conditioning trial.

Testing phase: on PD 17 subjects were tested in terms of consumption of an almond (Experiment 1a) or vanilla (Experiment 1b) solution. On the testing day, pups were separated from their mothers and an intraoral cannula (PE 10 polyethylene tubing, length: 5 cm, Clay Adams) was implanted in the right cheek of each pup, as described previously [13]. 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 [28]. Sixty minutes after cannulation, pups' 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 ($15 \times 7 \times 15$ cm) where they received an intraoral infusion of the odor solution [Experiment 1a: 0.1% of the almond scent; Experiment 1b: 10% solution of a vanilla scent (Condimentos Americanos S.A., Córdoba, Argentina) dissolved in distilled water]. The solution 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). With comparable infusion parameters, pups are capable of either consuming or rejecting the infused solution [15]. After the infusion procedure, subjects were weighed to estimate odor consumption scores. The difference between body weight before and after consumption was used as the dependent variable.

1.1.3. Data analysis

Intake scores from Experiment 1a and 1b were analyzed by means of a one-way between-factor ANOVA including group as the only factor. This factor had 3 levels [CS-only (vehicle), amphetamine or LiCl)]. The significant main effects obtained in these and subsequent experiments were further analyzed by means of follow-up ANOVAs and post hoc analyses (Newman–Keuls). All inferential analyses conducted in the present study employed an α level equal to 0.05.

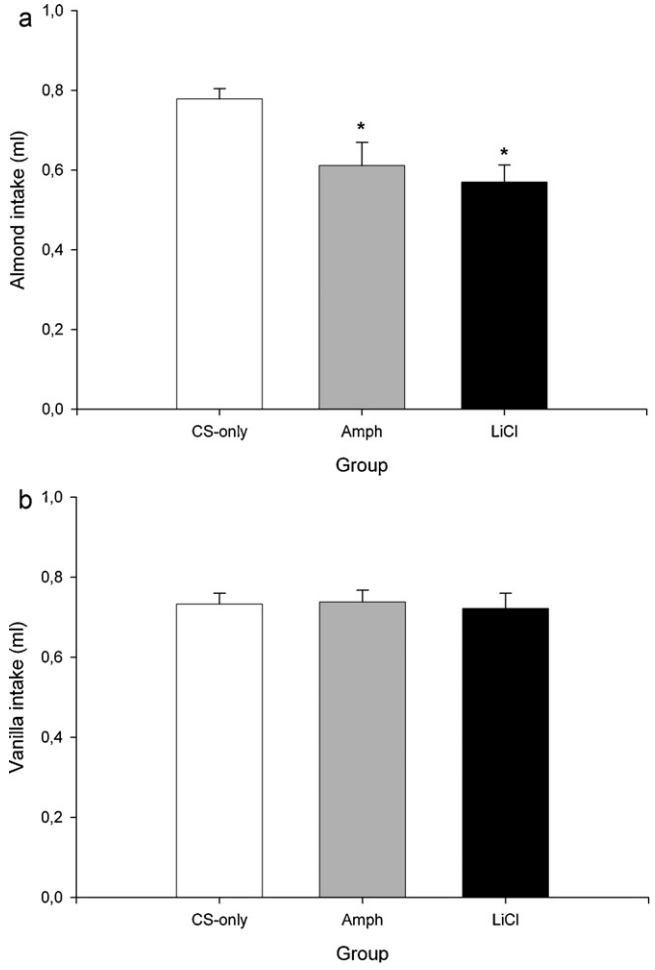

Fig. 1. (a) Consumption of the almond solution as a function of the conditioning treatment [CS-only, 1 mg/kg amphetamine (Amph) or 1% of body weight of a 0.3 M LiCl (LiCl). Vertical lines represent standard errors of the means. *Significant difference with the control group (CS-only).
(b) Vanilla consumption as a function of the conditioning treatment [CS-only, 1 mg/kg amphetamine (Amph) or 1% of body weight of a 0.3 M LiCl (LiCl). Vertical lines represent standard errors of the means.

1.1.4. Results

1.1.4.1. Experiment 1a. The results from Experiment 1a are shown in Fig. 1a. According to the ANOVA, consumption of the almond odor solution varied significantly as a function of the conditioning treatment [$F_{(2,18)} = 4.91$, $p < 0.05$]. Post hoc analyses revealed that subjects given LiCl or amphetamine at conditioning consumed less of the almond solution than the other groups. This result indicates that both drug treatments induced aversive odor conditioning in preweanling rats.

1.1.4.2. Experiment 1b. The one-way ANOVA failed to find any significant differences between groups (see Fig. 1b), thus indicating that the intake suppression of the almond solution observed in Experiment 1a reflects aversive conditioning.

2. Experiment 2

In Experiment 2 we evaluated odor avoidance in an alternative task that we shall call the locomotor avoidance test. In this test, subjects were able to avoid the conditioned odor (almond odor; Experiment 2a) or an alternative odor (vanilla; Experiment 2b) by moving out of an environment containing the odor into another area of the testing chamber with no explicit odor. Our working hypothesis is that pups given LiCl at conditioning will avoid the environment with the conditioned odor. This hypothesis is based on the fact that environmental cues [29], including odors [30,31], can acquire aversive properties after being paired with LiCl. However, if the amphetamine-mediated odor avoidance found in Experiment 1a was due to the fact that rats cannot vomit, we expect that, in the locomotor avoidance test, animals given amphetamine will not avoid the environment impregnated with the conditioned odor. Furthermore, it is possible that subjects trained with amphetamine shows odor preference. In this case, amphetamine-treated pups should spend more time in the environment with the conditioned odor than vehicle-treated controls. Experiment 2b tested whether or not contingent exposure to almond odor and LiCl or amphetamine affects the response to a novel odor, vanilla.

Table 1
Latency to escape from the odor side (seconds) and the number of crossings through the hole as a function of the conditioning treatment [CS-only, Amph (1 mg/kg) or LiCl (1% of body weight of a 0.3 M LiCl)] obtained in Experiment 2a (almond odor) and Experiment 2b (vanilla odor). Values represent means ± standard errors of the mean. *Significant difference with the control group (CS-only).

Experiment 2a (almond odor)

Group	Latency to escape	Number of crossings	*n*
CS-only	36.50 ± 11.12	6.40 ± 0.49	10
Amph	122.40 ± 36.76*	3.90 ± 1.07	10
LiCl	57.10 ± 10 18	4.40 ± 1.18	10

Experiment 2b (vanilla odor)

Group	Latency to escape	Number of crossings	*n*
CS-only	41.83 ± 19.47	5.66 ± 1.64	12
Amph	33.91 ± 11.81	4.92 ± 1.41	12
LiCl	81.16 ± 30.53	3.33 ± 0.96	12

2.1. Materials and methods

2.1.1. Subjects

In this Experiment 2a we employed 30 rats derived from 11 litters, while 36 rats derived from 12 litters were employed in Experiment 2b.

2.1.2. Procedures

The procedure employed at conditioning was exactly the same as the one described for Experiment 1, but at testing we varied the way pups were evaluated. As mentioned above, subjects were tested in a locomotor avoidance test. The apparatus employed consisted in two polyethylene environments (16 × 12 × 18 cm) connected by a small hole (2 × 1.5 cm) located in the middle. This hole was just large enough to allow pups to pass through in order to switch environments. One of the environments was covered with a plastic top in which we placed a piece of cotton with the almond odor (1 ml of a 0.1% solution of the almond scent for Experiment 2a, and 1 ml of the vanilla scent for Experiment 2b). The top of the alternative environment was not covered. At the beginning of the test pups were placed in the environment containing the odor, and their behavior was videotaped for 5 min. Two trained researchers blind to the experimental conditions analyzed the videos, measuring the following dependent variables: percentage of time spent in the environment with the odor, number of times that the animal crossed through the hole, and latency to escape from the environment with the odor.

2.1.3. Data analyses

Percentage scores, number of crossings and latencies were analyzed by means of a one-way between-factor ANOVA including group (CS-only, amphetamine or LiCl) as the only factor.

2.1.4. Results

2.1.4.1. Experiment 2a. Fig. 2a shows the percentage of time spent on the odor side as a function of the conditioning treatment. The ANOVA revealed a significant effect of group [$F_{(2,27)} = 14.32$, $p < 0.05$]. Post hoc analyses indicated that pups treated with LiCl avoided the environment containing the almond odor more than vehicle-control pups (CS-only group). Interestingly, subjects treated with amphetamine spent more time on the side containing the almond odor than the remaining groups. Groups also differed in their latency to escape from the odor side [$F_{(2,27)} = 3.82$, $p < 0.05$]. Post hoc analysis showed higher latencies in subjects treated with amphetamine than in those from the remaining conditions. The ANOVA failed to find differences in the number of crossings (see Table 1).

These results show that, in this test, rats conditioned with LiCl also expressed aversion, which is congruent with results obtained in the intake test. However, in the locomotor avoidance test, amphetamine-treated subjects behaved in the opposite way than in the consumption test. They showed preference towards the conditioned odor in comparison with the control group.

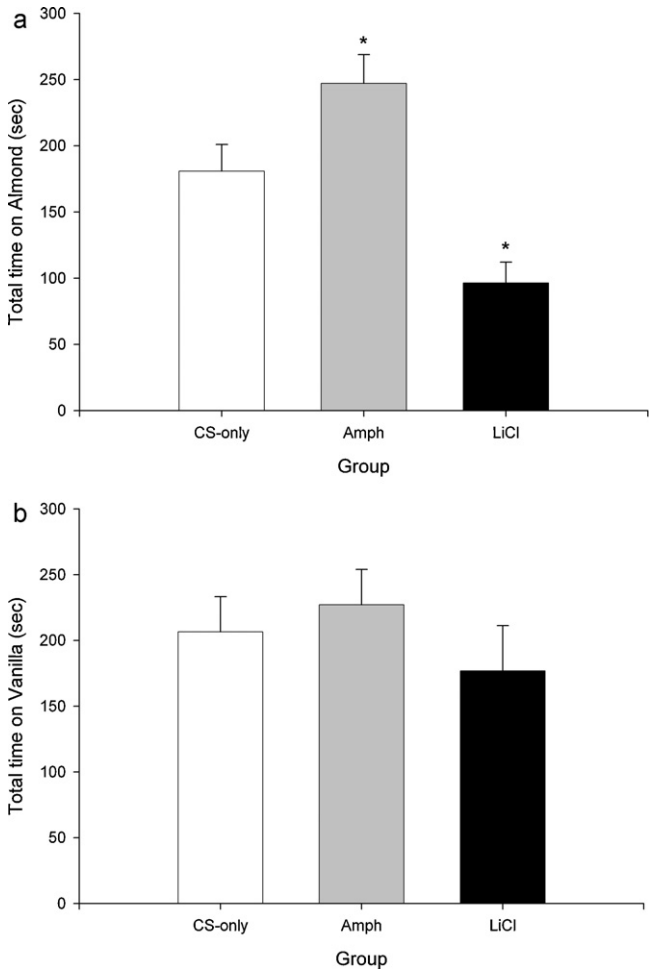

Fig. 2. (a) Total time spent on the almond odor side as a function of the conditioning treatment [CS-only, 1 mg/kg amphetamine (Amph) or 1% of body weight of a 0.3 M LiCl (LiCl). Vertical lines represent standard errors of the means. *Significant difference with the control group (CS-only).
(b) Total time spent on the vanilla odor side as a function of the conditioning treatment [CS-only, 1 mg/kg amphetamine (Amph) or 1% of body weight of a 0.3 M LiCl (LiCl). Vertical lines represent standard errors of the means.

2.1.4.2. Experiment 2b. The results of Experiment 2b are shown in Fig. 2b (percentage of time spent on the odor side) and Table 1 (latencies and number of crossings). The ANOVAs did not reveal any significant effect in the dependent variables analyzed. The conditioning of the almond odor was not generalized to the vanilla odor in this test.

3. Experiment 3

Results from the previous experiments showed differences between amphetamine- and LiCl-induced odor avoidance. According to these results, the expression of the aversive effects of a low amphetamine dose depends on the testing modality, and it requires ingestion of the CS. Riley purposes that drugs of abuse are capable of induce aversive and appetitive learning [8]. The unpleasant effects of psychostimulant drugs compete with their rewarding properties and that the aversive effects are more evident the higher is the dose of the drug [8]. In according with this hypothesis, it has been found that a 1 mg/kg, but not 5 mg/kg amphetamine, induced conditioned place preference in adult rats. Presumably, with the highest dose the appetitive and aversive effects may be competing [32]. In Experiment 3 we compared the hedonic value of a higher amphetamine dose (5 mg/kg) with the LiCl dose employed in the previous experiments in both, the intake (Experiment 3a) and the locomotor avoidance (Experiment 3b) tests.

Table 2
Latency to escape from the odor side (seconds) and the number of crossings through the hole as a function of the conditioning treatment [CS-only, Amph (5 mg/Kg) or LiCl (1% of body weight of a 0.3 M LiCl)] obtained in Experiment 3b. Values represent means ± standard errors of the mean. *Significant difference with the control group (CS-only).

Experiment 3b (almond odor)			
Group	Latency to escape	Number of crossings	*n*
CS-only	51.09 ± 21.23	3.14 ± 0.64	11
Amph	42.32 ± 8.88	6.09 ± 0.84*	11
LiCl	52.00 ± 13.59	3.75 ± 0.72	10

3.1. Experiment 3a

3.1.1. Material and methods

3.1.1.1. Subjects. A total of 32 Wistar rats derived from 8 litters were employed in Experiment 3a, while 47 rats derived from 11 litters were employed in Experiment 3b. In some of these litters more than one subject was assigned to the same experimental condition. As mentioned, to avoid overrepresentation of a given litter in any particular treatment, scores from subjects assigned to the same independent group were averaged. Hence, we analyzed 23 scores (from 8 litters) in Experiment 3a and 32 scores (from 11 litters) in Experiment 3b.

3.1.1.2. Procedure. The procedure in Experiment 3a was similar to the one employed in Experiment 1, but in this case the amphetamine dose employed was 5 mg/kg. This amphetamine dose is considered high by other authors [7]. In this experiment subjects were tested in terms of almond intake. In Experiment 3b, the procedure was similar to the one employed in Experiment 2, but in this case rats were evaluated in the locomotor avoidance test.

3.1.1.3. Data analyses. Intake (Experiment 3a) and data from the locomotor activity test (Experiment 3b) were analyzed by means of a one-way between-factor ANOVA including group (CS-only, amphetamine or LiCl) as the only independent variable.

4. Results

4.1. Experiment 3a

Results from Experiment 3a are shown in Fig. 3a. According to the ANOVA, consumption of the almond odor solution varied significantly as a function of the conditioning treatment [$F_{(2,20)} = 4.45$, $p < 0.05$]. Post hoc analyses revealed that subjects given LiCl or amphetamine at conditioning consumed less of the almond solution than controls (CS-only group).

4.2. Experiment 3b

Fig. 3b shows results from Experiment 3b. According to the ANOVA, the time spent on the side containing the almond odor varied significantly as a function of the conditioning treatment [$F_{(2,29)} = 6.11$, $p < 0.05$]. Post hoc analyses revealed that subjects treated with LiCl or amphetamine spent less time on odor side than controls (CS-only group). No significant differences between groups were detected in the analysis of latencies. The ANOVA conducted with the number of crossings revealed a significant main effect of group [$F_{(2,29)} = 4.56$, $p < 0.05$], indicating that subjects treated with amphetamine (5 mg/kg) crossed more times through the hole than the remaining conditions (Table 2).

5. Discussion

The present study shows that amphetamine supports appetitive and avoidance conditioning in preweanling rats. The expression of these responses was modulated by the modality of the test and the dose of the drug. The positive rewarding effect was observed only with the lower dose (1 mg/kg) and exclusively in the locomotor test through two measures, the time spent on the odor side and the latency to leave the odor side. In contrast, the highest amphetamine

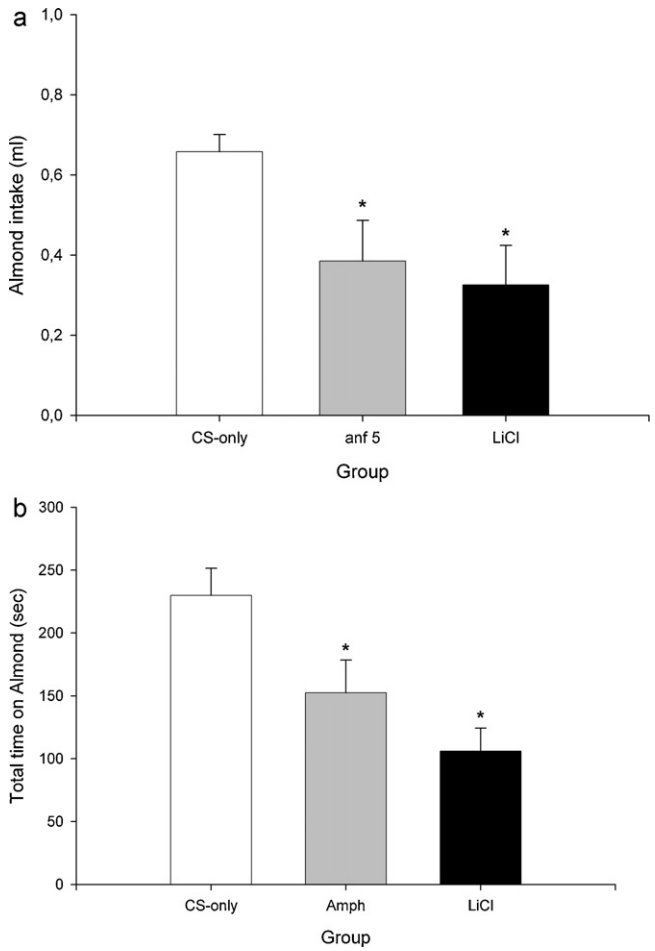

Fig. 3. (a) Consumption of the almond solution as a function of the conditioning treatment [CS-only, 5 mg/kg amphetamine (Amph) or 1% of body weight of a 0.3 M LiCl (LiCl). Vertical lines represent standard errors of the means. *Significant difference with the control group (CS-only).
(b) Total time spent on the almond odor side as a function of the conditioning treatment [CS-only, 5 mg/kg amphetamine (Amph) or 1% of body weight of a 0.3 M LiCl (LiCl). Vertical lines represent standard errors of the means. *Significant difference with the control group (CS-only).

dose (5 mg/kg), similar to LiCl, induced odor avoidance in the consumption and locomotor test.

In adult rats, amphetamine-induced conditioned place aversion has been rarely found, even employing high doses. For instance, in one study using different amphetamine doses (between 0.626 and 5 mg/kg), place preference was found with most of them, except with the highest one (5 mg/kg), with which no effect was observed. The failure to find conditioned place preference with this high dose, was interpreted as a possible competition between the appetitive and the aversive effects of amphetamine [32]. However our results with preweanlings showed odor avoidance in the locomotor test when the higher amphetamine dose (5 mg/kg) was used as US. There are important procedural differences between our study and the typical place preference paradigm employed with adult rats. The CS in the present study was and odor, while with adults usually is a context (without an explicit odor). Furthermore, we employed a delay conditioning procedure, which seems to facilitate the detection of the aversive effects of amphetamine in adult rats in place conditioning paradigms [33].

When animals were evaluated in the intake test, amphetamine (1 or 5 mg/kg) and LiCl induced odor avoidance (Experiments 1a and 3a). It is striking that the lower amphetamine dose (1 mg/kg) induced appetitive conditioning in the locomotor activity test, and avoidance in the intake test. Several hypotheses have been raised to explain why rats avoid intake of a flavor that predicts the effects of psychostimulants. As mentioned above, Parker and other authors argued that, because rats cannot vomit, they have evolved a highly sensitive defense barrier which is activated by chemosensory receptors [1]. Hypothetically, this defensive system protects the organism against the ingestion of toxins. When the animal is re-exposed to a given food that generated an important change in its homeostatic state, this system is activated and it signals danger to the animal. Consequently, the animal will reject the flavor. Our results obtained with the low amphetamine dose support this hypothesis. When rats were tested in the intake test, this hypothetical defensive system should have been activated to prevent the ingestion of the potentially toxic solution. Consistently with this hypothesis, in this test we observed that animals avoided the odor solution paired with amphetamine. However, during the locomotor avoidance test, rats were exposed to the ambient odor, but in this case they did not need to reject it because they were not going to ingest it. In this case, subjects trained with amphetamine showed conditioned odor preference. Hence, it seems that perception of the odor is not sufficient to induce avoidance. In other words, the hypothetical protective system seems to require ingestion of the flavor in order to be activated.

There is one study that reported a similar pattern of results to those observed with the low amphetamine dose. Smith and Holman [34] found that infant rats that received paired presentations of almond odor and amphetamine on PD2, showed odor avoidance in a consumption test, but odor preference in a place test. There are several critical procedural differences between this and our study, particularly as regards the age of conditioning, the interval between conditioning and testing, and the liquid and maternal deprivation schedules before testing. Conditioning in Smith and Holman's study took place on PD2. In a recent study we have shown that there is a marked resistance before PD10 to acquire conditioned taste avoidance induced by amphetamine [15]. However Smith and Holman observed a rejection of the odor in the intake test, despite the long interval between conditioning and testing. Their results suggest that the mechanisms regulating conditioned odor aversion may mature earlier than those mediating conditioned taste aversions. This is congruent with the fact that before PD10, infants seems to acquire faster odor-LiCl [35] than taste-LiCl aversions [15]. It is also likely that Smith and Holman's results were influenced by the severe deprivation schedule utilized before testing (PD18). During three days, the mother was removed from the home-cage in the morning and returned each evening. The fourth day, the day before testing, the mother was removed from the home cage and pups had no access to water until testing during at least 24 h [34]. There are many evidences showing that maternal separation is an important stressor for the infant rat [36,37]. Despite these procedural differences, our pattern of results was almost identical to the one reported by these authors, indicating that the odor preference and avoidance induced by amphetamine is a robust phenomenon, at least in preweanling rats.

As we have mentioned in the introduction, an alternative hypotheses raised to explain the paradoxical avoidance learning induced by positively reinforcing drugs, is based on the anticipatory contrast effect. According to this hypothesis, taste avoidance induced by these drugs is generated because, at conditioning, the hedonic value of the taste is reduced by the presence of the highly rewarding drug [11]. This hypothesis seems insufficient to account for our results. Firstly, the anticipatory contrast effect seems to depend on the rewarding properties of the CS. In current scientific literature we found no studies showing evidence of an anticipatory contrast using odor cues as a CS. In any case, even considering the almond odor as an appetitive stimulus able to participate in this kind of learning process, this hypothesis fails to predict the

appetitive response observed in the locomotor avoidance test. The hedonic value of the CS (the almond odor) should have been modified during conditioning trials by the presence of the more significant rewarding stimulus (amphetamine). Hence, rats should have avoided the odor not only in the intake test, but also in the locomotor test.

The neurobiological mechanisms underlying flavor avoidance induced by rewarding drugs are still a subject of debate. There is convergent evidence showing that the nucleus accumbens participates in the positive, but not in the aversive, effects of amphetamine [for example, 38]. However, it is not so clear which brain areas are involved in amphetamine-induced taste avoidance. Some brainstem nuclei seem to participate in both, amphetamine- and LiCl-induced taste aversion, such as area postrema, lateral parabrachial nucleus and nucleus of the solitary tract [38–41]. Area postrema detects emetic toxins from the bloodstream, and it mediates conditioned nausea, while nucleus of the solitary tract processes sensory and hedonic properties of the taste, projecting to the parabrachial nucleus where this information is integrated [42]. The amygdala participates in taste avoidance induced by amphetamine or LiCl, but not in conditioned disgust reactions [43]. This structure seems to participate in flavor avoidance induced by amphetamine (and different drugs of abuse) by means of a mechanism similar to the one underlying fear conditioning, processing danger rather than illness, and relatively independent from conditioned nausea [43]. This structure may mediate flavor avoidance when the low amphetamine dose was employed as the US. Odor avoidance induced by the high amphetamine dose (5 mg/kg), which was expressed also in the locomotor test, may be caused by different unconditioned effects that can produce psychostimulants at high dosage, such as high blood pressure, anxiety or dysphoria, rather than nausea [see 10].

In a recent review, Riley discussed the role that the pleasant and unpleasant effects of a given drug may play in drug seeking behavior [8]. This author proposes that the effect of a drug can be perceived as appetitive or aversive depending on many factors, including dosage. Riley also points out in this review that the aversive and appetitive effects of drugs of abuse are usually revealed by different tests in response to different CSs. Usually, the appetitive effects of psychostimulants are associated with environmental cues, while tastant CSs are easily associated with their aversive effects. The present series of experiments represents a valuable methodological strategy, showing a procedure sensitive to reveal both, appetitive and aversive conditioned responses induced by drugs of abuse employing a single CS. The paradigm presented here may help us to understand how the appetitive and aversive effects of different drugs compete to promote drug-seeking behaviors.

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