Patricio E. Aranda-Fernandez[1]
Mirari Gaztañaga[1]
Carlos Arias[1,2]
M. Gabriela Chotro[1]

[1]Faculty of Psychology
University of the Basque Country UPV/EHU
Donostia-San Sebastián, Spain
E-mail: ezee.aranda@gmail.com

[2]Instituto de Investigación Médica Mercedes y Martín Ferreyra Córdoba, Argentina

Conditioned Inhibition in Preweanling Rats

ABSTRACT: *Inhibitory conditioning is a very well established phenomenon in associative learning that has been demonstrated in both humans and adult animals. But in spite of the fact that this topic has generated much empirical and theoretical work, there are no published studies assessing inhibitory learning during the early ontogeny of the rat. In this study we test the possibility of finding conditioned inhibition in infant rats (Day 10) using a conditioned taste aversion procedure. We tested whether the consumption of saccharin (A) was reduced when paired with a LiCl injection compared to the presentation of saccharin in compound with a lemon odor (AX) without any aversive consequence. After training, retardation, and summation tests were conducted in order to evaluate the inhibitory properties of the lemon odor (X). The results of this study showed that in male pups, after conditioned inhibition training, stimulus X passed both retardation and summation tests. These results indicate that conditioned inhibition can be established in the early development of the rat, suggesting that animals at this stage of ontogeny have the capacity to acquire and to express inhibitory conditioning, although this effect appears to be sex-dependent.*

Keywords: *conditioned inhibition; preweanling; conditioned taste aversion; rat; sex differences*

INTRODUCTION

Conditioned inhibition (CI) is a well-established phenomenon in adult rats and it is one of the most widely used protocols for studying inhibitory conditioning (Savastano, Cole, Barnet & Miller, 1999). A variety of experimental procedures have been exploited to demonstrate conditioned inhibition (see Savastano et al., 1999). In a typical Pavlovian conditioned inhibition training procedure (Pavlov, 1927) a stimulus, A, is presented on trials in which it is paired with an unconditioned stimulus (US) (A+) and afterwards it is presented in compound with another stimulus, X (AX), without being paired with the US (AX−). Repeated and interspersed presentations of A+/AX− induces a differential response depending on whether A, or AX is being presented to the subject (Savastano et al., 1999) or in other words, the organism behaves differently if X is present or not during the presentations of A, meaning that X has become a conditioned inhibitor.

On the basis of the assumption that a conditioned response (CR) to an inhibitory conditioned stimulus (CS) is opposite to that elicited by an excitatory stimulus, Rescorla (1969) proposed two specific tests in order to test the inhibitory properties of stimulus X, these being a retardation test and a summation test. In the first test, a US is directly paired with the hypothetical conditioned inhibitor. If that stimulus were a conditioned inhibitor, it would be relatively difficult to establish any further excitatory conditioning to that stimulus in comparison with a group that had received no previous experience with it. In the case of the summation test, a conditioned excitor is presented in compound with the proposed conditioned inhibitor in

Manuscript Received: 30 December 2014
Manuscript Accepted: 6 September 2015
Correspondence to: Patricio E. Aranda-Fernandez
Contract grant sponsor: Spanish "Ministerio de Economía y Competitividad"
Contract grant numbers: PSI2011-24231, PSI2012-38019
Contract grant sponsor: Basque Government
Contract grant number: IT-694-13
Article first published online in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/dev.21359 •

order to rule out other processes that could be occurring at the time of the test (i.e. a latent inhibition process or an attentional deficit) (Rescorla, 1969). If the presence of X (the proposed inhibitor) reduces the strength of the response that would otherwise be exhibited by the excitatory stimulus when presented alone, then stimulus X is said to possess inhibitory properties.

Conditioned inhibition has primarily been studied using a conditioned suppression procedure (Rescorla & Holland, 1977), but it has also been examined using other procedures such as conditioned taste aversion. Taukulis and Revusky (1975) trained rats with a conditioned inhibition procedure involving trials in which the taste of saccharin was followed by induced illness (by a LiCl injection), and trials in which that same taste was presented in compound with an amyl acetate odor but without any aversive consequence. After three tests—summation, enhancement of conditioning, and a retardation test, the authors concluded that the odor had become a conditioned inhibitor, thereby demonstrating that conditioned inhibition can be observed when using a conditioned taste aversion procedure.

The phenomenon of conditioned inhibition has been described and studied almost exclusively in adult subjects. To our knowledge, there are only two studies reporting conditioned inhibition during early ontogeny, using 4–12 week-old kittens as the subjects (Dess & Soltysik, 1989, 1993) whilst there are no published studies consistently demonstrating this phenomenon in infant rats. It has been well demonstrated that early in development the rat pup has the capacity to learn about stimuli present in the environment, the relations between them, and their associated consequences (Spear, 1994). Although in general, developing animals tend to learn slower and with more difficulties than adults, especially when complex stimuli or difficult learning tasks are involved (Spear, 1994), the capacity of preweanling rats to display associative and non-associative learning has been well documented. For instance, there are various studies in the literature showing that most of the basic learning processes observed in adults are also evident during the preweanling period. Such phenomena include habituation, sensitization, and sensory preconditioning (Heyser, Chen, Miller, Spear & Spear, 1990; Snyder, Katovica & Spear, 1998), as well as conditioned aversions and conditioned preferences (Hunt & Amit, 1987; Randall, Kraemer & Bardo, 1998), second order conditioning (Cheatle & Rudy, 1979), latent inhibition (Revillo, Gaztañaga, Aranda-Fernández, Arias & Chotro, 2014), extinction (Revillo, Paglini & Arias, 2014), potentiation, overshadowing (Kucharski & Spear, 1985), and context conditioning (Lariviere, Chen & Spear, 1990). However, there are other phenomena that have not yet been described in the preweanling rat, such as conditioned inhibition. Given that the infant rat has already developed the minimal behavioral repertoire needed to reveal inhibitory conditioning, i.e., they can discriminate between stimuli and their consequences (Chotro & Alonso, 1999, 2003), and they can acquire and express a conditioned taste aversion (Misanin, Blatt & Hinderliter, 1985), it follows that any ability to acquire inhibitory conditioning should be detectable with a conditioned taste aversion procedure.

The aim of the current study, therefore, was to determine whether Pavlovian conditioned inhibition training (A+/AX−) could be established in infant rats, starting from PD 9. In order to address this question, we designed an experiment using a conditioned taste aversion paradigm similar to that used with adult rats by Taukulis et al. (1975). In the discrimination phase, infant rats were trained on an A+/AX− schedule. Following the training phase, a retardation test was conducted for half of the subjects, in which X was paired with a US. For the remaining half of the subjects the inhibitory training was followed by a summation test in which an excitor was paired with the assumed conditioned inhibitor. In studies with adult subjects, it is considered that in order for X to be regarded as a conditioned inhibitor after conditioned inhibition training, the conditioned inhibition group should differ from the other groups on both the retardation and the summation tests. Therefore, we can expect that on the retardation test X will be retarded in its ability to acquire an excitatory conditioned response, whilst on the summation test the presence of X in compound with a pretrained excitatory stimulus should be effective in reducing the response that would otherwise be elicited by the excitor when presented alone.

METHODS

Subjects

A total of 96 Sprague-Dawley pups, representative of 16 litters, were used for the present experiment (for further details see Table 1). No more than one male and one female from a given litter were assigned to the same treatment condition, in order to avoid overrepresentation of a particular litter in any treatment (Holson & Pearce, 1992). Animals were born and reared at the vivarium of the Basque Country University (Spain) under conditions of constant room temperature (22 ± 1.0°C), on a 12-hr light–12-hr dark cycle. Births were examined daily and the Day of parturition was termed as postnatal Day 0 (PD0). Litters were culled to 10 pups within 24 hr after birth. Subjects were 9 Day old pups (PD9) at the start of this and the following experiments. European regulations for the care and treatment of experimental animals

Table 1. Experimental Design for this Study

		Retardation		Summation		n	
Group	Training	Conditioning	Test	Conditioning	Test	♂	♀
CI	A+/AX−	X+	X	B+	BX	8	8
						8	8
CCI	A−/AX−	X+	X	B+	BX	8	8
						8	8
UT	—	X+	X	B+	BX	8	8
						8	8

(A) saccharin; (X) lemon; (B) almond; (+) LiCl; (−) saline. Group names: (CI) Conditioned Inhibition group, (CCI) Control of Conditioned Inhibition group, (UT) Untreated group.

were followed, and procedures were controlled and approved by the Diputación Foral de Gipuzkoa, Spain, in compliance with the European Communities Council Directive (86/609/EEC).

Procedures

Training Phase. Pavlovian conditioned inhibition training was carried out between PDs 10 and 19. During this phase, two sessions were conducted on each day, one corresponding to Trials A+ (p.m.) and another corresponding to Trials AX− (a.m.). On the first training day, subjects were removed from their home cages and assigned to one of the three independent groups (Conditioned inhibition [CI], Control [CCI] or Untreated [UT]) and marked on the tail for identification. The group names refer to the treatment received by each group during the treatment phase (see Table 1). Subjects from group UT were returned to their home-cages and remained untreated until the retardation or summation phases. Pups from the remaining groups (CI and CCI) were intraorally cannulated. For cannulation, a polyethylene cannula (PE 10 polyethylene tubing, length: 5 cm, Clay Adams, Parsippany, NJ) was implanted intraorally using a procedure already described in previous studies (Díaz-Cenzano, Gaztañaga & Chotro, 2014). Cannulas were made from 7-cm sections of polyethylene tubing (i.d. = 0.28 mm). One end of the section was heated to form a small flange. A thin wire attached to the non-flanged end of the cannula was placed on the internal surface of the pup's cheek. The wire was then pushed through the oral mucosa until the flanged end of the cannula was positioned over the internal surface of the cheek while the remainder of the cannula exited from the oral cavity. The entire procedure takes no more than 10 s per pup and induces minimal stress (L. P. Spear, Specht, Kirstein, & Kuhn, 1989). These cannulae were later used to infuse saccharin into the oral cavity of the subjects.

Trials A+: Starting on the afternoon of PD9, and following cannulation, pups' bladders were voided by gentle brushing of the anogenital area, and their body weights were recorded. The subjects were then placed in a clear plastic box (15 × 15 × 20) where they received an intraoral infusion of saccharin (0.15% w/v) followed by a LiCl (0.15M, 1% of body weight) i.p. injection in Group CI, or a saline (1% of body weight) i.p. injection in Group CCI. Immediately after saccharin infusion, the body weights of the animals were again recorded in order to calculate the amount of liquid consumed.

Trials AX−: following the parameters used during Trials A+, on these trials the saccharin solution (A) was presented together with the lemon odor, which served as stimulus X. The odor consisted of 0.2 ml of a lemon scent (Vahine, Sabadell, Spain) and was presented on a small piece of cotton located on top of the plastic chamber. As on the A trials, the weights of each animal were registered before and after the saccharin infusion. After these trials, neither LiCl nor saline injections followed the presentations of the stimuli. During the time period before returning to their home-cages (30 min after each trial), the pups were placed in heated holding chambers (15 × 8 × 15) grouped by treatment and litter (i.e grouping of the animals was arranged by putting together animals of the same litter - males and females - and the same experimental treatment, CI, CCI, or UT).

Retardation Test. Half of the trained males and females entered the retardation treatment. Following the last A+ trial, a retardation test was carried out. In this retardation phase, the untreated group was used to assess whether conditioning of X was effective, and to demonstrate that differences in the conditioning scores with this group can be attributed to the conditioned inhibition training. If X has acquired inhibitory properties over the course of conditioned inhibition training, a retardation test should reveal a deficit in establishing excitatory conditioning in Group CI in comparison with Group CCI and Group UT. Whilst it may also be possible to find no differences between Group CCI and CI due to a latent inhibition process (Lubow, 1973; Lubow & Moore, 1959) occurring during the conditioned inhibition training, it is still expected that both groups should differ from Group UT, which had received no previous experience with X.

Two excitatory conditioning trials were conducted including all three groups (CI, CCI, UT), the first trial beginning on the afternoon of PD 21 and the second trial on the morning of PD 22. The test was conducted on the afternoon of PD 22. For this phase, lemon was presented intraorally, mixed in filtered water (the concentration of the emulsion was 0.2%), following the same procedure used during the presentations of saccharin during training. After this, an injection of LiCl was administered to all three groups (using the same dosage as that administered on the A+ trials) during both excitatory conditioning trials. On the test, as in the training phase, lemon intake was calculated by using body weight differences after the intraoral infusion of lemon.

Summation Test. For the remaining half of the subjects, after A+/AX− training, we conducted a summation test to assess the inhibitory status of X as well as to rule out other explanations of the effect, such as latent inhibition or loss of attention to the stimulus (Rescorla, 1969). A summation test consists of presenting the conditioned inhibitor in compound with a known excitor, and consequently tests the magnitude of the response elicited by the compound. If X has become a conditioned inhibitor during conditioned inhibition training, then, when presented in compound with an excitor, the magnitude of the response should be weaker in comparison with other groups in which X does not act as an inhibitor. In this case we used a stimulus (almond) denoted as B, which was excitatory for all groups. After two excitatory conditioning trials with B, this stimulus was presented in compound with X (lemon odor) to all three groups (CI, CCI, UT), the first conditioning trial beginning on the afternoon of PD 21, and the second trial on the morning of PD 22. The test was conducted on the afternoon of PD 22.

For the excitatory conditioning of B, a mixture of 0.2 ml of almond essence in 100 ml of filtered water was presented intraorally to the subjects, following the same procedure used during the presentations of the saccharin solution during conditioned inhibition training, whilst the same doses of LiCl were injected to all three groups at the two excitatory conditioning trials.

For the summation test, after B was rendered excitatory, we presented it in compound with the lemon odor (X) placed on a small piece of cotton located on top of the Plexiglas chamber.

Data Analysis. Intake data from the training phase, the retardation test and the summation test were analyzed separately for males and females. Saccharin intake scores during the training phase were analyzed using three-way mixed ANOVAs $[2 \times (2) \times (10)]$, in which Treatment (CI, CCI) was the between-subject variable, and Presence of X (A, AX) and Trial (1–10) were the within-subject repeated measures. Significant differences between treatments (CI vs. CCI) were explored on trials AX10 and A11 with planned comparisons, to determine the effectiveness of the discrimination training.

Lemon intake data from the retardation phase were analyzed with two-way mixed ANOVAs $[3 \times (2)]$, in which Treatment (CI, CCI, UT) was the between-subject variable, and Trial (conditioning trial 1 and Test) the within-subject repeated measure.

For the summation test, two analyses were run. First, consumption scores from the conditioning phase with B were analyzed using a two-way mixed ANOVA [3 x (2)] in which Treatment was the between-subjects variable and Trial the within-subject repeated measure. Intake of BX (almond in the presence of the lemon odor) was analyzed with a one-way ANOVA.

Significant effects of the between-subject variables were analyzed using Duncan's post-hoc tests. Significant interactions involving a within-subjects variable were further analyzed using planned comparisons and factorial ANOVAs for each trial. In these and all subsequent tests, we adopted a significance level of $p < .05$.

RESULTS

Training Phase

In order to know whether conditioned inhibition training was effective for the pups, planned comparisons were conducted with the data from the final two training trials (A+ and AX−) with consumption data from both the males and females (Fig. 1). The results of the male subjects revealed a statistically significant difference between groups CI and CCI on the last trial of A+ but not on the last trial of AX−. The analyses conducted with female subjectś consumption scores during training indicated a significant effect of Treatment $[F(1,31) = 8.39,\ p < .001]$, although neither an effect of Trial nor an interaction were observed. These results indicate that females displayed an aversion to both A and AX, suggesting that training was effective in inducing differential responding to the presence of X only in male subjects.

Retardation Test

The results of the retardation test with the lemon intake scores for groups CCI, CI, and UT are depicted in Figure 2. An ANOVA with consumption scores for male subjects during the three trials, revealed a main effect of Trial $[F(2,42) = 17.15,\ p < .001]$ and an interaction between Treatment and Trial $[F(2,42) = 3.96,\ p < .001]$. One-way ANOVAs for each of the three trials revealed a significant effect of Treatment on the third trial $[F(2,21)=7.35,\ p < .01]$. Duncan's post-hoc test revealed no significant difference between groups CI and CCI but that both differed from group UT. A similar analysis with the consumption scores for female subjects did not reveal any statistical differences between the examined variables.

Summation Test

The consumption scores for flavor B for groups CI, CCI, and UT as a function of trials are depicted in Figure 3. Analysis of the intake of this flavor for male subjects during conditioning indicated a main effect of Treatment $[F(2,20) = 10.37,\ p < .001]$, of Trial $[F(1,20) = 92.42,$

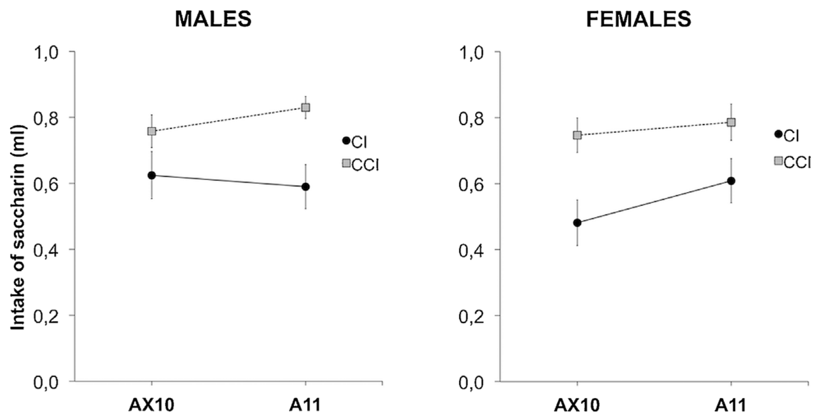

FIGURE 1 Mean (+/− SEM) intake of saccharin (stimulus A) during the last trials A+ and trials AX− of training, as a function of Treatment (CI, CCI). Male subjects (left panel), female subjects (right panel).

$p < .001$], and a significant interaction between Treatment and Trial [$F(2,20) = 11.21$, $p < .001$]. Planned comparisons between both trials for each treatment revealed a significant decline in consumption of almond for all three groups. A one-way ANOVA with the data from Trial 1 of conditioning to B indicated no differences between treatments, while the ANOVA for the second trial indicated a significant effect of Treatment on consumption of B. Duncan's post-hoc analysis of this effect revealed that both groups CI and CCI did not differ between them but both showed higher consumption of flavor B than group UT. These results indicate that, although all groups acquired a conditioned aversion to B, subjects with no previous experience of any taste (group UT) showed a stronger aversion to B than groups previously exposed to taste A. This effect can be interpreted as generalization of latent inhibition.

A one-way ANOVA with intake of BX (Fig. 4) for male subjects revealed a main effect of Treatment [$F(1,20) = 19.63$, $p < .001$]. Duncan's post-hoc test revealed that the males in Group CI consumed more of B in the presence of X than those in Groups UT and CCI.

Analysis of the intake of B for female subjects during conditioning indicated a main effect of Trial [$F(1,22) = 198.6$, $p < .001$], and a significant interaction between Treatment and Trial [$F(2,22) = 4.80$, $p < .001$]. Similar analyses to those conducted with the male subjects indicated that while all groups acquired an aversion, this aversion was stronger in the UT group compared to the CI and CCI groups, which can be taken to indicate that females also displayed generalization of latent inhibition [$F(2,22) = 5.26$, $p < 05$]. However, in contrast to what has been observed with male subjects, the one-way ANOVA with the consumption scores for BX revealed no significant effect of Treatment on consumption of this flavor, confirming what has been observed during both the training and retardation test. In contrast to the results observed for males, X did not become a conditioned inhibitor for the females during Pavlovian conditioned inhibition training.

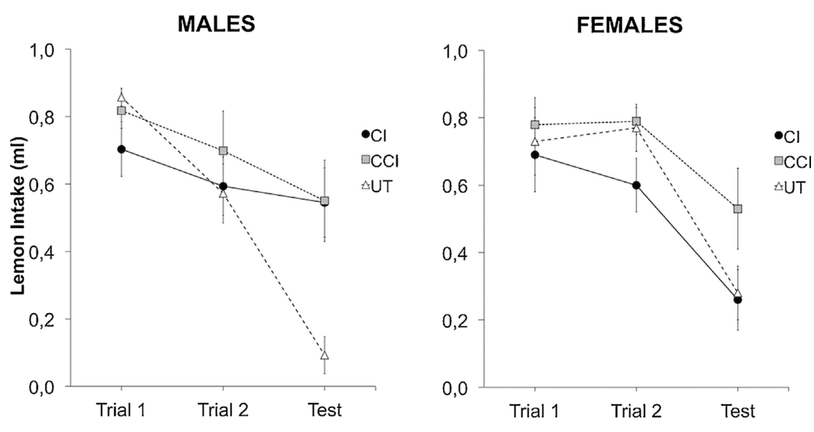

FIGURE 2 Mean (+/− SEM) intake of lemon (stimulus X) during conditioning trials and on the test trial as a function of Treatment (CI, CCI, UT). Male subjects (A), female subjects (B).

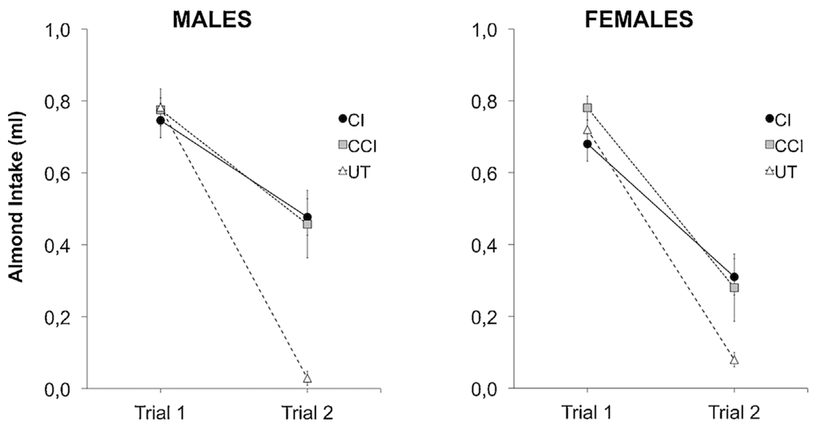

FIGURE 3 Mean (+/− SEM) intake of almond (stimulus B) during conditioning trials as a function of Treatment (CI, CCI, UT). Male subjects (A), female subjects (B).

GENERAL DISCUSSION

This study presents a series of experiments in which a Pavlovian conditioned inhibition procedure was conducted with preweanling rats (PD 9-22). Results show that after Pavlovian conditioned inhibition training (A+/AX−) in a taste aversion paradigm, excitatory conditioning of X was retarded in males (retardation test). Stimulus X also passed a summation test for this group, further confirming the inhibitory status of X after conditioned inhibition training. This same conditioned inhibition training was not effective for females, for which X showed no retardation in the establishment of excitatory conditioning nor a reduced response when X was paired with a known excitor (summation test). These results indicate that, at least at this early stage of development, there appears to be a sex difference in terms of the ability to express a discrimination response.

The clear finding to emerge from this study is that male subjects from group CI after A+/AX− training showed a differential response to stimulus A in the presence or absence of stimulus X, and that stimulus X passed both of the standard tests for inhibition, prompting the conclusion that X was indeed rendered inhibitory for this group. On the other hand, female subjects from group CI did not express the expected response to the inhibitory stimulus after training, and there were no clear differences between the response to the excitatory and inhibitory stimuli. That is, when A was followed by a LiCl injection a visible taste aversion was produced when compared with the CCI group. The results of the retardation and the summation tests confirmed this result, indicating that female rats at this age failed to acquire conditioned inhibition, or at least failed to express this type of learning under the present experimental conditions.

When considering the different tests to assess the inhibitory status of a given stimulus, Rescorla (1969) suggested that retardation in the acquisition of an excitatory conditioned response to that stimulus would constitute a satisfactory demonstration of conditioned

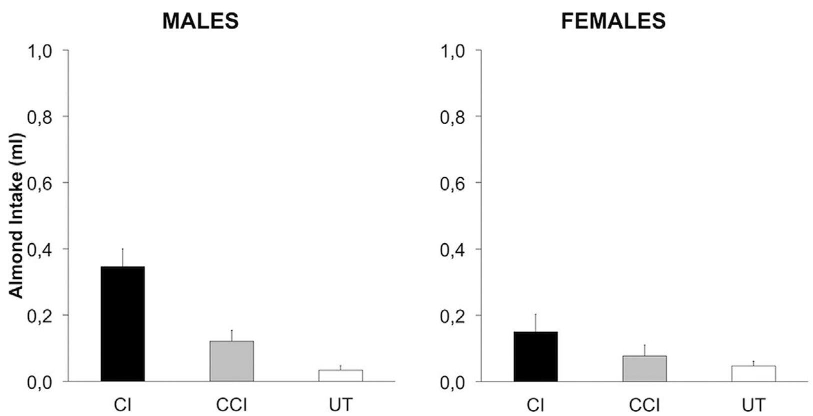

FIGURE 4 Mean (+/− SEM) intake of almond (B) in presence of lemon (X) as a function of Treatment (CI, CCI, UT). Male subjects (A), female subjects B).

inhibition. It is possible, however, that the learning decrement could also be due to different processes in which selective-attention mechanisms are implied, such as latent inhibition (Rescorla, 1969)—and it is reasonable to expect that after several training trials with the A+/AX− arrangement, pups from all groups would undergo latent inhibition of stimulus X. For this reason, the summation test plays an important role in detecting conditioned inhibition.

Other authors consider that the summation test is not the critical test for assessing conditioned inhibition, but rather a retardation test with the appropriate control conditions would constitute the best option for demonstrating the inhibitory properties of a stimulus (Papini & Bitterman, 1993). However, if the animals are only presented with a retardation test, an explanation in terms of a loss of attention to the inhibitor cannot be ruled out. Moreover, if only a summation test is presented, an account in terms of an increase in attention to the inhibitor at the expense of the excitor could also explain the outcome of this test. The use of both tests, therefore, helps to discard these two alternative explanations, since attention to the inhibitor cannot be increased and decreased at the same time by conditioned inhibition training (Savastano et al., 1999).

Results of the retardation test in this experiment clearly show no difference between groups CI and CCI. This could mean either that both groups showed retardation of conditioning because of the same phenomenon: latent inhibition (both groups received the same amount of preexposure to the critical stimuli); or that both groups are responding in a similar way to stimulus X but as a result of two different phenomena: Group CCI due to latent inhibition and Group CI due to conditioned inhibition. Conventionally, as explained by Rescorla (1969) there is no other valid way of ascertaining this but with a summation test. If Group CI responded to the excitatory conditioning of X in a retarded fashion because X had become a conditioned inhibitor during conditioned inhibition training, when a summation test was presented, this group would have shown a decrease in responding when the supposed conditioned inhibitor was paired with a known excitor. On the contrary, if both groups CI and CCI responded to the excitatory conditioning of X in a retarded fashion due to latent inhibition, their responses to the presence of X in compound with a known excitor (BX) would have been similar to the control group (UT). The present results showed that only Group CI responded differently to the UT group. This allows us to conclude that X has acquired inhibitory properties in male subjects after conditioned inhibition training.

With respect to the sex differences found in this study, the performance of females and males was found to be significantly different during training and on the summation test. During training and the retardation test, although not statistically significant, the males also appeared to behave differently to the females. Several studies with adult animals have shown sex differences in specific learning tasks (for a meta-analysis see Jonasson, 2005), males being more prone to displaying advantages on spatial learning and memory tasks (e.g. water maze and radial maze) when compared with females, a finding that has also been observed in classical fear-conditioning procedures, and conditioned taste aversion paradigms. Females, however, tend to be more responsive to the effects of psychomotor stimulant drugs, classical eyeblink conditioning, fear-potentiated startle, and many operant conditioning tasks (Dalla & Shors, 2009; Hawley, Grisson, Barratt, Conrad & Dohanich, 2012; Simpson & Kelly, 2012). With respect to conditioned inhibition, no sex differences have been reported in adults. It is worth noting, however, that the majority of the studies on conditioned inhibition in adults include only data of male subjects, and the generalities of this phenomenon are based primarily on data obtained with males. Traditionally, female adults are not included in learning studies chiefly to avoid behavioral variability related to hormonal changes. Some studies have demonstrated clear differences in the expression of learning as a function of the phase of estrous cycle in adult female rats, for example in latent inhibition (Quinlan, Duncan, Loiselle, Graffe & Brake, 2010). These authors did not find this same result when using 24-day old females, considered to be pre-pubertal. Thus, to our knowledge, there are no studies showing sex differences in learning tasks during the preweanling period of the rat, although the possibility of finding differences increases when approaching the weaning age (beginning of puberty). This could be the case for the present study, in which training finished on PD 21, and retardation and summation tests were run on PD 21-22.

To conclude, the results of central interest to emerge from the present study suggest that conditioned inhibition can be observed in preweanling rats when using a conditioned taste aversion paradigm. However, given the possibility that sex may play a role in the tendency to observe this effect in preweanlings, further research is needed to obtain a more in-depth analysis of this phenomenon across ontogeny. In addition, it would be interesting to test the development of this phenomenon using a range of other learning procedures involving stimulus modalities that mature later in ontogeny.

NOTES

This research was supported by grants from the Spanish “Ministerio de Economía y Competitividad” (PSI2011-24231 and PSI2012-38019), and from the Basque Government (IT-694-13), as well as by a predoctoral contract from the Basque Government to M.G. We would like to thank Dr. Michelle Symonds for her valuable assistance reviewing the manuscript, also to the anonymous reviewers for their careful review and respected recommendations, as well as to SGiker for personal and technical support.

REFERENCES

Cheatle, M. D., & Rudy, J. W. (1979). Ontogeny of second-order odor-aversion conditioning in neonatal rats. Journal of Experimental Psychology: Animal Behavior Processes, 5(2), 142–151.

Chotro, M. G., & Alonso, G. (1999). Effects of stimulus preexposure on the generalization of conditioned taste aversions in infant rats. Developmental Psychobiology, 35(4), 304–317.

Chotro, M. G., & Alonso, G. (2003). Stimulus preexposure reduces generalization of conditioned taste aversions between alcohol and non-alcohol flavors in infant rats. Behavioral Neuroscience, 117(1), 113–122.

Dalla, C., & Shors, T. J. (2009). Sex differences in learning processes of classical and operant conditioning. Physiology and Behavior, 97(2), 229–238. DOI: 10.1016/j.physbeh.2009.02.035.

Dess, N. K., & Soltysik, S. S. (1989). Ontogeny of conditioned inhibition of conditioned respiratory supression in kittens. Developmental Psychobiology, 22(3), 257–269. DOI: 10.1002/dev.420220306.

Dess, N. K., & Soltysik, S. S. (1993). Associative properties of a conditioned inhibitor as a function of age in kittens. Animal Learning & Behavior, 21(2), 138–144. DOI: 10.3758/bf03213393.

Díaz-Cenzano, E., Gaztañaga, M., & Gabriela Chotro, M. (2014). Exposure to ethanol on prenatal days 19–20 increases ethanol intake and palatability in the infant rat: Involvement of kappa and mu opioid receptors. Developmental Psychobiology, 56(6), 1167–1178. DOI: 10.1002/dev.21162.

Hawley, W. R., Grissom, E. M., Barratt, H. E., Conrad, T. S., & Dohanich, G. P. (2012). The effects of biological sex and gonadal hormones on learning strategy in adult rats. Physiology and Behavior, 105(4), 1014–1020. DOI: 10.1016/j.physbeh.2011.11.021.

Heyser, C. J., Chen, W. J., Miller, J., Spear, N. E., & Spear, L. P. (1990). Prenatal cocaine exposure induces deficits in Pavlovian conditioning and sensory preconditioning among infant rat pups. Behavioral Neuroscience, 104(6), 955–963.

Holson, R. R., & Pearce, B. (1992). Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. Neurotoxicology and Teratology, 14(3), 221–228.

Hunt, T., & Amit, Z. (1987). Conditioned taste aversion induced by self-administered drugs: paradox revisited. Neuroscience & Biobehavioral Reviews, 11(1), 107–130.

Jonasson, Z. (2005). Meta-analysis of sex differences in rodent models of learning and memory: A review of behavioral and biological data. Neuroscience & Biobehavioral Reviews, 28(8), 811–825. DOI: 10.1016/j.neubiorev.2004.10.006.

Kucharski, D., & Spear, N. E. (1985). Potentiation and overshadowing in preweanling and adult rats. Journal of Experimental Psychology: Animal Behavior Processes, 11(1), 15–34.

Lariviere, N., Chen, J., & Spear, N. (1990). The influence of olfactory context on Pavlovian conditioning and its expression in preweanling (16-day-old) and adult rats. Animal Learning & Behavior, 18(2), 179–190.

Lubow, R. E. (1973). Latent inhibition. Psychological Bulletin, 79(6), 398–407.

Lubow, R. E., & Moore, A. U. (1959). Latent inhibition: The effect of nonreinforced exposure to the conditioned stimulus. Journal of Comparative and Physiological Psychology, 52, 415–419.

Misanin, J. R., Blatt, L. A., & Hinderliter, C. F. (1985). Age dependency in neophobia: Its influence on taste-aversion learning and the flavor-preexposure effect in rats. Animal Learning & Behavior, 13, 69–76.

Papini, M. R., & Bitterman, M. E. (1993). The two-test strategy in the study of inhibitory conditioning. Journal of Experimental Psychology, 19, 342–352. DOI: 10.1037/0097-7403.19.4.342.

Pavlov I. P. (1927). Conditioned Reflexes: An Investigation of the Physiological Activity of the Cerebral Cortex (G.V. Anrep Ed.). London: Oxford University Press.

Quinlan, M. G., Duncan, A., Loiselle, C., Graffe, N., & Brake, W. G. (2010). Latent inhibition is affected by phase of estrous cycle in female rats. Brain and Cognition, 74, 244–248.

Randall, C. K., Kraemer, P. J., & Bardo, M. T. (1998). Morphine-induced conditioned place preference in preweanling and adult rats. Pharmacology Biochemistry and Behavior, 60(1), 217–222.

Rescorla, R., & Holland, P. (1977). Associations in Pavlovian conditioned inhibition. Learning and Motivation, 8(4), 429–447. DOI: 10.1016/0023-9690(77)90044-3.

Rescorla, R. A. (1969). Pavlovian conditioned inhibition. Psychological Bulletin, 72(2), 77–&. DOI: 10.1037/h0027760.

Revillo, D. A., Gaztañaga, M., Aranda, E., Paglini, M. G., Chotro, M. G., & Arias, C. (2014). Context-dependent latent inhibition in preweanling rats. Developmental Psychobiology, 56(7), 1507–1517. DOI: 10.1002/dev.21236.

Revillo, D. A., Paglini, M. G., & Arias, C. (2014). Spontaneous recovery from extinction in the infant rat. Behavioural Brain Research, 274, 149–157. DOI: 10.1016/j.bbr.2014.08.009.

Savastano, H. I., Cole, R. P., Barnet, R. C., & Miller, R. R. (1999). Reconsidering conditioned inhibition. Learning and Motivation, 30(1), 101–127. DOI: 10.1006/lmot.1998.1020.

Simpson, J., & Kelly, J. P. (2012). An investigation of whether there are sex differences in certain behavioural and neurochemical parameters in the rat. Behavioural Brain Research, 229(1), 289–300. DOI: 10.1016/j.bbr.2011.12.036.

Snyder, K. J., Katovic, N. M., & Spear, L. P. (1998). Longevity of the expression of behavioral sensitization to cocaine in preweanling rats. Pharmacology Biochemistry and Behavior, 60(4), 909–914.

Spear, L. P., Specht, S. M., Kirstein, C. L., & Kuhn, C. M. (1989). Anterior and posterior, but not cheek, intraoral cannulation procedures elevate serum corticosterone levels in neonatal rat pups. Developmental Psychobiology, 22(4), 401–411. DOI: 10.1002/dev.420220407.

Spear N. E., (1994). Memory: Phenomena and principles. In D. C. Riccio, (Ed.), Needham Heights. MA, US: Allyn & Bacon.

Taukulis, H. K., & Revusky, S. H. (1975). Odor as a conditioned inhibitor- applicability of Rescorla-Wagner model to feeding behavior. Learning and Motivation, 6(1), 11–27. DOI: 10.1016/0023-9690(75)90032-6.