Disentangling the Effects of Context Change and Context Familiarity on Latent Inhibition with a Conditioned Taste Aversion Procedure

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ABSTRACT

Contextual specificity of Latent Inhibition (LI) has been demonstrated using an ample range of experimental procedures. Context dependence has not been consistently obtained, however, when LI has been induced using a Conditioned Taste Aversion (CTA) procedure. This paper presents two experiments designed to analyze whether the context plays the same role in LI with a CTA paradigm as compared to other Pavlovian techniques. Experiment 1 compared the effect on LI of a context change between the conditioning and test stages as a function of whether the testing context was new or the animals' home cage. The results of this experiment showed that using the animals' home cage as context at testing enhanced the expression of LI. Experiment 2 manipulated context novelty and familiarity beforehand to introduce different context changes. The results indicate that, as compared to the no context change condition, the strength of LI increased when the conditioning context was different from that of preexposure and testing (ABA). Conversely, a context change from preexposure to conditioning/test stages (ABB) disrupted LI, but only when the animals had been pre-familiarized with the new context introduced at conditioning. These results are similar to those obtained with other conditioning procedures different from CTA.

Keywords: Latent Inhibition, Home cage, Novelty, Context change

When a neutral stimulus is repeatedly presented without consequences it results in one or more of a range of processes, including a progressive decrease of orienting responses (Sokolov, 1963; Turpin, 1983), the gradual reduction of attentional responses elicited by the stimulus (Lubow, 1989), the reduction of stimulus associability (e.g., Mackintosh, 1975; Pearce & Hall, 1980), the development of an association between the context and the stimulus (e.g., Wagner, 1978), and/or an association between the stimulus and the absence of following consequences (e.g., Bouton, 1993; Hall & Rodriguez, 2010). Some of the aforementioned processes, or a combination of them, determines that, when the preexposed stimulus is subsequently presented again and is followed by an Unconditioned Stimulus (US), the resulting Conditioned Response (CR) is weaker as compared to that elicited by a stimulus that has not been preexposed before conditioning. This phenomenon, termed Latent Inhibition (LI), is easily obtained in laboratory conditions but seems to be extraordinarily complex when considering its underlying processes (for a recent review, see Lubow & Weiner, 2010).

One factor modulating the intensity of the LI effect is related to the context present during the different experimental stages (e.g., Hall & Channell, 1985, 1986). More specifically, in experiments with fear conditioning procedures, when stimulus exposure is conducted in the presence of one context but conditioning and testing are conducted in a separate context, LI is disrupted relative to when there is no change of context across stages (e.g., Lovibond, Preston, & Mackintosh, 1984; Westbrook, Jones, Bailey, & Harris, 2000). This LI contextual dependence has been interpreted from different

theoretical perspectives as a result of an external inhibition process induced by context novelty (Lubow, Weiner, & Schnur, 1981; Schmajuk, Lam, and Gray, 1996), as the result of an association established between the preexposure context and the preexposed stimulus (Miller, Kasprow, & Schachtman, 1986; Wagner, 1978, 1981), or as the result of the contextual control over a stimulus-no consequence association established during non-reinforced stimulus presentations at the preexposure stage (Bouton, 1993; Hall & Rodriguez, 2010). Whatever the mechanism underlying the contextual specificity of LI is proven to be, it is reasonable from an adaptive point of view that processing of the preexposed stimulus varies when the stimulus is presented in a different context, since it is possible that a stimulus that is irrelevant in some specific spatial-temporal coordinates becomes relevant when the temporal and/or spatial frame changes.

Although LI context specificity has been repeatedly demonstrated using an ample set of experimental procedures, the results when a Conditioned Taste Aversion (CTA) procedure is used have been seemingly contradictory and they remain difficult to interpret (Lubow, 2009). In order to obtain LI using the CTA procedure, three independent experimental stages are typically programmed: i) Preexposure, which involves repeated presentations of a flavor, usually dissolved in water, for a Preexposed (PE) group, and water access in similar conditions for a Non-preexposed (NPE) group; ii) Conditioning, with all animals being exposed to the flavored solution followed by the induction of gastric malaise that acts as the US; and iii) a Test stage, with all animals having access to the flavored solution, and fluid consumption being considered as an index of conditioning. The usual result after this treatment is an increased consumption

of the flavor-Conditioned Stimulus (CS) for the animals in the PE as compared to those in the NPE group, in spite of a conditioning stage involving similar manipulations for both groups. As noted above, the results of contextual modulation of LI with the CTA procedure have been mixed, with some experiments resulting in LI attenuation after a context change (Hall & Channell, 1986; Quintero, Díaz, Vargas, Schmajuk, Lopez, & De la Casa, 2011), and others showing unchanged, or even increased LI after a context change (Chamizo, 1996; Moron, Manrique, Molero, Ballesteros, Gallo, & Fenton, 2002; Quintero, Diaz, Vargas, Schmajuk, Lopez, & De la Casa, 2011). Table 1 summarizes the available experimental evidence on context-dependence LI using 3-stage procedures. It includes those experiments with CTA procedures published to the date and the results from Westbrook et al. (2000, Exp. 2) with a fear conditioning procedure (freezing), because it is the only systematic attempt to identify the role of different context change combinations on LI intensity. As can be seen in the table, the context combinations are complicated by the use of the animal's home cage as experimental context, and by the novelty or familiarity of the new context introduced at the corresponding stage. An inspection of the results reveals a complex pattern that is difficult to interpret, with similar context changes (as in the ABA condition) either inducing a reduction of LI (Manrique et al., 2004), unchanged LI (Chamizo, 1996; Moron et al., 2002) or even increased LI (Westbrook et al., 2000, Exp. 2).

Table 1 about here

As mentioned, these contradictory results contrasts with the consistent LI context dependence observed with other pavlovian preparations. One difference that could form the basis of the mixed results reported with CTA has been the use of the animals' home cage as the experimental context (e.g., Hall & Channell, 1986; Quintero et al., 2011). It is possible that conducting the different experimental treatments in the presence of home contextual cues favors the introduction of some sources of confusion such as the familiarity of the contextual cues (McLaren, Bennet, Plaisted, Aitken, & Mackintosh, 1994), or a possible safety effect that can occur in the home cage and is related to its previous historical lack of association with aversive experiences (Quintero et al., 2011). A second relevant difference between CTA and other procedures used to reproduce the LI effect is the number of experimental stages involved. Some non-CTA LI procedures use a preexposure stage and a conditioning stage that also serves as a test for conditioning (e.g., McLaren et al., 1994; Weiner, Feldon & Katz, 1987). Obviously, context changes between phases in a 3-stage procedure allow for many more combinations as compared to procedures which include only two stages. Finally, it is possible that differences in experimental parameters such as preexposure number or duration, stimuli type or intensity, etc. were on the basis of the discrepancies between the experimental results described in Table 1.

The present experiments were designed to identify the effects of several context change combinations on LI intensity – keeping constant the remaining experimental parameters- with a 3-stage CTA procedure. More specifically, we focused on the effect on LI intensity of a context change between the

conditioning and test stages (Experiments 1 and 2), and between preexposure and conditioning (Experiment 2). Additionally, we will check for a possible interaction between context change and the presence of new vs. familiar contexts at testing. To this end, context novelty and familiarity was manipulated by using the animals' home cages as one of the experimental contexts in Experiment 1, and two experimental contexts—one familiar and one new, but neither the home cage—in Experiment 2. From previous research using CTA procedures to induce LI, we expected an increase in the LI effect when the test context was the animal's home cage in Experiment 1 (Quintero et al., 2011). As data from CTA using different combinations of new contexts are far from consistent (see Table 1), the results from non-CTA LI experiments (e.g., Hall & Chanell, 1985; Lovibond et al., 1984; Westbrook et al., 2000) led us to predict an attenuation of LI in Experiment 2 only when the context of preexposure was different from the conditioning and test contexts (ABB condition).

Experiment 1

Previous LI experiments with a CTA procedure have analyzed the effect of a context change using the animals' home cages as one of the experimental contexts. These experiments found that preexposing the stimulus at the home cage and conducting the conditioning and test phases in a new experimental context—or a HAA configuration, where H is the home cage and A is a distinctive experimental context— caused LI to either remain unaffected (Best & Meachum, 1986; Hall & Channell, 1986, exp. 1), or be slightly reduced (Quintero et al., 2011, Experiment 2). When preexposure and conditioning are conducted in an experimental context, however, and the test stage in the

animals' home cage—an AAH arrangement—LI was seen to increase (Quintero et al., 2011, Experiment 2). This enhancement of the preexposure effect has also been observed using the same pattern of context change but using new experimental contexts—AAB—with a fear conditioning procedure (Westbrook et al., 2000, Experiment 2).

The main purpose of this experiment was to replicate the enhancement of LI previously reported when preexposure and conditioning are conducted in a new experimental context but testing occurs in the animal's home cages (Quintero et al., 2011, Experiment 2). In their experiment, Quintero et al. programmed three context configurations to analyze the effect of preexposure in a familiar context (HHH, HAA, and AAH), and they observed a reduction of the LI effect in the HAA condition, but an enhanced LI effect in the AAH as compared to the HHH condition. The LI enhancement was attributed to the fact that a familiar and safe context could be triggering mechanisms different from those occurring when testing is conducted in the presence of a novel context. This interpretation can be compromised by the lack of appropriate control conditions for the AAH groups. Thus, for instance, it could be argued that preexposure might have been more effective for the animals in the novel context (AAH condition) as compared to the animals in the familiar context (HAA). Alternatively, it can be considered that there were differences in generalization between the contexts, with effective transfer of learning from the novel context to home cages, but not in the opposite direction. In order to control for these possibilities, proper control groups were included in the present experiment that were intended to evaluate whether the expected increased LI

effect for the AAH as compared to the remaining conditions depends solely on the properties of the home cage.

A second objective of this experiment was to check if the predicted LI enhancement manifests only when the test is conducted at the home cage or if it is also expressed in the presence of a new experimental context. If the context change effect depends exclusively on a change occurring between the conditioning and testing phases, LI should be more intense in the AAH condition as compared to the AAB condition independently of the test context novelty or familiarity. Conversely, if context familiarity is a relevant factor in the effect of context change, as proposed by Quintero et al. (2011), LI increase should be restricted to the group tested in the presence of the familiar context.

Method

Subjects

The subjects were sixty-four adult male Wistar rats with a mean weight of 261 g (range of 252–345 g). The animals were individually housed in plexiglas cages in a temperature-controlled room at 21º C on a 12:12 hr light-dark cycle. Standard feed was continuously available. All procedures were conducted in accordance with the guidelines established by Directive 86/609/CEE of the European Community Council, as well as Spanish R.D. 223/1988.

Apparatus and stimuli.

Three unique contexts, A, B and the animals' home cages—H—were used in this experiment. Contexts A and B were established in rooms other than the vivarium. Specifically, context A was located in a temperature-controlled 3 x 4 m room illuminated by a single 75 W red light. The floor and walls of the 40 x

20 x 19 cm experiment boxes used in context A were made of plexiglas, and the floor was layered with cardboard. The ceiling was an aluminum grating. Context B was located in a different room, measuring 3 x 3 m. A single 54 W fluorescent white light illuminated the room, and the temperature was again held at 21ºC. The experimental boxes used in context B were similar to those used in context A, except that the floor in context B boxes was covered with a green plastic grating. Contexts A and B were counterbalanced between groups. The home cages, measuring 35 x 20 x 14 cm, were located in the 2.5 x 3.5 m colony room, which was illuminated by four 100- W bulbs. The floor and walls of these cages were made of plexiglas, with wood shavings as bedding.

As context includes the time of day, and LI is sensitive to changes in this variable (e.g., Manrique, Molero, Ballesteros, Moron, Gallo, and Fenton, 2004), all experimental sessions were conducted starting at the same time each day (10:00 AM) to avoid any effect of temporal factors on LI.

The solutions were presented in 150 ml graduated glass bottles with fitted stainless steel spouts. The bottles were attached to the front of each cage during liquid presentation. The amount of fluid consumed was calculated as the difference between bottle weight before and after consumption. The taste used as the CS was a 0.04% saccharin solution. The US was an intraperitoneal (IP) injection of LiCl (0.2-M, 0.5% of body weight).

Procedure.

A summary of the procedure is provided in Table 2. After seven days on a 23.5 hr water deprivation schedule, which was maintained for the duration of the experiment, the animals were matched for body weight and assigned to 8

groups, each with 8 subjects. Each animal was handled for 2 min four days prior to beginning the preexposure stage. After each experimental session, the animals had an additional period of 25 min to access water in order to complete the daily required period of 30 min water access.

Table 2 about here

The procedure consisted of the following stages:

Preexposure. Over a four day period, the animals were given 5 min of access to either the saccharine solution (PE condition) or to water (NPE condition) each day. Each session began with introducing the animals into the corresponding context (H, A or B, the last two being counterbalanced) 10 min prior to fluid consumption, in order to allow the animals to become habituated to the experiment context. The animals were then allowed to drink the corresponding solution—saccharine or water—for a period of 5 min, after which the bottles were removed. The animals remained in the context for 5 min with no additional stimulation.

Conditioning. This stage lasted one day and was conducted on the day following the last preexposure trial. The procedure was similar to that described for preexposure, albeit with two differences: all animals consumed the saccharine solution and, after fluid consumption, each animal received the IP injection. For all groups, the conditioning context was the same as that experienced during preexposure.

Test. This stage comprised a single trial scheduled to occur on the day following the conditioning stage. The test stage lasted 5 min and the process

was similar to that described for preexposure. For HHH and AAA groups, testing was conducted in the same context as had been used for preexposure and conditioning. For Group AAB, testing took place in an experimental context different from that of the preexposure and conditioning stages. Animals in the AAH group experienced the testing stage at their home cages.

Results

A 4 x 8 mixed ANOVA (Trials x Groups) conducted on the mean amount of saccharine consumed across preexposure trials revealed a significant main effect of Trials, $F(3,156)= 16.71$, $p<0.001$, that reflects the progressive increase in consumption across preexposure trials as a result of neophobia habituation to the saccharine solution. Neither the main effect of Groups nor the two-way interaction was significant (both ps>0.18).

A one-way ANOVA with Group as main factor conducted on mean consumption during the conditioning stage revealed significant differences, $F(7,63)=6.18$; $p<0.001$. Post-hoc comparisons between groups (Tukey's HSD tests, $p<0.05$) revealed lower rates of saccharine consumption in those groups that underwent conditioning in a new context as compared to those in the home cages (Mean = 7.95 ml., SD = 1.87, and Mean = 10.59 ml., SD = 1.93, respectively).

Figure 1 depicts mean saccharine consumption at test trial as a function of Preexposure (NPE vs. PE) and Context (Home vs. Experimental) for those groups without context change (Section A), and those with a different context at testing (Section B). As can be seen in the figure, the LI effect (as measured for the difference between NPE and PE intake) was evident for all comparisons,

irrespective of the context condition. In addition, context change resulted in a stronger LI effect when testing was conducted in the animals' home cages than in the presence of a new experimental context.

Figure 1 about here

An one-way ANCOVA with Groups as the main factor conducted on consumption at testing, with intake at conditioning as a covariate, revealed significant differences between groups, $F(7,55)=10.68$; $p<0.001$. *A priori* comparisons between groups based on our hypotheses (t-tests, $p<.05$, one-tailed) revealed significant LI effects for all conditions when comparing PE vs. the corresponding NPE groups. Therefore, a context change between conditioning and testing, irrespective of whether the test context was a new experimental context or the animals' home cages, did not disrupt LI. The difference between PE/HHH and PE/AAH groups was non significant. Therefore, the enhancement of the LI effect reported by Quintero et al. (2011, Exp. 2) was not replicated.

In order to test our specific hypothesis on the increased LI when the context change at testing involves the animals' home cages as compared to a new experimental context, two independent 2 x 2 ANCOVAs (Test context: Home vs. Experimental x Preexposure: PE vs. NPE), including consumption at conditioning as a covariate, were conducted on No context change (Section A, Figure 1) and Context change groups (Section B, Figure 1). The ANCOVA for those groups that maintained the same context across the different stages revealed a significant main effect of Preexposure, $F(1,27)=22.16$; $p<0.001$, due

to the overall LI effect. Neither the main effect of Test context nor the 2-way interaction was significant (both ps>.25). The ANCOVA for those groups with a context change at testing revealed a significant main effect of Preexposure, $F(1,27)=52.03$; $p<0.001$, indicating that the context change was not effective in disrupting LI, and a significant Test context x Preexposure interaction, $F(1,27)=4.38$; $p<0.05$. The main effect of Test context was non-significant, $F(1,27)=2.59$; $p<0.11$. As can be seen in Figure 1, Section B, the source of the 2-way interaction was an increase in the LI effect in the AAH as compared to the AAB condition. Comparison between groups (t-tests, one tailed, $p<.05$) revealed that the increased LI effect comes from higher consumption in the AAH/PE as compared to the AAB/PE group. This difference indicates that testing LI at the home cage facilitated the expression of learning acquired at preexposure (Quintero et al. 2011). There were no significant differences between the AAH/NPE and the AAB/NPE groups.

Experiment 2

Experiment 1 revealed a significant LI effect with a CTA procedure when the test stage was conducted in a context different from that present at the preexposure and conditioning stages, a result which replicates previous observations with CTA (Quintero et al., 2011, Exp. 1) and fear conditioning (Westbrook et al., 2000, Exp. 2). A possible explanation for this result is the lack of information that the context introduced at testing offers to disambiguate the meaning of the preexposed and conditioned CS in the AAB condition (Bouton, 1993, 1994). On the other hand, we found an increase in the expression of LI that was independent of the context change when testing occurred at the

animals' home cages. Such increase was not observed when testing was conducted in an experimental context different from the home cage.

Whether the effect of testing LI in the home cages depends on previous context familiarization or any other mechanisms triggered by context safety remains unclear. To address this question, and to evaluate possible differential effects of context novelty vs familiarity on LI context dependence, Experiment 2 involves a more detailed study of LI modulation by including all possible combinations of between-stages context change—AAA, ABB, AAB, and ABA. All contexts in this experiment were different from the animals' home cages, but they were novel for half of the animals and previously familiarized for the second half. All subjects used in this experiment were preexposed to the to-be-conditioned flavor for two reasons: 1) Experiment 1 identified differences only between the preexposed groups; and, 2) our hypotheses center on the changes that can be induced by contextual manipulations after preexposure to the to-be-conditioned stimulus. If we were thus to assume that a familiar context will lead the subject to act in the same way as they would in their home cages, we would expect increased LI for the AAB Group, but only when the animals have been previously familiarized with the testing context. Conversely, we would expect a disruption of LI expression for ABB groups, when both conditioning and testing are conducted in a new but familiar context (e.g., Hall & Channell, 1986), and when the respective stages are conducted in a new and unfamiliar context (Quintero et al., 2011). The predicted outcomes are less clear for the ABA group, as previous results using the CTA procedure with such context manipulation revealed a significant LI effect (Chamizo, 1996; Moron et al., 2002), but those using a fear conditioning procedure showed the LI effect to be

enhanced (Westbrook et al., 2000). Finally, a control group was introduced in Experiment 2, one which did not experience context changes—AAA—in order to obtain an index of regular LI.

Method

Subjects

Fifty-six male Wistar rats with a mean weight of 315 g and a range of 268–435 g were used in this experiment. The animals were individually housed in plastic cages in a temperature-controlled room kept at 21ºC on a 12:12 hr light-dark cycle. Standard food was continuously available. As in Experiment 1, all procedures were conducted in accordance with the guidelines established by Directive 86/609/CEE of the European Community Council, and with Spain's R.D. 223/1988.

Apparatus and stimuli

Two sets of 8 boxes each were used. The boxes of context A were made of transparent plexiglas and measured 18 x 24 x 43 cm. The floors were formed by parallel steel bars, measuring 0.4 mm in diameter and spaced 1.4 cm from center to center. These boxes were located in a room illuminated by four 100 W fluorescent white bulbs. A 100 dB, 5000 Hz PC-generated white noise was continuously present during all experimental manipulations conducted in this context. Context B consisted of 8 circular boxes measuring 30 cm high x 30 cm in diameter and made of black plastic. The floor of these boxes was identical to those of the context A boxes, with parallel, 0.4 mm diameter steel bars spaced 1.4 cm from center to center. The lights were kept off in context B and there

were no sounds introduced. Contexts A and B were counterbalanced between groups.

The solutions were presented in 150 ml graduated glass bottles with fitted stainless steel spouts. The bottles were attached to the front of each cage during liquid presentation. The amount of fluid consumed was calculated as the difference between bottle weight before and after consumption. As in Experiment 1, the taste used as the CS was a 0.04% saccharin solution and the US was an i.p. injection of LiCl (0.2-M, 0.5% of body weight).

Procedure.

A summary of the procedure can be seen in Table 2. After seven days on a 23.5 hr water deprivation schedule, which was maintained for the entire duration of the experiment, the animals were matched for body weight and assigned to 7 groups, each with 8 subjects. As described for Experiment 1, each animal was handled for 2 min four days prior to beginning the preexposure stage. The rats had an additional period of 25 min access to water after each experimental session.

Table 2 about here

As indicated for Experiment 1, all experimental sessions started at the same time each day (10:00 AM) to avoid any effect of temporal factors on LI.

The procedure consisted of the following stages:

Preexposure. This stage lasted for 8 days. On even days, all animals were preexposed to the to-be-conditioned flavor in the corresponding context.

On odd days, those rats in the familiar condition were allow to drink water in the alternative context introduced at time of context change, while the animals in the new and the AAA conditions remained in their home cages. Each trial began with a 10 min period spent in the corresponding context, during which time there was no programmed activity to allow the rats to adapt to the new context. Afterward, the rats, still in the corresponding context, received 5 min of access to either water or to the flavor. After this 5 min period, the bottles were removed, and the rats remained in the context an additional 5 min before being returned to their home cages.

Conditioning. The conditioning stage was conducted on day 9, and comprised a single trial. Those animals in groups AAA and AAB underwent conditioning in the same context as that in which they had experienced preexposure, while the animals in groups ABB and ABA underwent conditioning in the alternative context. As in the preexposure stage, the conditioning stage began with a 10 min acclimation period in the experimental boxes. Following that, the saccharin solution was made available for a 5 min period. The rats were then i.p. injected with the LiCl, after which they remained in the experimental context for an additional 5 min before being returned to the home cage.

Test. The test stage lasted one day, beginning 24 hours after the conclusion of the conditioning stage. During the test trial, all animals had access to the saccharin solution for 5 min. In the AAA and ABA groups, the test stage occurred in the same context as the preexposure stage; in the ABB and AAB groups, the test stage occurred in a context different from that of the preexposure stage. The sequence of events in the test stage was similar to that

described for the previous stages: 10 min of adaptation, 5 min of flavor presentation, and 5 min spent in the experimental context before being returned to the home cage.

Results

A 4 x 7 mixed ANOVA (Trials x Groups) conducted on the mean amount of saccharine consumed across preexposure trials revealed a significant main effect of Trials, $F(3,129)= 58.01$, $p<0.001$, reflecting the progressive increase in consumption across preexposure trials as a result of neophobia habituation to the saccharine solution. Neither the primary effect of Groups nor the two-way interaction were significant (both ps>0.09).

A one-way ANOVA with Group as the main factor conducted on mean consumption during the conditioning stage revealed significant differences, $F(6,49)=4.01$; $p<0.01$. Post-hoc comparisons between groups (Tukey's HSD tests, $p<0.05$) revealed lower rates of saccharine consumption in those groups which underwent conditioning in a new context (ABA/new and ABB/new).

Figure 2 depicts mean saccharine consumption during the test trial as a function of Groups. A one-way ANCOVA with Groups as the main factor conducted on these data, with consumption at conditioning as a covariate, revealed significant differences among groups, $F(6,48)=5.65$; $p<0.01$. A priori comparisons between groups based on our hypotheses (t-tests, two-tailed) revealed significant differences between AAA vs. ABA/fam and AAA vs. ABA/new, $t(14)=2.17$; $p<.05$, and $t(14)=3.72$; $p<.01$, respectively, due to an increase in the LI effect which was detectable in both ABA groups. The difference between AAA and ABB/fam was also significant, $t(14)=3.22$; $p<.01$,

reflecting a reduction of LI in the ABB/fam group. Conversely, the difference between AAA and ABB/new was non-significant, $t(14)=1.01$; $p>.30$, revealing that LI reduction after context change was restricted to the condition in which the context at the conditioning and testing stages was different but familiar from that used during preexposure. No other relevant comparisons were seen to be significant.

Figure 2 about here

General Discussion

This research was aimed at analyzing contextual modulation of LI with a CTA procedure because, in contrast to the internally consistent data obtained using other conditioning procedures, the available data in this field were rather inconsistent (for a review, see Lubow, 2009). The experimental results can be summarized in two main findings: 1) The use of the animal´s home cage as an experimental context at testing reduced the expression of the conditioned response for the preexposed groups (Experiment 1). More specifically, a context change at testing resulted in higher consumption (i.e., less conditioning) when the test was conducted in the home cage as compared to a new experimental cage. 2) CTA-LI is context-dependent in the same way as observed with other non-CTA procedures (e.g., Westbrook et al., 2000), but only when the experimental stages were conducted in familiar contexts that were different from the animals' home cages. Thus, a context change at time of testing (AAB condition) did not affect LI intensity (Experiments 1 and 2),

changing context at conditioning (ABA condition) resulted in an enhanced LI effect (Experiment 2), and a context change between preexposure and conditioning/test (ABB condition) disrupted LI when the new context introduced at conditioning was already familiar for the animal (Experiment 2).

Regarding the results of Experiment 1, the enhancement of LI when testing was conducted in the animals' home cages indicates that the home cage has some peculiarities that favor the recovery of the memory of the flavor as a safe stimulus established during stimulus exposure without relevant consequences (Bermudez-Rattoni, 2004; Quintero et al., 2011). The results from groups in the AAB condition from Experiment 2 argue against the possibility that the differences detected in Experiment 1 were due only to the familiarity of the home cage; the use of two experimental contexts, both different from the home cage, but one being new at the start of the context change, and the second having been previously familiarized, revealed that, irrespective of the context novelty or familiarity, a change undertaken between the conditioning and test stages did not affect LI intensity. Specifically, the significant LI observed in the AAB/fam group contrasts with the increased LI observed when testing was conducted at home cages in Experiment 1—the AAH group. This result indirectly supports the idea that the home cage has some additional properties beyond familiarity that favor the recovery of the CS-no consequences association established through preexposure.

As we mentioned above, Experiment 2 revealed that changing context at the conditioning stage while maintaining constant preexposure and test contexts—the ABA condition—results in an increased expression of the LI effect that was independent of the novelty or familiarity of the contexts (for a

similar result with a freezing procedure, see Westbrook et al., 2000). Additionally, the results of those groups that underwent a context change after preexposure and before conditioning and test stages—the ABB condition—were critical in demonstrating that such a change disrupts LI when using a CTA procedure. Contrary to our expectations, however, the context change was only effective in interrupting LI when the conditioning and testing context was familiar, but not when it was new at the time of conditioning. This result was consistent with the results of previous, similar experiments with CTA (Hall & Channell, 1986; Kurz & Levitski, 1986), and with other aversive and appetitive preparations (e.g., McLaren et al., 1994), but contrasted with the reduction of LI reported by Quintero et al. (2011, Experiment 1) when using an unfamiliar context. A detailed inspection of the contexts employed in Quintero et al. reveals that the experimental boxes they used to create different contexts were similar except for the material covering the floor—cardboard vs. green plastic grating—and the illumination of the experimental room. The fact that the boxes were similar in size and materials, however, could have resulted in, or contributed to, a generalization process which rendered the new context introduced at the conditioning and testing stages functionally familiar to the rat.

A possible factor that could be affecting the results observed at testing is the difference in consumption registered at conditioning, both for Experiments 1 and 2, with those animals receiving conditioning at home in familiar cages drinking significantly more than those conditioned in the unfamiliar cages. More specifically, higher rates of consumption during the conditioning trial could be increasing conditioned taste aversion as compared to lower rates. From this perspective, the differences in consumption at conditioning would have resulted

in an increased conditioned response for those groups tested at home in familiar cages. Considering that our prediction regarding the effect of context familiarity on LI expression was the opposite, namely reduction in the expression of conditioned aversion, we can conclude that the possible differences in conditioning, if any, would have contributed to minimizing the size of the predicted effect at testing.

The modulation of CTA-LI by the different context combinations observed in our experiments can be interpreted in light of those theories which consider LI to be the result of two competing associations at the time of testing: a CS-no consequences association established at preexposure, and a CS-US association formed during conditioning (e.g., Bouton, 1993; Hall & Rodriguez, 2010). More specifically, when the animals are confronted with conflicting information at testing—due to the presence of the CS, which predicts both no consequences and the aversive US—they use the context to disambiguate the meaning of the stimulus (Bouton, 1993, 1994). When the context is the same for all stages, a primacy effect is therefore seen to determine the CR reduction which characterizes LI (Bouton, 1993; Lubow & De la Casa, 2005). In the AAB condition, LI remains unchanged because the context at testing does not favor the recovery of any of the learned associations, so the recovery of the first-learned association prevails. When the context of preexposure and testing is the same, but different from that of the conditioning stage—the ABA condition—the CS-no consequence association is recovered, thereby resulting in a stronger LI effect. Conversely, when the context for conditioning and testing are identical but the context of preexposure is different—the ABB condition—the CS-US association is recovered, thereby inducing a strong CR during the

testing stage. This LI reduction, however, was only apparent in Experiment 2 when both the conditioning and testing contexts were familiar to the animals (e.g., Hall & Channel, 1986).

This difference observed between the ABB/new and the ABB/fam is better predicted by a traditional associative view, by considering that the context and the flavor would have competed to gain associative strength with the US during conditioning (e.g., Rescorla & Wagner, 1972). Considering that context exposure meant to familiarize the animal with the context before the conditioning stage can retard the association of the context with an US (e.g., Hall & Symonds, 2006; Hall, Symonds, & Rodriguez, 2009), it is possible that the flavor presented in the familiar context will have gained more associative strength than the flavor presented in the new context, resulting in a stronger CR to the flavor—that is, in a weaker LI effect—in the former than in the latter.

Finally, the enhanced LI observed in Experiment 1 when the animals' home cages were used as the experimental context during testing (see also Quintero et al., 2011, Experiment 1) requires a specific analysis, because it did not fit well with any of the above mentioned theories. Although admittedly speculative, we are tempted to apply the idea of learned safety developed by Rozin and Kalat (1971) to the reported context effects, to explain the effect of long delays on taste aversion learning. When the animal encounters a new context, it produces a set of responses (e.g., Timberlake, 2001) which in turn allow it to determine whether such a context is safe or potentially dangerous. The more time the animal is given to explore the context without experiencing aversive consequences, the higher the safety value the animal will attach to the context. From this perspective, context familiarization would result in safety

learning, just as Rozin and Kalat (1971) propose that a flavor gains safety value when it is exposed without consequences. The maximum expression of context safety would appear in the animals´ home cages, because the long exposure without aversive consequences (or even with the appetitive consequences derived from the constant temperature, the presence of food and water, the absence of predators, etc.) reaches maximum expression. Thus, the extensive familiarization will turn the home cage into a safe context that will induce an increase in the consumption of any flavor presented in this context, regardless of the previous associative history of the flavor. De la Casa and Diaz (2012) provide evidence of this possibility by demonstrating that both neophobia habituation and flavor consumption after an episode of CTA were seen to increase when tests for consumption were conducted at home cages as compared to new or familiar experimental contexts.

Conclusions

It can be therefore concluded that LI with CTA is modulated by a context change, but in a fairly complex manner: i) LI is disrupted by a context change only when conditioning and testing are conducted in a different but familiar context to that presented at preexposure; ii) LI remains intact when conditioning and testing are conducted in a different and new context in relation to the preexposure context; iii) LI is enhanced when conditioning is conducted in a context different to that of preexposure and testing; iv) LI remains unchanged when preexposure and conditioning are conducted in a context different from that of testing; and v) LI increases when testing is conducted in the animals' home cage.

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Table 1: Effects on LI of contextual changes in 3-stage procedure experiments. CTA: Conditioned Taste Averion. A and B: Experimental contexts; H: Home cage

Preexposure/Condi-tioning/Test context	**Context B novelty/familiarity**	**Experiments**	**Procedure**	**Result**
ABB	Novel	Quintero et al., 2011	CTA	Reduced LI
	Familiar	Westbrook et al, 2000 (Exp. 2)	Freezing	Reduced LI
	Familiar	Hall & Channell, 1986 (Exp. 3)	CTA	Reduced LI
	Novel	Kurz and Levitsky (1982)	CTA	LI
HBB	Novel	Quintero et al., 2011	CTA	Reduced LI
	Novel	Hall & Channell, 1986 (Exp. 2)	CTA	LI
	Novel and Familiar	Best and Meachum (1986)	CTA	LI
ABA	Familiar	Westbrook et al., 2000 (Exp. 2)	Freezing	Enhanced LI
	Familiar	Chamizo, 1996	CTA	LI
	Familiar	Manrique et al. (2004)	CTA (time)	Reduced LI
	Novel	Moron et al. (2002)	CTA	LI
AAB	Novel	Quintero et al., 2011	CTA	LI
	Familiar	Westbrook et al, 2000 (Exp. 2)	Freezing	Enhanced LI
BBH	Novel	Quintero et al., 2011	CTA	Enhanced LI

Table 1. Summary of design for Experiment 1. PE: Preexposed; NPE: Non-Preexposed; W: Water; Sac: Saccharine. A, B, and H (Home) refer to three separate contexts (A and B were counterbalanced, see text for details).

Group	Preexposure (4 trials)	Conditioning (1 trial)	Test (1 trial)
AAA/NPE	A: W	A: Sac-LiCl	A: Sac
AAA/PE	A: Sac	A: Sac-LiCl	A: Sac
AAB/NPE	A: W	A: Sac-LiCl	B: Sac
AAB/PE	A: Sac	A: Sac-LiCl	B: Sac
AAH/NPE	A: W	A: Sac-LiCl	Home: Sac
AAH/PE	A: Sac	A: Sac-LiCl	Home: Sac
HHH/NPE	Home: W	Home: Sac-LiCl	Home: Sac
HHH/PE	Home: Sac	Home: Sac-LiCl	Home: Sac

Table 2. Summary of design for Experiment 2. All animals were preexposed to saccharine during preexposure. W: Water; Sac: Saccharine. H: Home cage; A and B refer to two different, counterbalanced contexts (see text for details).

Group	Preexposure (even days - 4 trials)	Familiarization (odd days- 4 trials)	Conditioning (1 trial)	Test (1 trial)
AAA	A: Sac	H: W	A: Sac-LiCl	A: Sac
ABB/New	A: Sac	H: W	B: Sac-LiCl	B: Sac
ABB/Fam	A: Sac	B: W	B: Sac-LiCl	B: Sac
ABA/New	A: Sac	H: W	B: Sac-LiCl	A: Sac
ABA/Fam	A: Sac	B: W	B: Sac-LiCl	A: Sac
AAB/New	A: Sac	H: W	A: Sac-LiCl	B: Sac
AAB/Fam	A: Sac	B: W	A: Sac-LiCl	B: Sac

Figure captions

Figure 1: Mean saccharine consumption at test trial as a function of Preexposure (NPE vs. PE) and Context (Home vs. Experimental). Section A depicts those groups without context change, and Section B those groups with a context change at testing. Error bars represent SEMs.

Figure 2. Mean saccharine consumption at test trial as a function of context familiarity (new vs. familiar) and context change (the first letter is for preexposure context, the second for conditioning context, the third for test context). Error bars represent SEMs.

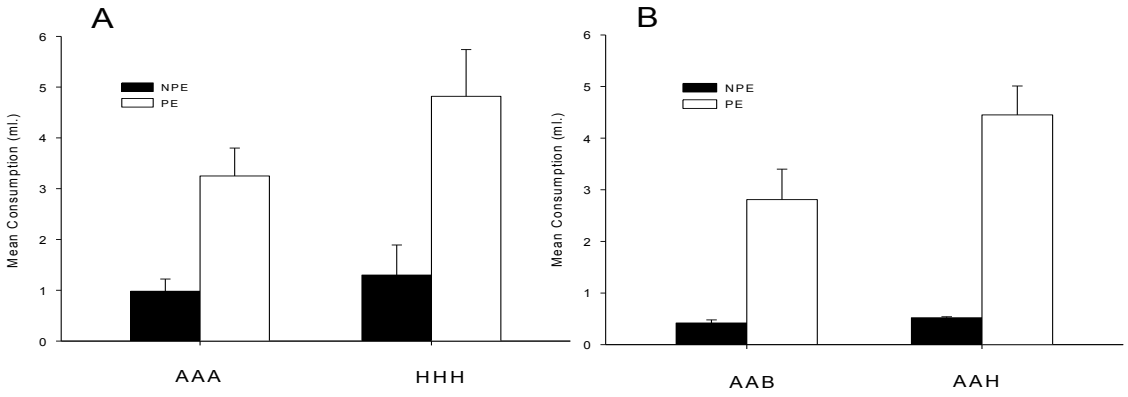

Figure 1.

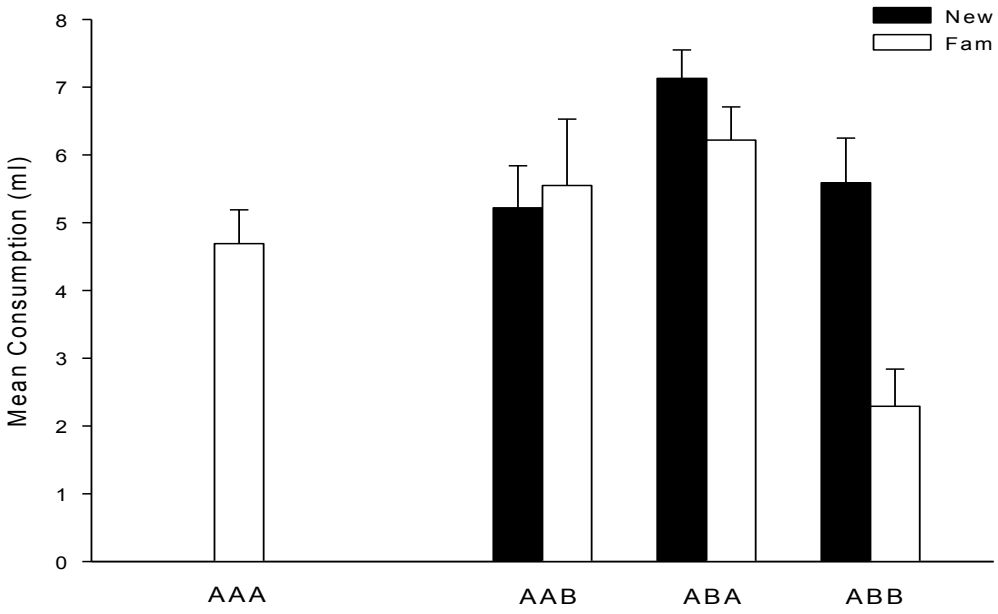

Figure 2.