RESEARCH PAPER

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Temporal specificity of latent inhibition in rats with daily water restriction prior to taste conditioning

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Temporal specificity of latent inhibition of conditioned taste aversion (CTA) has been demonstrated after prolonged habituation to temporal contexts in the stages preceding conditioning, and it has been eliminated by restricting consumption during conditioning. However, it is not known if latent inhibition of CTA is still dependent on the temporal context when fluid consumption is limited in the stages prior to conditioning. We tested temporal specificity of latent inhibition in rats with (different time of day for the conditioning stage) and without (same time of day for pre-exposure and conditioning stages) temporal changes on the conditioning day. All animals had limited access to water in the morning sessions of the stages prior to the conditioning day and 15 min of free access to fluid in the evening sessions of these stages. Compared to animals without temporal changes between stages, animals with a different temporal context during conditioning did not show evidence of latent inhibition. Unlike the effects observed after taste stimulus restrictions during conditioning, these results suggest that the temporal specificity of latent inhibition of CTA is not abolished when access to water is limited in the stages preceding conditioning.

Key words: rats, taste aversion, taste stimulus restriction, temporal context, time of day

INTRODUCTION

The formation of latent inhibition (LI), that is a reduced conditioned response due to pre-exposures to the future conditioned stimulus (CS) before conditioning, is sensitive to certain contextual changes between different stages in the procedure (Hall and Channell, 1986; Escobar et al., 2002). Particularly, LI of taste aversion learning (CTA) can be modulated by contextual changes to the external or physical environment (De la Casa and Lubow, 1995; 2001; Lukoyanov et al., 2002; Quintero et al., 2011; 2014; Gonzalez et al., 2015) and the internal environment, induced by the time of day (Manrique et al., 2004; Molero et al., 2008; Molero-Chamizo, 2013).

In rats, it was shown that introducing contextual changes in the time of day between the pre-exposure and conditioning stages reduced the magnitude of LI in the CTA paradigm (Manrique et al., 2004). Nevertheless, the key elements of the procedure that induce the temporal specificity of LI of CTA have not been fully established. The period of habituation to different temporal contexts of drinking (morning *vs.* evening) in the stages preceding conditioning appears to be an important factor for properly detecting temporal specificity of LI. Thus, long periods of habituation to temporal contexts (more than two days) prior to conditioning facilitate the temporal specificity of LI of CTA (Molero-Chamizo and Rivera-Urbina, 2017). The characteristics of the differing CS used in the paradigm are an additional relevant factor. Saline, as well as saccharin, sucrose and other sweet flavors are frequently used as a CS in taste aversion protocols (Bures et al., 1991; Rodríguez and Alonso, 2002; Flores et al., 2016). Although CTA (Nowlis et al., 1980; Morón et al., 2002; Lubow, 2009) and LI of CTA (De la Casa and Lubow, 1995; Rodríguez and Alonso, 2002; Lubow, 2009) have been demonstrated using various types of stimuli, neural processing and homeostatic regulation

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of water and nutritional balance can differ depending on the stimulus used (Yamamoto and Yuyama, 1987; Mark et al., 1991; Yamamoto, 1993; Yamamoto et al., 1994; Heyer et al., 2004; Smith et al., 2004). Temporal specificity of LI of CTA has been demonstrated using saline as a CS (Manrique et al., 2004; Molero et al., 2008; Molero-Chamizo, 2013; 2017; 2018). Therefore, the inclusion of different taste stimuli as a CS might be another important procedural element for inducing temporal specificity. Restriction of the amount of different fluids consumed by animals throughout the procedure could also be a key variable in the modulation of LI induced by changes in the time of day. Relating to consumption of the future CS, the influence of restriction on consumption of the taste stimulus might even differ depending on the procedural stage in which it is applied. Particularly, the elimination of temporal specificity of LI of CTA observed after limited access to the taste stimulus during conditioning (Molero-Chamizo, 2017) could be reversed if the restriction on consumption is applied only during the stages prior to conditioning. This assumption may be based on previous findings that showed LI is a function of total exposure and consumption of the future CS (De la Casa and Lubow 1995; 2001; Lubow and De La Casa, 2005; Lubow, 2009, Lubow and Weiner, 2010). Regarding the amount of water or other non-conditioned fluids consumed during the procedure, the effect of restriction on consumption also needs to be explored to fully understand the relevance of the stimulus restriction in different stages. Since limited consumption of the taste stimulus on the conditioning day blocks the temporal specificity of the LI of CTA (Molero-Chamizo, 2017), in the present study we explored in Wistar rats whether selective restrictions on water consumption during the stages prior to conditioning also eliminate this phenomenon. Conversely, a temporal specificity of LI would be evident if these selective restrictions do not influence the temporal dependency of this learning.

METHODS

Subjects

Forty adult male Wistar rats, weighing between 285-300 g, were individually housed in boxes measuring 30 cm × 15 cm × 30 cm. All the animals were exposed to a daily 12 h light/dark cycle (lights on from 9:00 to 21:00), and the temperature was kept constant at 23°C. Throughout the procedure, food was provided ad libitum and the fluid (water or saline, depending on the group and the specific stage of the procedure, as described below) was provided in two daily 15 min sessions, one in the morning (10:00) and one in the evening (20:00), to all animals. The procedure was approved by the Ethics Committee for Animal Research of the University of Granada and was conducted in accordance with both the NIH Publications (Nº 8023) of the National Institute of Health Guide (United States) for the Care and Use of Laboratory Animals (2015 revision, Office of Laboratory Animal Welfare, Health Research Extension Act of 1985, Public Law 99-158, November 20, 1985, "Animals in Research") and the European Community Council Directive 2010/63/EU. The National Legislation, in agreement with this Directive, is defined in R.D. 53/02013, Law 32/2007.

Procedure

Rats were randomly distributed among the following four groups (10 per group, for an optimal sample size): PE-D (pre-exposed to the taste – PE, and different time of day for conditioning and testing – D; *n*=10), PE-S (pre-exposed to the taste – PE, and the same time of day for conditioning and testing – S; *n*=10), NPE-D (non-pre-exposed to the taste – NPE, and different time of day for conditioning and testing – D; *n*=10), NPE-S (non-pre-exposed to the taste – NPE, and the same time of day for conditioning and testing – S; *n*=10).

The entire procedure (baseline, pre-exposure, conditioning and testing) was conducted in the same room for all groups. All animals received two 15 min sessions of access to water per day (at 10:00 and 20:00) over 3 consecutive baseline days to facilitate the differentiation of the temporal contexts (morning *vs.* evening), as previously described (Molero-Chamizo 2017; Molero-Chamizo and Rivera-Urbina, 2017). The water consumption during the 15 min of the morning session was restricted to 8 ml for all groups. During the 15 min of exposure to water, the consumption of the animals was concentrated in the first minutes in both sessions, morning and evening, which indicated that their hydration was maintained in a reasonably acceptable status during this deprivation procedure. After this period, the procedure had three main stages (pre-exposure, conditioning and testing). All of the rats had access to water in the morning session (15 min, with a restriction of 8 ml) for two days during the pre-exposure stage. The non-pre-exposed groups (NPE-D and NPE-S) also received water during the evening session (15 min) of these two days, whereas the pre-exposed groups (PE-D and PE-S) were exposed to a sodium chloride solution dissolved in water (saline 1%) for 15 min, as a novel taste (Falk and Titlebaum, 1963). The difference in this study, with respect to previous designs, was that in the

morning sessions of the baseline and the pre-exposure stage, all animals had limited access to water during the drinking period (as mentioned above), to explore whether this limitation in the stages prior to conditioning affects the phenomenon of temporal specificity as it occurs when a taste restriction is introduced on the conditioning day (Molero-Chamizo, 2017). The amount of restricted water in the present study was 8 ml. Restriction was determined relative to the highest volumes of water typically consumed during a 15 min drinking session (Molero-Chamizo, 2017). On the day following the last pre-exposure, conditioning was conducted in the morning session for the PE-D and NPE-D groups. These groups were exposed to saline 1% (which served as the CS) for 15 min during the morning session and the ingested amounts were recorded. Twenty minutes later, the animals from these groups received an injection of lithium chloride (LiCl 0.15 M, 2% of body weight, intraperitoneally, which served as the unconditioned stimulus, US). Water was available for 15 min during the evening session. In the PE-S and NPE-S groups, the procedure was the same except that the conditioning was conducted in the evening session and water was available for 15 min during the morning session. After a day of recovery with water (15 min) during the morning and evening sessions, the animals' response to the CS was recorded in the evening session for the following five days (testing stage). Water was available for 15 min in the morning sessions of the testing stage. We included five testing days to ensure the extinction of the taste aversion and considered a consumption greater than 30% of the amounts recorded on the conditioning day as indicative of extinction. Thus, once aversion was extinguished in all the groups under this criterion, a renewal test was conducted on the sixth day to analyze for temporal specificity of the extinguished aversion. In this renewal test, the response to the CS was analyzed during the opposite session to that of the testing days (i.e., the morning session). The water and saline were administered throughout the procedure by calibrated burettes to facilitate recording the ingested amounts. Fig. 1 represents the behavioral procedure.

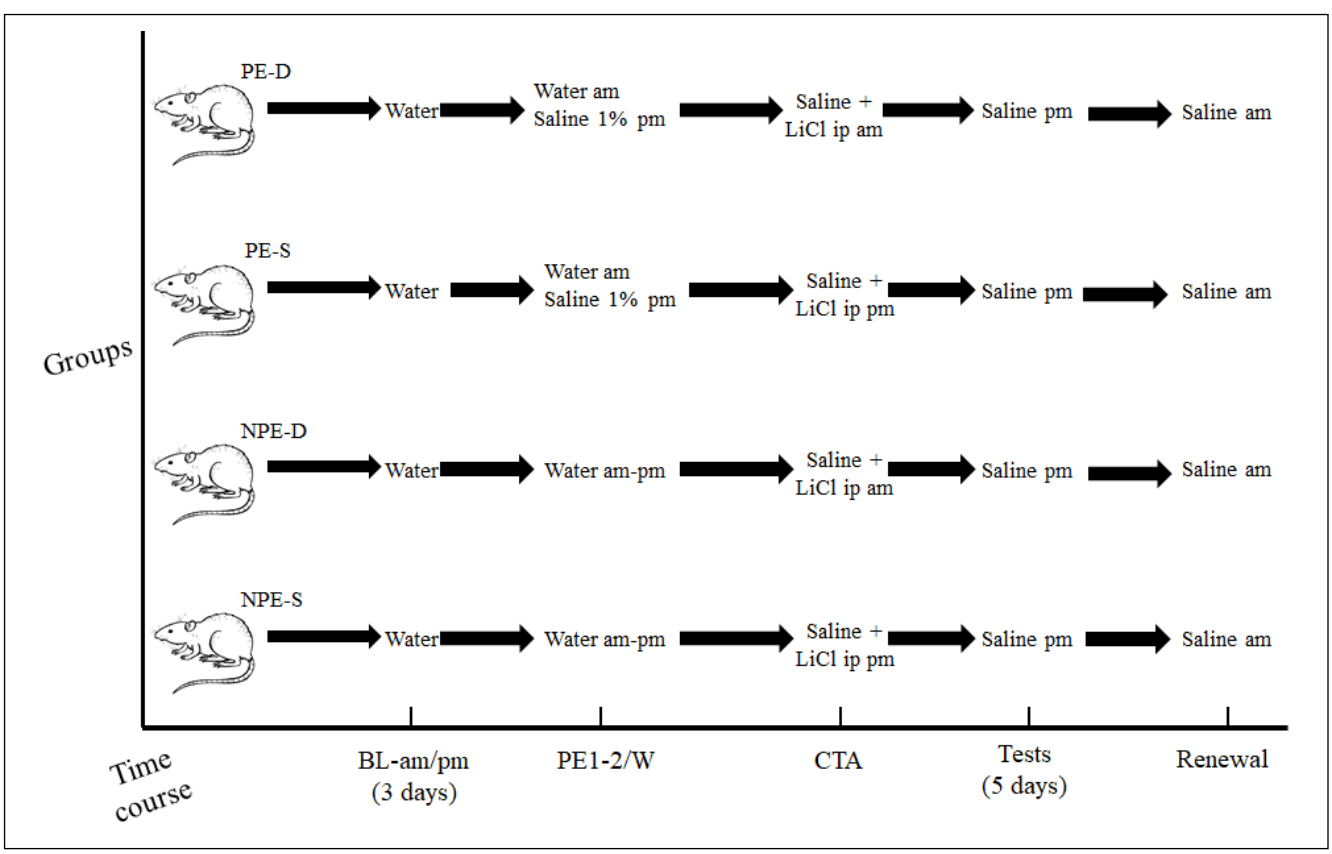

Fig. 1. Behavioral procedure. BL (baseline), three days of habituation to restricted (8 ml) and free water consumption in the morning (10:00) and evening (20:00) drink sessions (15 min), respectively; PE1-2/W-pm, days 1 and 2 of pre-exposure to saline or water (depending on the group) in the evening session (consumption during the morning sessions of this stage was limited to 8 ml in all groups); CTA, conditioning day; PE-D, pre-exposed group in the different (D) context (CTA in the morning session); PE-S, pre-exposed group in the same (S) context (CTA in the evening session); NPE-D, non-pre-exposed group in the different (D) context; NPE-S, non-pre-exposed group in the same (S) context. LiCl i.p., lithium chloride intraperitoneal. For brevity, the morning consumption after CTA is not represented.

Statistical analysis

The saline consumption on the conditioning day was analyzed using a 2 × 2 factorial design with two between-subjects factors, the first being pre-exposure (pre-exposure *vs.* non-pre-exposure) and the second factor being context (different, D, *vs.* same, S, temporal context of conditioning). The CS consumption across the test days was analyzed by a 2 × 2 × 5 repeated-measures ANCOVA, with the consumption during the conditioning day as covariate. The differences between groups in the renewal test were analyzed by a 2 × 2 ANCOVA, with the CS consumption during the last test day (fifth day) as covariate. When the interactions were significant, Newman-Keuls *post-hoc* tests were applied to analyze the differences. We also conducted within-subject analyses of the consumptions of each group on the fifth test day and the renewal day. In all the tests, the critical level of significance for differences was set to $p<0.05$. The analyses were carried out using SPSS software.

RESULTS

Excluding taste aversion, no important adverse events of the intervention were observed. Table I shows the results of the ANCOVAs. Fig. 2 represents the mean consumption by the groups for the baseline, pre-exposure, conditioning and testing stages. The results indicate that conditioning at a different time of day in the pre-exposure (PE-D group) disrupted LI of CTA compared to the pre-exposed group with no temporal changes between the stages (PE-S group) and the non-pre-exposed groups. Latent inhibition was acquired when pre-exposure and conditioning were performed at the same time of day (PE-S group).

Table II depicts the mean consumption and standard deviation of the groups during the procedure. No significant differences were found in the baseline and pre-exposure stages, except for a significant effect of the pre-exposure factor in the evening session of the second day of pre-exposure ($F_{1,35}$=36.93, $p<0.01$, η2=0.493), associated with higher consumption in the pre-exposed groups. An ANOVA of the mean consumption on the conditioning day showed a significant effect of the pre-exposure factor ($F_{1,35}$=28.17, $p<0.01$, η2=0.425) due to the expected higher consumption of the pre-exposed groups. Also, there was a significant effect of the context factor ($F_{1,35}$=7.98, $p<0.01$, η2=0.173), but there was no significant interaction between the two factors ($F_{1,35}$=0.07, p=0.79, η2=0.001). The three-way repeated-measures ANCOVA, conducted to evaluate the mean consumption of each group throughout the five test days, indicated a significant effect of the interaction between context, pre-exposure and test day ($F_{4,35}$=9.492, $p<0.01$, η2=0.2). Post-hoc tests revealed that there was no significant effect of the pre-exposure factor in the different context (p=0.34), which indicates that there was no LI in the

Table I. Results of the ANCOVAs.

	df	*F*-value	*p*-value	η^2
2 × 2 × 5 ANCOVA				
Context	1	19.386	<0.001*	0.338
Pre-exposure	1	3.674	0.063	0.088
Test day	4	104.43	<0.001*	0.733
Context × Pre-exposure	1	25.901	<0.001*	0.405
Context × Test day	4	6.749	<0.001*	0.150
Pre-exposure × Test day	4	0.747	0.561	0.019
Context × Pre-exposure × Test day	4	9.492	<0.001*	0.200
2 × 2 ANCOVA				
Context	1	0.395	0.533	0.010
Pre-exposure	1	0.542	0.466	0.014
Context × Pre-exposure	1	0.017	0.894	0.001

A three-way repeated-measures ANCOVA (context *vs.* pre-exposure *vs.* test day) was calculated to analyze differences in the consumptions throughout the test days. A two-way ANCOVA (group *vs.* pre-exposure) was conducted to analyze differences in the renewal test. *$p<0.05$. d.f., degrees of freedom. η2, Eta Squared.

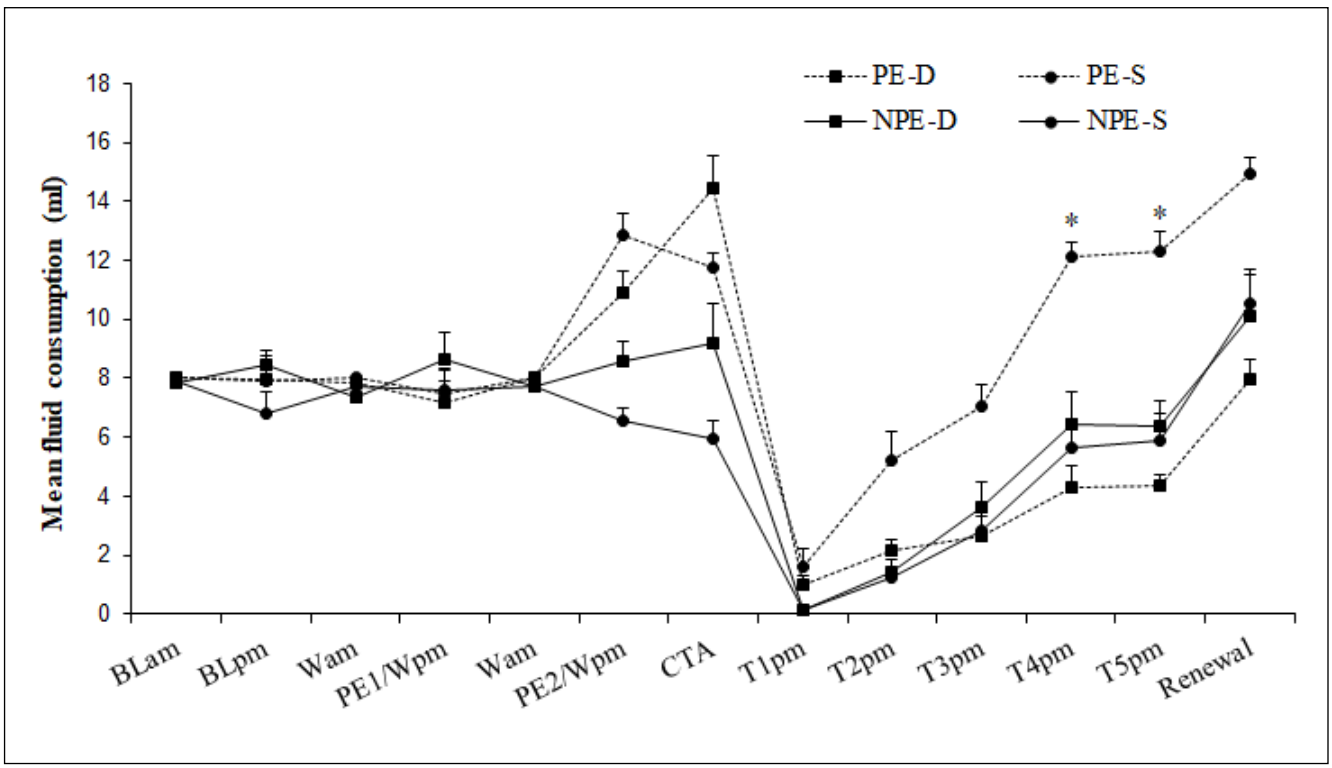

Fig. 2. Mean consumption of fluid by the groups at different stages of the behavioral procedure and standard deviations (spread bars). BL, third and last day of habituation to restricted (8 ml) water consumption in the morning session (am) and free water consumption in the evening session (pm); Wam, restricted (8 ml) water consumption in the morning session of the pre-exposure days; PE1/Wpm and PE2/Wpm, days 1 and 2 of pre-exposure to saline (pre-exposed groups) or water (non-pre-exposed groups) in the evening session; CTA, conditioning day; T1-5pm, testing days in the evening session (extinction tests); Renewal, sixth testing day (morning session); PE-D, pre-exposed group in the different (D) context (CTA in the morning session); PE-S, pre-exposed group in the same (S) context (CTA in the evening session); NPE-D, non-pre-exposed group in the different (D) context; NPE-S, non-pre-exposed group in the same (S) context. There was no significant effect of pre-exposure in the D-groups, indicating that the PE-D group did not acquire latent inhibition. No temporal specificity of the extinction (that is, of the extinguished aversion) was found when the mean consumption of each group was analyzed on the renewal day (sixth testing day) regarding the fifth testing day. *p<0.05.

Table II. Average consumption of the groups (in ml) throughout the procedure, and standard deviation.

Consumption	BLam	BLpm	Wam	PE1/ Wpm	Wam	PE2/ Wpm	CTA	T1pm	T2pm	T3pm	T4pm	T5pm	Renewal
PE-D	8	7.96	7.84	7.2	8	10.88	14.43	1.01	2.13	2.67	4.27	4.33	7.96
PE-S	8	7.91	8	7.49	8	12.84	11.76	1.63	5.23	7.03	12.14	12.3	14.94
NPE-D	7.85	8.44	7.34	8.63	7.73	8.59	9.17	0.12	1.43	3.62	6.46	6.36	10.13
NPE-S	7.88	6.78	7.72	7.6	7.7	6.56	5.94	0.13	1.22	2.83	5.63	5.88	10.56
SD													
PE-D	0	0.80	0.10	0.73	0	0.72	1.10	0.27	0.41	0.65	0.74	0.37	0.65
PE-S	0.04	0.52	0.52	0.91	0.17	0.65	1.38	0.05	0.43	0.87	1.04	0.89	1.38
NPE-D	0.02	0.61	0	0.85	0	0.75	0.49	0.56	0.94	0.77	0.47	0.70	0.52
NPE-S	0.02	0.76	0.26	0.68	0.21	0.45	0.61	0.10	0.34	0.68	0.99	0.90	1.12

BLam/pm (baseline: habituation to restricted (8 ml) water consumption in the morning session -am- and free water consumption in the evening session -pm-); Wam, restricted water consumption in the morning session of the pre-exposure days; PE1/Wpm and PE2/Wpm, days 1 and 2 of pre-exposure to saline (pre-exposed groups) or water (non-pre-exposed groups) in the evening session; CTA, conditioning day; T1-5pm, test days in the evening session; PE-D, pre-exposed group in the different (D) context (CTA in the morning session); PE-S, pre-exposed group in the same (S) context (CTA in the evening session); NPE-D, non-pre-exposed group in the different (D) context; NPE-S, non-pre-exposed group in the same (S) context; SD, standard deviation.

PE-D group. Conversely, there was a significant effect of the pre-exposure factor in the same context (p<0.01), indicating that the PE-S group acquired LI. Post-hoc tests also revealed a significant effect of the context factor in the pre-exposed animals (p<0.05) and comparisons showed that the mean consumption of the PE-S group was higher than that of the PE-D group (which did not exhibit LI) throughout the test days (p<0.01). No significant effect of the context factor was found in the non-pre-exposed animals (p>0.05) and comparisons indicated that there were no differences between the mean consumptions of the NPE-S and NPE-D groups (p=0.91), which was expected because they were both non-pre-exposed groups. The Newman-Keuls tests also showed that the mean consumption of the PE-S group was higher than that of the PE-D, NPE-S and NPE-D groups (which did not exhibit latent inhibition) on the third (p<0.05), fourth (p<0.05) and fifth (p<0.05) test days. Throughout the test days, no significant differences were found between the mean consumption of the PE-D, NPE-S and NPE-D groups (p>0.05 in all cases).

The 2 × 2 ANCOVA conducted to analyze for temporal specificity of the extinguished aversion (renewal test) with respect to the last testing day revealed a non-significant effect of the context (p=0.533) and pre-exposure (p=0.466) factors. The interaction between these two factors was also non-significant (p=0.894). The within-subject analysis of consumption by each group on the fifth test day and the renewal day revealed an increase in the amounts ingested in the renewal test in all groups (p<0.01 in all cases). This increase may suggest that the extinguished response is not dependent on the temporal context.

DISCUSSION

Latent inhibition of CTA may be reduced after temporal-contextual changes between pre-exposure and conditioning (Manrique et al., 2004; Molero et al., 2008). The critical factors for inducing temporal specificity of LI in this paradigm are not well established. For example, this phenomenon has been demonstrated independently of the direction of the circadian change between stages (from morning to evening, or vice versa) (Molero-Chamizo, 2018), suggesting that it is the change of temporal context, and not the specific time of day of testing (morning *vs.* evening) (Pace-Schott et al., 2013; Stryjek et al., 2013), which determines the effect on LI. Moreover, long periods of habituation to the temporal contexts before conditioning seem to facilitate the temporal specificity of LI with respect to short periods (Molero-Chamizo and Rivera-Urbina, 2017). Therefore, we included a long period of habituation prior to conditioning to make the procedure sensitive to this phenomenon. Additionally, a restriction on consumption during conditioning impairs the temporal specificity of LI of CTA compared to free access to the fluid during conditioning (Molero-Chamizo, 2017). However, it has not yet been demonstrated if the restrictions on consumption during the stages prior to conditioning have an effect on the ability of time of day to modulate LI of CTA. Several possibilities arise here. In various paradigms, contextual change effects are reduced under contextual habituation prior to conditioning (De La Casa and Lubow, 2001; De la Casa et al., 2003; Lubow and De La Casa, 2005; Quintero et al., 2011; De la Casa and Díaz, 2013; Molero-Chamizo and Rivera-Urbina, 2017). Thus, the fluid restriction applied during the stages prior to conditioning might not affect the temporal specificity of LI if this restriction makes the temporal context more salient. Nevertheless, since the procedure to induce temporal specificity includes several stages, and fluid restriction during conditioning has been shown to reduce this phenomenon (Molero-Chamizo, 2017), another possibility might be that fluid restriction during the stages prior to conditioning also reduces the context effect on LI. In addition, the water restriction in the present study may not only have had an effect on the temporal discrimination but could also have induced a different hydric state prior to conditioning that would have influenced the results differently compared to a selective restriction during conditioning (Molero-Chamizo, 2017). With the procedure and animals used in the present study, a temporal specificity of LI was evident because a different temporal context during conditioning (PE-D) eliminated LI of CTA relative to the non-pre-exposed groups and compared to a typical LI group without temporal changes between stages (PE-S). The increased consumption observed in the PE-S group throughout the testing days (i.e., LI) was significant after the second test. It suggests that limited water consumption for all animals during baseline and pre-exposure does not prevent the modulatory effect of the time of day on this learning nor the temporal-contextual dependence of LI of CTA. On the other hand, the renewal test conducted on the sixth testing day did not provide evidence for temporal specificity of the extinguished aversion, and the extinction of the acquired response was evident in all groups after a new temporal-contextual change. Renewal of the acquired taste aversion has been described when tested in the context of conditioning after extinction in a different context (Rosas and Bouton, 1997). Considering our results, it can be argued

that the extinguished aversion is not dependent on the temporal context, unlike LI.

The mechanisms by which restricting consumption during the stages preceding conditioning fails to abolish temporal specificity of LI may be disparate. First, a restriction in the fluid equates the intakes and physiological states of the groups before conditioning, which may promote the phenomenon of temporal specificity of LI of CTA by eliminating possible baseline motivational differences (Benstaali et al., 2001; Lukoyanov et al., 2002). Additionally, differences in hydration balance induced by water restriction in the morning sessions prior to conditioning may be a cue identifying the different temporal contexts and consequently facilitate the temporal specificity of the latent inhibition. In previous CTA studies where temporal specificity was induced, water restriction was not included in these stages of the procedure (Morón et al., 2002; Manrique et al., 2004; Molero et al., 2008; Molero-Chamizo, 2013; 2017; 2018), which means hydration balance is also a differential factor with respect to previously described protocols. Finally, reduced hydration under this water restriction schedule could induce a higher intake during conditioning, which also could influence the effect of temporal context on LI of CTA. Such mechanisms assume the importance of the consumption variables and hydration balance during the different stages of the behavioral procedure in detecting temporal dependence of LI. Accepting these mechanisms as being highly probable, the effects of stabilizing the intake of the groups and their physiological states may not be the only influence that temporal specificity of LI is sensitive to. The reason for this is that limited consumption during conditioning, though an essential variable for quantifying the magnitude of taste aversion (Bernstein, 1999; Lubow, 2009), does not facilitate the temporal dependence of LI but instead eliminates it (Molero-Chamizo, 2017). Hence, the specific stages where restrictions are applied might be another critical factor for inducing temporal specificity in this paradigm. Limiting consumption during conditioning can interfere with the role of the time of day in modulating LI, but restrictions during the stages preceding conditioning (De la Casa and Lubow, 1995) might not disrupt the discrimination between different temporal contexts (morning *vs.* evening) thereby allowing the modulatory effect of the time of day on learning. The potential improvement observed in discriminating temporal contexts after selective restrictions of the taste stimulus in one of these contexts (the morning) could be related to attentional processes. This improved discrimination between contexts would allow the temporal-contextual dependence of LI to act as a supplementary mechanism to the equalized physiological state resulting from a restriction on the amounts consumed. The restrictions of water (or other non-conditioned fluids) in the stages before conditioning might increase the attentional processes of animals and consequently the associative power of stimuli would increase, as described for other variables in associative learning (Pearce and Hall, 1980; Hall and Channell, 1986; Pearce and Bouton, 2001). In the LI of CTA paradigm, the increase in attentional processes may facilitate contextual associations during conditioning and promote a modulation in learning. According to this idea, previous studies have suggested that LI is a direct function of the strength of the association between CS and context which occurs during pre-exposure (Escobar et al., 2002). Finally, the restrictions on the fluid amounts or even the duration of the stimulus exposure may be salient cues that interact with the motivational state and modify the absolute salience of the stimuli. In this study, the restriction of water consisted of a limited amount (8 ml) for a limited time (15 min). Therefore, both conditions might be considered part of the restriction factor.

Some aspects of the present investigation should be systematically explored in future studies. We used saline as the CS because its preference has been demonstrated in rats (Falk and Titlebaum, 1963) and because temporal specificity of LI of CTA has been shown using this taste stimulus (Morón et al., 2002; Manrique et al., 2004; Molero et al., 2008; Molero-Chamizo, 2013; 2017; 2018). However, saline is a solution affecting the homeostatic regulation of sodium and water and that could influence important metabolic processes that regulate fluid intake differently depending on the time of day. Consequently, the effect of the time of day on the LI of CTA should also be evaluated using non-saline taste stimuli. On the other hand, no direct comparisons with non-restricted groups were made when the restriction was applied in the stages prior to conditioning. A direct comparison between restriction and non-restriction has been made when this factor was introduced during conditioning (Molero-Chamizo, 2017), but in the present study all animals were exposed to a limited quantity of water in the stages prior to conditioning. This fact may restrict the scope of the results because typical comparisons between control (same context) and experimental (different context) groups were made in this study, which have been demonstrated as effective in revealing the temporal specificity of LI in procedures without fluid restriction (Molero-Chamizo and Rivera-Urbina, 2017); however, direct comparisons between restriction and non-restriction during the previous stages to conditioning were not tested. Therefore,

these direct comparisons should be included in future procedures to validate the findings. Despite this limitation, these results may help to understand how fluid restrictions in different stages of the procedure may or may not influence the temporal specificity of LI of CTA and may expand our understanding of the influence of consumption variables on this phenomenon. The inclusion of direct comparisons between restriction and non-restriction conditions in future studies may clarify the role of the restriction factor in the contextual discrimination process and consequently in the context specificity of LI of CTA.

CONCLUSIONS

The findings of this study reveal a clear temporal specificity of LI of CTA after limited consumptions in the stages prior to conditioning. It can be concluded that limited availability of the stimuli during specific stages of the behavioral procedure is a relevant factor for consideration while investigating contextual influences on LI and, generally, associative learning. Further studies are required to elucidate the function of the stimulus restrictions prior to conditioning in the contextual modulation of learning and its relevance among other species.

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