Excitotoxic lesion of the hippocampus of Wistar rats disrupts the circadian control of the latent inhibition of taste aversion learning

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ABSTRACT

Previous experiments have shown that changes in the time of day between taste pre-exposure and conditioning prevent the latent inhibition of conditioning taste aversion. The effect of these changes in circadian context between pre-exposure and conditioning on the magnitude of the learned aversion appears to be similar to the effect of changes in spatial context on this type of learning. To elucidate the brain areas involved in this circadian dependence of latent inhibition of conditioning taste aversion, the effect of excitotoxic lesions of the hippocampus, a region related to spatial-contextual modulation in this learning process, was analyzed. The latent inhibition of conditioning taste aversion in animals with hippocampal lesions, that were pre-exposed and conditioned to the same or different time of day, was compared with the response of animals exposed to either conditions ("same" or "different") but had undergone amygdala lesions or sham lesions. The results showed that selective dorsal hippocampus lesion eliminated the circadian dependence of latent inhibition of taste aversion. A change in the time of day between pre-exposure and conditioning did not prevent latent inhibition in animals with hippocampal lesions. In contrast, this change prevented latent inhibition in the amygdala-lesioned and sham groups. These findings suggest that the hippocampus contains a selective mechanism that modulates the contextual dependency of the latent inhibition of conditioning taste aversion without interfering with the effect of taste pre-exposure itself. This study may help to understand the possible common involvement of the hippocampus in different types of contextual control of associative learning.

Keywords: amygdala; conditioning; context; hippocampus; pre-exposure

1. Introduction

In associative learning, context can be associated directly with the unconditioned stimulus or may modulate the recovery of the learned response. Furthermore, physical and interoceptive contextual cues are especially able to influence the association between stimuli (Bouton, 1993; Bouton et al., 2006). Physical changes in the experimental box in which animals are tested have been the most frequently used contextual cues. Therefore, the spatial contexts of physical stimuli have traditionally been considered in conditioning (Innis and Mills, 1985). Latent inhibition of fear conditioning (Escobar et al., 2002) and latent inhibition of conditioning taste aversion (CTA) (Hall and Channell, 1986; Quintero et al., 2011) are sensitive to the effects of experimental changes in spatial contexts. Moreover, conditioning taste aversion (González et al., 2012) and its extinction (Rosas and Bouton, 1997) are also sensitive to the spatial context of learning. The influence of interoceptive cues on associative processes, for example, those related to temporal context changes (Lukoyanov et al., 2002), could also serve as a useful model for understanding the mechanisms of learning and memory (Pearce and Bouton, 2001). Specifically, the time of day relative to the sleep/wake cycle has proven to be a valid contextual cue that modulates latent inhibition of CTA (Manrique et al., 2004) and CTA retrieval (Morón et al., 2002).

The brain mechanisms involved in the effects of context on associative learning seem to be different depending on the contextual cues and learning paradigm used. The involvements of the hippocampus and the amygdala in specific contextual effects depend on specific learning procedures. The hippocampus is necessary for memory processes, and its activity is crucial in contextual learning (Holland and Bouton, 1999; Mitsushima et al., 2011). The involvement of the hippocampus in contextual learning

has mainly been demonstrated in fear conditioning (Anagnostaras et al., 2001; Hamilton et al., 2011). Similarly, the amygdala underlies the contextual modulation of fear conditioning (Cangioli et al., 2002) and place conditioning (Hetzel et al., 2012).

The hippocampus also seems to be necessary for the dependence of latent inhibition on spatial context (Maren and Holt, 2000), the dependence of CTA on temporal context (Gámiz and Gallo, 2011) and the latent inhibition of CTA (Molero et al., 2005). However, to the best of knowledge, there have been no studies on the possible role of the amygdala in the temporal modulation of taste learning. The amygdala may be necessary for proper acquisition of CTA (Yamamoto and Ueji, 2011), although the latent inhibition of CTA and the contextual dependence of latent inhibition of CTA do not require the activity of this structure. In contrast, the hippocampus may be selectively involved in the contextual specificity of taste learning (Molero et al., 2005), although the integrity of this structure is not necessary for the acquisition of CTA or the latent inhibition of CTA (Gallo and Cándido, 1995). Two experiments were completed to analyze this possible differential involvement of the hippocampus and the amygdala. The aim of this study was to evaluate the effects of a change in circadian context made on the day of conditioning in rats with bilateral excitotoxic lesions of the amygdala or hippocampus in a latent inhibition of CTA paradigm.

2. Results

2.1. Anatomical

The neurotoxin induced lesion in the cell bodies of both the hippocampus and amygdala (see Fig. 1 and 2). Rats with unsuitable lesions (n=4) were excluded from the final

sample. Animals with hippocampal lesions exhibited bilateral cell loss mainly in CA1, CA2 and the dentate gyrus. In larger lesions, neurodegeneration of pyramidal and polymorphic cells was also observed in the CA3 hippocampal area. In all cases, the cellular degeneration was surrounded by intensely stained glial proliferation. Microglia plates were also formed around the damaged granular cells of the dentate gyrus. The amygdala lesions induced bilateral cell loss mainly in the pyramidal cells of the basolateral, central and medial nuclei, the lateral amygdala and, to a lesser extent, the basomedial amygdala. The cell damage induced by amygdala lesions was also surrounded by intensely stained glial proliferation. There was no cellular degeneration in animals that received sham lesions.

FIG. 1 AND 2 APPROXIMATELY HERE, PLEASE

2.2. Behavioral

2.2.1. Experiment 1

An ANOVA of the average consumption on the second day of pre-exposure to saline revealed no significant effect of group ($F[1,42]=0.16$, $p = 0.69$) or lesion ($F[2,42]=1.77$, $p = 0.18$). The interaction between group and lesion was also non significant ($F[2,42]=0.37$, $p = 0.69$). An ANOVA of the average consumption on the conditioning day indicated a significant effect of group ($F[1,42]=13.14$, $p<0.01$). Animals in the "different" group consumed significantly more saline (11.77±1.5) than those in the "same" group (7.47±1), probably because consumption was usually greater in the

morning sessions. There was no effect of lesion ($F[2,42]=2$, $p = 0.15$) or interaction between group and lesion ($F[2,42]=1.16$, $p = 0.32$).

Because there were differences between groups on the conditioning day, a 2x3x3 repeated measures ANCOVA was performed on consumption over the three test days. The analysis indicated significant effects of group ($F[1,41]=13.7$, $p<0.01$), lesion ($F[2,41]=4.1$, $p<0.02$) and test ($F[2,100]=6.32$, $p<0.01$), and a significant interaction between group and lesion ($F[2,41]=5.8$, $p<0.01$). Analysis of the interaction revealed a significant effect of lesion in the "different" groups ($F[2,21]=8.75$, $p<0.01$). The PD HC group consumed more saline (10.01±1.14), and therefore showed less aversion, than the PD AM (6.63±0.69) and PD Sham (5.4±0.7) groups. In contrast, there was no significant effect of lesion in the "same" groups ($F[2,21]=3.01$, $p = 0.07$). Analysis of the interaction also showed that there was no significant effect of group on consumption among animals with hippocampal lesions ($F[1,16]=0.01$, $p = 0.97$), indicating that the change in the time of day between pre-exposure and conditioning did not alter the effect of the pre-exposure in the hippocampal lesion group. However, a significant group effect was found in groups with amygdala lesions ($F[1,16]=26.99$, $p<0.01$). The PS AM group consumed more saline (14.44±1.29) than the PD AM group (6.63±0.69), i.e., the PS AM group showed less aversion. There was also a significant effect of group in sham animals ($F[1,10]=21.06$, $p<0.01$). The PS Sham group exhibited less aversion because this group consumed more saline (11.03±1.33) than the PD Sham group (5.4±0.7). Table 1 shows the average consumption of each group on the pre-exposure, conditioning and test days. Fig. 3 indicates the average consumption of each group over the days.

TABLE 1 APPROXIMATELY HERE, PLEASE

FIG. 3 APPROXIMATELY HERE, PLEASE

In summary, all pre-exposed groups in the "same" condition, except the animals with hippocampal lesions, showed weaker aversion throughout the tests as indicated by the observation that saline consumption was significantly higher in these groups than in the groups that were pre-exposed and conditioned at different times of day (Fig. 3). On the other hand, animals in the "different" condition with hippocampal lesions (PD HC) consumed significantly more saline along the tests than those in the "different" condition with amygdala or sham lesions. The consumption of the PD HC group was similar that of the group in the "same" condition with hippocampal lesions (PS HC) (Fig. 3). Thus, hippocampal lesions blocked the effect of changes in the time of day between pre-exposure and conditioning. This change in temporal context induced greater aversions in the other groups. Although hippocampal lesions eliminated the effects of changes in temporal context on taste aversion, it is necessary to compare pre-exposed and non-pre-exposed groups to conclusively demonstrate that hippocampal lesions prevent the temporal dependence of latent inhibition of CTA. This was the aim of experiment 2.

2.2.2. Experiment 2

An ANOVA of the average consumption on the second day of pre-exposure to saline revealed no significant effects of pre-exposure ($F[1,27]=3.05$, $p = 0.1$), lesion ($F[1,27]=0.26$, $p = 0.61$) or interaction between pre-exposure and lesion ($F[1,27]=1.27$,

p = 0.27). An ANOVA of the average consumption on the conditioning day indicated a significant effect of pre-exposure (F[1,27]=9.56, p<0.01); pre-exposed animals consumed significantly more saline (13.17±1.3) than controls that were not pre-exposed (9.29±1) and for whom the flavor was unknown. There was no significant effect of lesion (F[1,27]=0.53, p = 0.47) or interaction (F[1,27]=0.14, p = 0.71). Because there was a pre-exposure effect on the conditioning day, covariance analyses were performed for the test days. A 2x2x3 ANCOVA indicated a significant interaction of pre-exposure and lesion (F[1,26]=5.42, p<0.02). Analysis of this interaction revealed a significant effect of pre-exposure in the hippocampal lesion group F[1,14]=4.49, p<0.05); the PD HC group consumed more saline (6.83±1.12) than the CD HC group (3.28±0.78), which indicates that the PD HC group exhibited latent inhibition. There was no significant effect of pre-exposure in sham animals (F[1,11]=0.04, p = 0.83), i.e., these animals did not exhibit latent inhibition. Analysis of the interaction also indicated a significant effect of lesion in the pre-exposed animals (F[1,13]=14.39, p<0.01); the PD HC group consumed more saline (6.83±1.12) than the PD Sham group (2.29±0.68). There was no significant effect of lesion in non-pre-exposed control animals (F[1,12]=1.18, p = 0.29). Table 2 shows the average consumption of the groups on pre-exposure, conditioning and test days. Fig. 4 represents the average consumption of each group over the days.

TABLE 2 APPROXIMATELY HERE, PLEASE

FIGURE 4 APPROXIMATELY HERE, PLEASE

These results indicate that the PD HC group acquired latent inhibition compared to the CD HC group. However, the PD Sham group did not show latent inhibition. Saline intake on test days was significantly higher in the PD HC group compared to all other groups (Fig. 4). On the other hand, sham animals that were pre-exposed in a temporal context that was different from that used in conditioning showed a consumption pattern during the tests that was similar to that of the non-pre-exposed control subjects (Fig. 4). This loss of latent inhibition of CTA can certainly be attributed to the change in temporal context that the pre-exposed animals experienced. However, hippocampal lesions blocked this interruption as evidenced by the fact that animals in the "different" condition showed latent inhibition in spite of being pre-exposed in a temporal context that differed from the temporal context of conditioning. Therefore, hippocampal lesions prevented the effect of changes in temporal context on latent inhibition of CTA.

3. Discussion

In experiment 1, all groups pre-exposed and conditioned in different temporal contexts (except the hippocampal lesion group) consumed significantly less saline on test days than the "same" groups. Therefore, a change in the time of day between pre-exposure and conditioning disrupted the effect of pre-exposure. The hippocampal lesion group did not show this temporal context specificity, and the saline intake of these animals after conditioning was similar to that of the "same" groups. This loss of the pre-exposure effect did not seem to be due to a defect in the recovery process. Because the temporal context on test days was the same as the context of the pre-exposure, the lesion may have selectively interrupted the effect induced by a context change on

associative processes. On the other hand, because the saline consumption of the sham animals on the days of pre-exposure was not significantly different from those of the groups with amygdaloid or hippocampal lesions, it seems that lesions of these structures have no influence on a possible attenuation of neophobia.

Experiment 2 seemed to demonstrate that lesions of the hippocampus selectively eliminated the temporal specificity of the latent inhibition of CTA. Processing of a context other than pre-exposure context on the day of conditioning only eliminated latent inhibition in sham animals. Nevertheless, the new context during learning acquisition did not induce a loss of latent inhibition in animals with hippocampal lesions. Thus, the hippocampal lesions seemed to selectively interfere with the circadian control of latent inhibition of CTA and not with the phenomenon of latent inhibition.

In general, these results demonstrate that the hippocampus is necessary for the circadian specificity of latent inhibition of CTA. Hippocampal lesion does not seem to affect the phenomenon of latent inhibition in the conditioning taste aversion paradigm (Buhusi et al., 1998; De Oliveira et al., 1999; Gallo and Cándido, 1995), and therefore, our results can be explained through the selective function of this structure on the effects of changes in temporal context on latent inhibition of CTA. In contrast, recent research suggests that the amygdala is selectively involved in the acquisition of taste aversion (Garcia-Delatorre et al., 2012; Yamamoto and Ueji, 2011) and not in the latent inhibition of CTA (St. Andre and Reilly, 2007). Additionally, our research has shown that the amygdala is not necessary for the contextual modulation of the latent inhibition of CTA. These findings demonstrate the differential functions of the hippocampus and amygdala in the complex phenomena of associative learning. The current study also shows that interoceptive cues may be considered as contextual stimuli that modulate

associative processes. The temporal and spatial modulation of learning might share common mechanisms (Morón et al., 2002), and data suggest that, in both cases, this modulation requires the integrity of the hippocampus.

The role of the hippocampus in mediating the effect of cues on subsequent taste learning has been interpreted in different ways. One method for analyzing the selective function of the hippocampus in taste learning phenomena is the study of aged animals. In these animals, deficits in hippocampus-dependent learning and some memory tasks have clearly been observed (Manrique et al., 2007). More specifically, the circadian modulation of CTA has been described even in developing rats (Manrique et al., 2009a) during specific stages in which hippocampal function emerges. These studies are consistent with the hypothesis that the hippocampus selectively participates in the modulation of taste learning by previous experiences, although this structure is not necessary for the acquisition of taste associations. The specific circadian control of taste aversion has been attributed to the ability of the hippocampus to form separate memories in associative learning (Manrique et al., 2009b). In this sense, given that the acquisition of taste aversion appears to be independent of the hippocampus, the results of our study also suggest that this structure can form a circadian representation that is independent of aversive taste associations. This conclusion is reinforced by the analysis of our behavioral procedure. When aversions were tested in the pre-exposure context, animals with hippocampal lesions responded as if there was no context change on the day of conditioning. In short, the behavioral pattern observed in our experiments indicated that the hippocampus is necessary for the selective representation of interoceptive contextual cues that can modulate taste associations.

We conclude that this research provides new evidence about the differential functions of the amygdala and hippocampus in the complex phenomena of taste learning. Both structures are involved in the contextual dependency of some types of associative learning. The amygdala is also necessary for the correct acquisition of taste learning (Yamamoto and Ueji, 2011), but our experiment 1 showed that the contextual dependence of the latent inhibition of CTA does not require the integrity of the basolateral amygdala. Nonetheless, experiments 1 and 2 showed that the activity of the hippocampus is required for this contextual dependency, although this structure does not appear to be necessary for the acquisition of latent inhibition or taste aversion (Gallo and Cándido, 1995).

4. Experimental procedures

4.1. Subjects

Eighty-four adult male Wistar rats (280-300 g) were individually housed in boxes measuring 30x15x30 cm. Throughout the behavioral procedure, the animals were exposed to a daily 12 hours light-dark cycle (lights on from 9:00 to 21:00), and the temperature conditions were kept constant at 23º C. Food was provided *ad libitum*, and the availability of fluid was restricted to two daily 15 min sessions, one in the morning and one in the evening. The procedure was approved by the Ethics Committee for Animal Research of the University of Granada and was conducted in accordance with both the Guide for the Care and Use of Laboratory Animals of the NIH of the United States and the European Communities Council Directive of 24 November 1986 (86/609/EEC).

4.2. Surgery

Animals were anaesthetized with an i.p. injection of sodium pentobarbital (1 mL/kg), and placed in a stereotaxic apparatus for surgery (Stoelting Co. Instruments, Wood Dale, IL, U.S.A). After a longitudinal incision was made to expose the skull, bregma and lambda points leveled in the horizontal plane, and two craniotomies were made in each hemisphere using bregma as a reference point. Bilateral lesions to the hippocampus or amygdala were made through these craniotomies. The stereotaxic coordinates were taken from the atlas of Paxinos and Watson (1996) (Table 3). All animals, except those that received "false" lesions (Sham group), received two successive injections of N-methyl-D-aspartate (NMDA) (0.6 μL; 0.077 M) in each hemisphere, through an injection cannula (0.3 mm exterior x 0.15 mm interior) connected to a micro-syringe (Hamilton, 10 μL). The neurotoxin was injected at a rate of 1 μL/min using an injection pump (Harvard, USA). The cannula remained at the lesion site for 2 min after the injections to allow for diffusion of the neurotoxin. Immediately thereafter, the cannula was removed and the incision sutured. The procedure was identical for sham groups, except that no neurotoxin was injected through the cannula. Rats were allowed postoperative recovery period of seven days in which water and food was available *ad libitum*.

TABLE 3 APPROXIMATELY HERE, PLEASE

4.3. Procedure

4.3.1. Experiment 1

The objective of this experiment was to elucidate the involvements of the hippocampus and amygdala in the effect of changes in temporal context between pre-exposure and conditioning in a taste aversion learning paradigm.

The "different" condition entailed conditioning and pre-exposure to the taste taking place at different times of day. When pre-exposure and conditioning were conducted at the same time of day, it was considered the "same" condition. Forty-eight rats were distributed among the following six groups: PD HC, "different" condition and hippocampal lesion (n = 9); PD AM, "different" condition and amygdaloid lesion (n = 9); PD Sham, "different" condition and false lesion in the amygdala or hippocampus (n = 6); PS HC, "same" condition and hippocampal lesion (n = 9); PS AM, "same" condition and amygdaloid lesion (n = 9); and PS Sham, "same" condition and false lesion in the amygdala or hippocampus (n = 6).

Animals were water restricted and received two 15 min sessions of access to water per day, one at 10.00 and the other at 20.00, over four days, to facilitate the differentiation of the temporal contexts (Morón et al., 2002). After period of habituation to fluid restriction in both temporal contexts, all rats received water in the morning session (15 min) and were exposed to a sodium chloride solution dissolved in water (saline 1%) in the evening session (15 min) for two days. On the day following the last pre-exposure, conditioning took place in the morning session for all "different" groups (PD HC, PD AM and PD Sham). These animals were exposed to saline for 15 min in the morning, and the amounts ingested were recorded. Twenty minutes later, they received an injection of lithium chloride (LiCl) (0.15 M, 2% of body weight, i.p.). Water was available for 15 min in the evening session. In contrast, all "same" groups

(PS HC, PD HC and PS Sham) received conditioning in the evening session, and water (15 min) in the morning session. After one day of recovery with water (15 min) in the morning and evening sessions, the response to saline (conditioned stimulus) was tested in the evening session in all groups for three days. Water was available for 15 min in the morning session. Table 4 summarizes the behavioral procedure of experiment 1.

TABLE 4 APPROXIMATELY HERE, PLEASE

4.3.2. Experiment 2

In the previous experiment, animals conditioned and pre-exposed in different temporal contexts acquired greater taste aversion compared to animals that were conditioned and pre-exposed at the same time of day. Therefore, a change in temporal context interfered with the extent of aversion. Amygdaloid or sham lesions had no effect on this circadian dependence of learning. In contrast, hippocampal lesions abolished the temporal specificity of the pre-exposure effect. Nevertheless, in experiment 1, all groups were pre-exposed to the taste. Thus, to elucidate whether changes in temporal context really affected the phenomenon of latent inhibition, it was necessary to compare the behavior of pre-exposed animals to that of non-pre-exposed controls. This comparison allowed us to analyze the magnitude of the effect of changes in temporal context on taste aversion and to clarify whether circadian manipulation induced the attenuation or elimination of latent inhibition. This comparison between pre-exposed and non-pre-exposed groups also gave us an opportunity to examine the precise effect of hippocampal lesion on the latent inhibition of CTA. Thus, to affirm that hippocampal lesions prevented the

dependence of latent inhibition on temporal context, this experiment 2 was performed on pre-exposed animals in a context that was different from that of the conditioning and on non-pre-exposed animals; some of the animals had received hippocampal lesions and other had received sham lesions. If hippocampal lesions disrupted the temporal dependence of the latent inhibition of CTA, the animals of experiment 2 with this lesion should display latent inhibition despite having been pre-exposed in a temporal context that was different from that of the conditioning. For the sham group, a change in circadian context should prevent the latent inhibition of CTA.

The behavioral procedure was the same as in experiment 1, except that all groups were conditioned in temporal context that was different from that of the pre-exposure and test (pre-exposed groups) or the test (non-pre-exposed groups). Thirty-two rats were distributed into four groups: PD HC, pre-exposed, "different" condition and hippocampal lesion (n = 9); CD HC, control non-pre-exposed, "different" condition and hippocampal lesion (n = 8); PD Sham, pre-exposed, "different" condition and "false" lesion in the hippocampus (n = 8); and CD Sham, control non-pre-exposed, "different" condition and "false" lesion in the hippocampus (n = 7). Non-pre-exposed control animals received *ad libitum* access to water for 15 min in the evening session of the pre-exposures to saline. Table 5 summarizes the behavioral procedure of experiment 2.

TABLE 5 APPROXIMATELY HERE, PLEASE

4.4. Histology

When the experiments were completed, brains were removed and stored in 10% formalin solution and subsequently cryo-sectioned (Erma-422, Tokyo) at 45 μm. The slides were stained with cresyl violet and examined under an optical microscope (CH-30, Olympus). Images of the slides were captured with a TV Olympus camera (U-PMTVC, Olympus).

4.5. Data analysis

4.5.1. Experiment 1

Fluid consumption on the water baseline and taste pre-exposure days was analyzed in a 2x3 factorial design, with one inter-group factor with two levels ("different" and "same") and another inter-group factor with three levels (hippocampal, amygdaloid and sham lesions). A one-way ANOVA was used to analyze group differences. A 2x3x3 design was used to determine differences across test days. Saline consumption over test days was analyzed with covariance analysis (ANCOVA). Where interactions were significant, Newman-Keuls post-hoc tests were applied to establish differences.

4.5.2. Experiment 2

A 2x2 factorial design, with an inter-group factor with two levels (pre-exposure and non-pre-exposure) and another inter-group factor with two levels (hippocampal lesion and sham lesion), was used to determine differences in fluid consumption on the day of baseline and on pre-exposure days. A one-way ANOVA was used to analyze group differences. The differences across test days were determined using a 2x2x3 design.

Similar to experiment 1, saline consumption over the test days was analyzed by covariance analysis (ANCOVA). Where interactions were significant, Newman-Keuls post-hoc tests were applied to establish differences.

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Figure legends

Fig. 1. Brain section of an animal with a sham hippocampal lesion (above) showing the intact cells of the CA1 hippocampal region. Below is a section of brain from an animal with a hippocampal lesion showing the cell loss in the CA1 hippocampal region induced by the neurotoxin.

Fig. 2. Brain section of an animal with a sham lesion of the basolateral amygdala (above) showing the intact cells of this nucleus. The bottom image is a section of the brain of an animal with a lesion of the basolateral amygdala showing cell destruction and microglia proliferation in this nucleus.

Fig. 3. Representation of the average consumption of the groups (in mL) over the days in experiment 1. P1 pm and P2 pm, first and second days of pre-exposure to saline in the evening session, respectively; C am/pm, conditioning day in the morning ("different" groups) or evening ("same" groups), respectively; T1-3 pm, first, second and third days of testing (evening session). PD, pre-exposed group in the "different" condition (different temporal context on the day of conditioning); PS, pre-exposed group in the "same" condition (same temporal context on the day of conditioning); HC, hippocampal lesion group; AM, amygdaloid lesion group; Sham, animals without real lesion in the amygdala or hippocampus; am, fluid exposure at 10.00; pm, fluid exposure at 20.00. A significant group effect ("different" versus "same" groups) across test days was found for the groups with amygdaloid lesions ($F[1,16]=26.99$, $p<0.01$). There was also a significant group effect across test days in sham animals ($F[1,10]=21.06$, $p<0.01$).

However, there was no significant group effect across test days in animals with hippocampal lesions ($F[1,16]=0.01$, $p = 0.97$). There was also a significant effect of lesion across test days in the "different" groups ($F[2,21]=8.75$, $p<0.01$). No significant effect of lesion across test days was found in the "same" groups ($F[2,21]=3.01$, $p = 0.07$), indicating that hippocampal lesions prevented the dependence of taste aversion on temporal context.

Fig. 4. Representation of the average consumption of the groups (in mL) over the days in experiment 2. P1-W pm and P2-W pm, first and second days of pre-exposure to saline (pre-exposed groups) or water (non-pre-exposed groups) in the evening session, respectively; C am, conditioning day in morning session; T1-3 pm, first, second and third days of testing in evening session; PD, pre-exposed group in the "different" condition (different temporal context on the day of conditioning); CD, non-pre-exposed group and "different" condition; HC, hippocampal lesion group; Sham, animals with no real lesion in the hippocampus; am, fluid exposure at 10.00; pm, fluid exposure at 20.00. There was a significant pre-exposure effect across test days in the hippocampal lesion group $F[1,14]=4.49$, $p<0.05$); the PD HC group consumed more saline (6.83±1.12) than the CD HC group (3.28±0.78). There was no significant effect of the pre-exposure factor across test days in sham animals ($F[1,11]=0.04$, $p = 0.83$), indicating that hippocampal lesion did not prevent latent inhibition although the conditioning was acquired in a different temporal context.

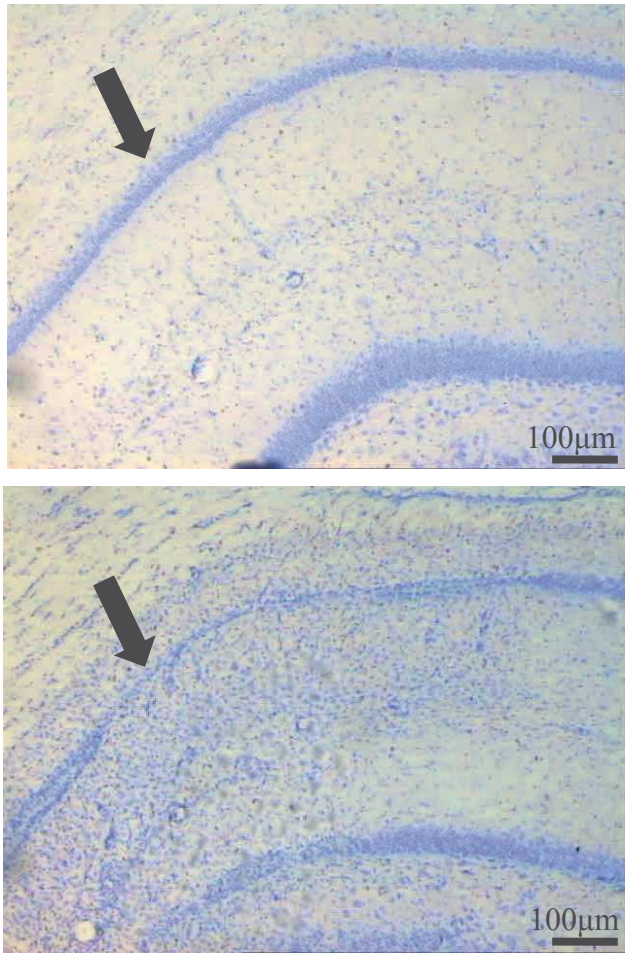

Fig. 1.

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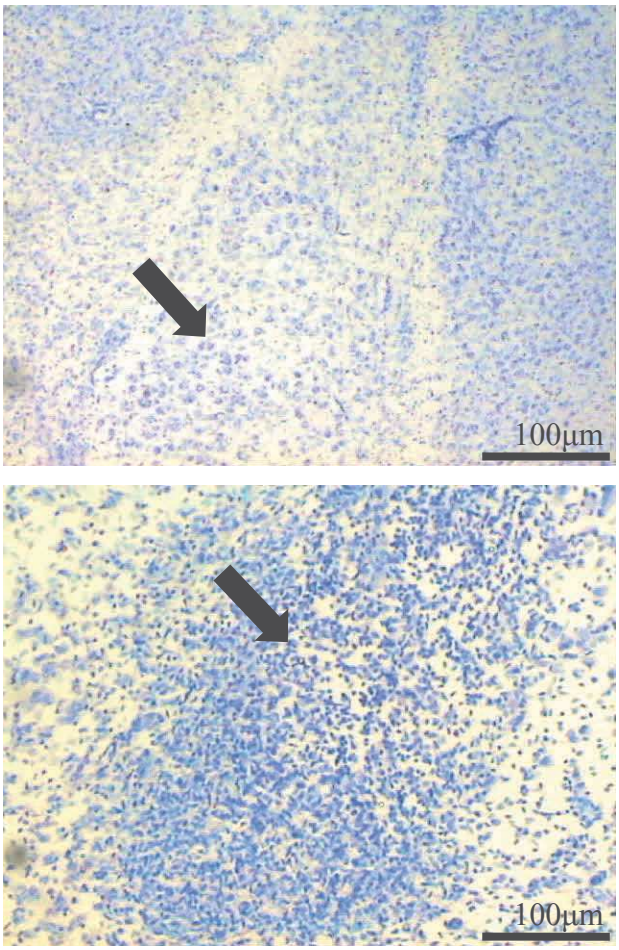
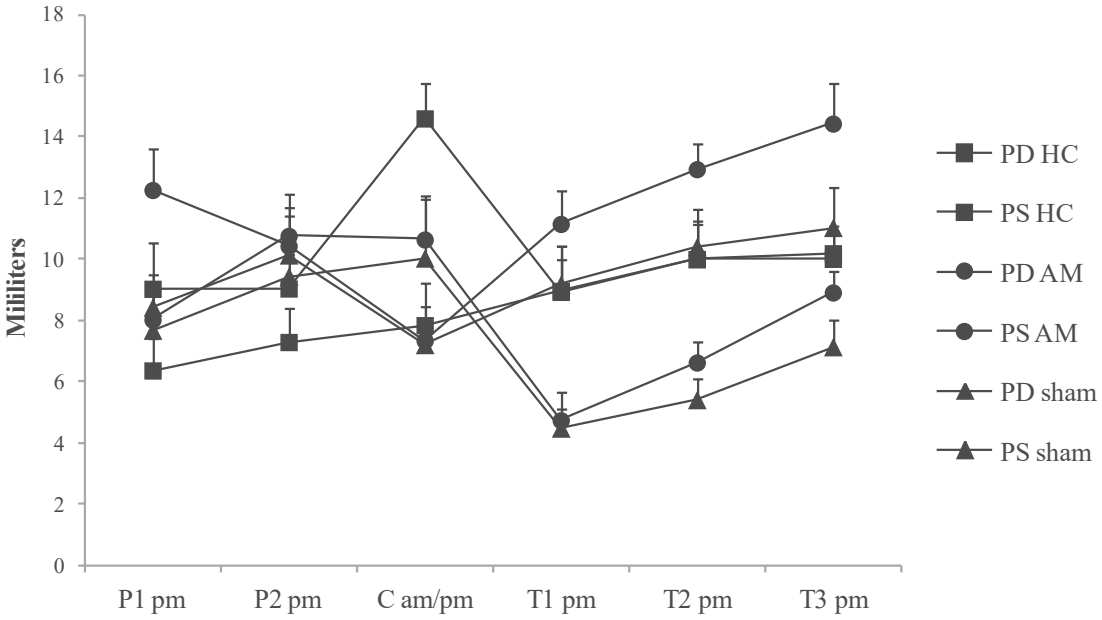


Fig. 2.



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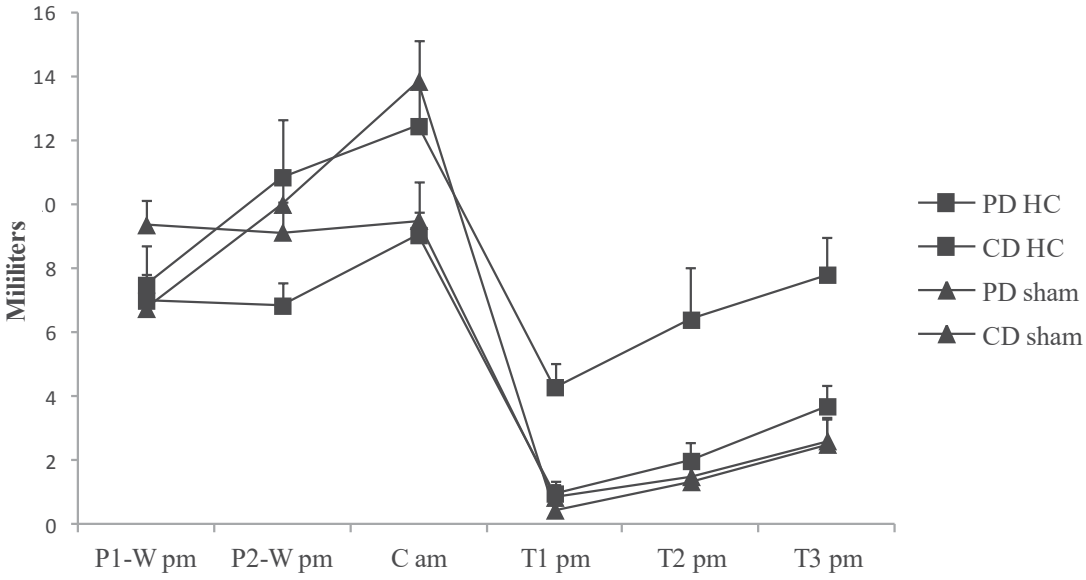

Fig. 4.

TABLE 1. Average consumption of groups (in mL) along the behavioral procedure of experiment 1, and error. The water consumption during habituation is not shown

Average	P1 pm	P2 pm	C am/pm	T1 pm	T2 pm	T3 pm
PD HC	9.04	9.05	14.61	8.94	10.01	10.2
PS HC	6.37	7.3	7.85	8.97	10.01	10.02
PD AM	8.03	10.77	10.65	4.74	6.63	8.92
PS AM	12.27	10.44	7.35	11.16	12.95	14.44
PD Sham	7.67	9.43	10.03	4.47	5.4	7.13
PS Sham	8.42	10.13	7.2	9.17	10.4	11.03
Error						
PD HC	1.49	1.09	1.14	1.07	1.14	0.89
PS HC	1.03	1.1	1.35	1.45	1.65	0.9
PD AM	0.97	0.94	1.44	0.39	0.69	0.66
PS AM	1.35	1.7	1.13	1.09	0.84	1.29
PD Sham	1.22	1.5	1.95	1.16	0.7	0.87
PS Sham	1.07	1.31	0.67	1.24	0.87	1.32

P1 pm and P2 pm, first and second days of pre-exposure to saline in the evening session; C am/pm, conditioning day in the morning ("different" groups) or evening ("same" groups); T1-3 pm, first, second and third days of testing (evening session). PD, pre-exposed group in the "different" condition; PS, pre-exposed group in the "same" condition; HC, hippocampal lesion group; AM, amygdaloid lesion group; Sham, animals with "false" lesion in the amygdala or hippocampus.

TABLE 2. Average consumption of groups (in mL) along the behavioral procedure of experiment 2, and error. The water consumption during habituation is not shown

Average	P1-W pm	P2-W pm	C am	T1 pm	T2 pm	T3 pm
PD HC	7.5	10.87	12.47	4.3	6.41	7.82
CD HC	7.01	6.85	9.06	0.97	1.99	3.7
PD Sham	6.75	10.01	13.86	0.44	1.33	2.5
CD Sham	9.37	9.14	9.51	0.84	1.49	2.61
Error						
PD HC	1.21	1.81	1.3	0.74	1.63	1.17
CD HC	0.39	0.72	0.7	0.38	0.56	0.68
PD Sham	1.06	1.09	1.28	0.2	0.46	0.78
CD Sham	0.75	0.96	1.23	0.43	0.5	0.74

P1-W pm and P2-W pm, first and second days of pre-exposure to saline in the evening session vs. water for non-pre-exposed groups; C am, conditioning day in the morning; T1-3 pm, test days (evening session). PD, pre-exposed group in “different” condition; CD, non-pre-exposed group and “different” condition; HC, hippocampal lesion group; Sham, animals with “false” lesion in the hippocampus.

TABLE 3. Stereotaxic Coordinates used in experiments 1 (HC, AM and Sham groups) and 2 (HC and AM groups)

GROUP	A-P 1	A-P 2	M-L 1	M-L 2	D-V 1	D-V 2
HC	-2.8	-3.6	±1.6	±2.2	+3.4	+3.4
AM	-2.8	-3.3	±4.6	±4.6	+8.2	+8.8
Sham	-2.8/-2.8	-3.6/-3.3	±1.6/±4.6	±2.2/±4.6	+3.4/+8.2	+3.4/+8.8

A-P, anterior-posterior axis; M-L, medial-lateral axis; D-V, dorsal-ventral axis; HC, hippocampal lesion group; AM, amygdaloid lesion group; Sham, "false" lesion group (the first coordinate of each axis in this group corresponds to the "false" lesion in the hippocampus, and the second one corresponds to the "false" lesion in the amygdala).

TABLE 4. Behavioral procedure of experiment 1

GROUP	Habituation (4 days)	Pre-exposure (2 days)	Conditioning	Test (3 days)
PD HC	Water am-pm	Saline 1% pm	Saline+ LiCl am	Saline pm
PS HC	Water am-pm	Saline 1% pm	Saline+ LiCl pm	Saline pm
PD AM	Water am-pm	Saline 1% pm	Saline+ LiCl am	Saline pm
PS AM	Water am-pm	Saline 1% pm	Saline+ LiCl pm	Saline pm
PD Sham	Water am-pm	Saline 1% pm	Saline+ LiCl am	Saline pm
PS Sham	Water am-pm	Saline 1% pm	Saline+ LiCl pm	Saline pm

PD, pre-exposed group in the "different" condition; PS, pre-exposed group in the "same" condition; HC, hippocampal lesion group; AM, amygdaloid lesion group; Sham, animals with "false" lesion in the amygdala or hippocampus; am, fluid exposure at 10.00; pm, fluid exposure at 20.00; LiCl, intraperitoneal lithium chloride.

TABLE 5. Behavioral procedure of experiment 2

GROUP	Habituation (4 days)	Pre-exposure (2 days)	Conditioning	Test (3 days)
PD HC	Water am-pm	Saline 1% pm	Saline+ LiCl am	Saline pm
CD HC	Water am-pm	Water am-pm	Saline+ LiCl am	Saline pm
PD Sham	Water am-pm	Saline 1% pm	Saline+ LiCl am	Saline pm
CD Sham	Water am-pm	Water am-pm	Saline+ LiCl am	Saline pm

PD, pre-exposed group in the "different" condition; CD, non-pre-exposed group and "different" condition; HC, hippocampal lesion group; Sham, animals with "false" lesion in the hippocampus; am, fluid exposure at 10.00; pm, fluid exposure at 20.00; LiCl, intraperitoneal lithium chloride.