Neuroscience Letters 703 (2019) 184–190

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Lateral habenula lesions disrupt appetitive extinction, but do not affect voluntary alcohol consumption

Rocio Donaire[a], Ignacio Morón[b], Santos Blanco[c], Alvaro Villatoro[c], Fernando Gámiz[d], Mauricio R. Papini[e], Carmen Torres[a,*]

[a] Department of Psychology, University of Jaén, 23071, Spain
[b] Department of Psychobiology, Research Center for Mind, Brain, and Behavior (CIMCYC), University of Granada, Spain
[c] Department of Experimental Biology, University of Jaén, Spain
[d] Department of Psychobiology, Biomedic Research Center (CIBM), Neuroscience Institute "Federico Olóriz", University of Granada, Spain
[e] Department of Psychology, Texas Christian University, Fort Worth, USA

ARTICLE INFO

Keywords:
Lateral habenula
Reward loss
Consummatory extinction
Instrumental extinction
Voluntary alcohol consumption

ABSTRACT

This study analyzed the effects of LHb lesions on appetitive extinction and alcohol consumption. Eighteen male Wistar rats received neurochemical lesions of the LHb (quinolinic acid) and 12 received a vehicle infusion (PBS). In a runway instrumental task, rats received acquisition (12 pellets/trial, 6 trials/session, 10 sessions) and extinction training (5 sessions). In a consummatory task, rats had daily access to 32% sucrose (5 min, 10 sessions) followed by access to water (5 sessions). Then, animals received 2 h preference tests with escalating alcohol concentrations (2%–24%), followed by two 24 h preference tests with 24% alcohol. Relative to Shams, LHb lesions delayed extinction, as indicated by lower response latencies (instrumental task) and higher fluid consumption (consummatory task). LHb lesions did not affect alcohol consumption regardless of alcohol concentration or test duration. The LHb modulates appetitive extinction and needs to be considered as part of the brain circuit underlying reward loss.

1. Introduction

In rodents, the lateral habenula (LHb) receives afferents, via the stria medullaris and the inferior thalamic peduncle, from the entopeduncular nucleus, lateral hypothalamus, lateral preoptic area, medial prefrontal cortex, lateral septum, ventral pallidum, raphe nuclei, locus coeruleus, bed nucleus of the stria terminalis, and ventral tegmental area (VTA). LHb outputs join the fasciculus retroflexus to send projections to the rostromedial tegmental nucleus (RMTg), VTA, substantia nigra pars compacta, raphe nuclei, laterodorsal tegmentum, locus coeruleus, hypothalamus, several thalamic nuclei, and the nucleus accumbens [1–4]. This complex connectivity enables the LHb to integrate motivational and emotional states crucial for survival [5]. Several studies highlight a role of the LHb in reward loss (rewards whose magnitude or quality is worse than expected). Single-cell recordings in macaque monkeys found that LHb neurons were activated by stimuli predicting a small reward and by the small reward itself, as long as it was unexpected [6]. One of the consequences of this activation is the inhibition of the dopaminergic neurons in the brain reward system, an action that depends on the connections between the LHb and the RMTg (an inhibitory nucleus that projects on VTA [3]). The involvement of LHb in non-reward processing has also been observed in lesion studies showing delayed extinction after cocaine [7] or sucrose self-administration [8]. Human neuroimaging studies reveal increased LHb activity in tasks involving response errors, missing rewards and negative feedback [9–12]. However, the specific function of the LHb in reward loss situations is largely unknown.

Recent studies suggest a connection between reward loss and drug intake [13]. Rats exposed to unexpected reward omission or devaluation exhibit increased consumption of anxiolytics (alcohol, benzodiazepines [14–16]). This loss-induced increase in anxiolytics intake (referred to as emotional self-medication, ESM) could also be mediated by the LHb, since its functional manipulation affects alcohol consumption. Rats with LHb lesions given intermittent access to 20% alcohol increased voluntary intake more rapidly than sham animals [17]. This effect was dependent on lateral hypothalamus projections to the LHb [18]. Inhibition of LHb activity by high-frequency stimulation reduced voluntary alcohol consumption under similar conditions [19]. Lesions of the LHb also induced high rates of responding for alcohol in an operant self-administration task and blocked yohimbine-induced alcohol-

* Corresponding author.
E-mail address: mctorres@ujaen.es (C. Torres).

https://doi.org/10.1016/j.neulet.2019.03.044
Received 24 December 2018; Received in revised form 14 March 2019; Accepted 25 March 2019
Available online 27 March 2019

seeking reinstatement [17]. Although these results suggest that the LHb regulates voluntary alcohol consumption, whether this influence depends on alcohol concentration has not been explored. This question would clarify whether LHb modulates the rewarding vs. the aversive effects of alcohol [20], since these are dose-dependent effects [21].

This study explored the role of the LHb in appetitive extinction and alcohol intake. Animals were exposed to two appetitive tasks, consummatory and instrumental (counterbalanced), and subsequently given extinction training. Two tasks were included to assess the generality of the lesion effects and also because consummatory and instrumental tasks not always produce the same results [22]. After completing these tasks, animals underwent preference testing with escalating concentrations of alcohol (from 2 to 24%). Based on previous studies, increased resistance to extinction and increased consumption of high alcohol concentrations were expected in LHb-lesioned animals compared with sham controls.

2. Method

2.1. Subjects

Thirty 90 day-old, male, Wistar rats, weighing 388.9 ± 4.3 g, served as subjects (Harlan Laboratories, Barcelona, Spain). Rats were housed individually in polycarbonate cages with water continuously available, in a room with constant temperature (18–22 °C) and humidity (50–60%), and lights on between 08:00 and 20:00 h. Animals were food deprived and maintained within 82–85% of their ad lib weight. The experiment followed the European Union directive guidelines for the use of animals in research (2010/63/EU) and Spanish Law (6/2013; R.D.53/2013).

2.2. Apparatus

Consummatory training involved 3 Plexiglas boxes (30 × 15 × 30 cm, LxWxH). The sipper tube of a graduated cylinder was inserted through a hole located in the front wall. The 32% sucrose solution was prepared w/w by mixing 32 g of sucrose for every 68 g of distilled water. A magnetic mixer (Nahita, 680-9, Beriain, Spain) was used to dissolve the sucrose. Session length was measured with a manual stop-watch (Extech, 365510, Madrid, Spain).

For instrumental extinction training, a straight runway was used (245 × 12 × 12 cm, LxWxH), divided into three sections by two Plexiglas guillotine doors. Two sections (start, goal) were 20-cm long, the running section was 205-cm long. The walls and floor of the runways were made of black Plexiglas (0.7-mm thick). The entire length of the runway was covered by clear Plexiglas lids. Food pellets (45 mg, formula P; Research Diets, Lancaster, NH, USA) were used as the reward. Time to run through the runway was manually registered with a manual chronometer (see above). Trials began when the start door was raised and ends when the rat entered the goal section with its four paws.

Access to alcohol and water in the preference tests was provided in home cages (32 × 15 × 30 cm, LxWxH) with the floor covered with saw dust and containing two 150-ml plastic bottles. Fluid consumption was measured by weighing the bottles before and after each preference session (Cobos, JT-300C Digital Scale, Barcelona, Spain). Alcohol (96%, Panreac, Castellar del Vallés, Spain) was diluted in tap water on a v/v basis. Animals were weighed daily (Baxtran, BS3, Girona, Spain).

2.3. Procedure

Fig. 1 shows a timeline of the general procedure.

2.3.1. Surgery

Animals were randomly assigned to the LHb (n = 18) or sham (n = 12) condition, and anesthetized with ketamine (150 mg/kg, ip) and xylazine (5 mg/kg, ip). Once anesthetized, the rat's head was shaved and cleaned with betadine, and then set in a stereotaxic apparatus (Digital Lab Standard Stereotaxic, Stoelting, Dublin, Ireland). A midline incision was made, the skull was scrapped clean of connective tissue, and bregma was located. Quinolinic acid (Sigma Aldrich, Madrid, Spain), 0.12 M, dissolved in a 10% phosphate buffered saline (PBS) solution, titrated to pH 7.4 with sodium hydroxide, was used as neurotoxin and administered with an infusion pump (Harvard Apparatus, 11 Elite, Holliston, MA, USA). The neurotoxin was infused in a volume of 0.175 µL, at a rate of 0.1 µL/min, over a period of 1:40 s. The injection cannula was kept at the lesion site for 1:30 min to facilitate the flow of the toxin. Four infusions were made, two in each hemisphere: AP: −3.1, ML: ± 0.7, DV: −4.7, and AP: -3.6, ML: ± 0.75, DV: −5 [23]. Once the infusion procedure was concluded, the incision was closed with stitches, the suture was cleaned, and the animal was placed in a polycarbonate box under a light providing heat until the anesthesia wore off. Behavioral tests began when all animals were recovered and completed their food deprivation schedule.

2.3.2. Behavior

Two (counterbalanced) appetitive extinction tasks and an alcohol preference test were conducted. Animals were fed at least 30 min after the end of the corresponding test.

For the consummatory task, animals were placed in the box for a 5-min habituation session without fluids. On Days 1–10 (acquisition) animals had free access to 32% sucrose. On Days 11–15 (extinction), animals received water. Each session lasted 5 min starting from the first contact with the sipper tube. Rats were transported in squads of 3 animals, all from the same group, with the order of squads randomized across days.

For the instrumental task, animals were transported in squads of 5 animals, with the squad order randomized across days. Rats received three habituation sessions (see [14] for details). Training began on Day 4: animals were placed in the start box, the start door was opened and the rat was allowed to run down the runway to obtain the reward (12 pellets). A maximum time of 40 s was allowed to complete the trial. As soon as the rat finished eating or 30 s had elapsed, it was placed back in its home cage for a 10-min intertrial interval. Each rat received 6 trials per day during 10 acquisition sessions. In extinction (5 sessions), no food was present and rats were enclosed in the goal box for 30 s.

The preference test started after completing extinction training. Animals were habituated on Days 1–4 to the two-bottle procedure with both bottles containing tap water [15]. On Days 5–20, animals received increasing concentrations of alcohol in one bottle and water in the other. The concentrations of alcohol were 2, 4, 6, 8, 10, 12, 16, and 24%. Each concentration was presented for two consecutive days. On Days 1–20, alcohol preference tests were 2-h long. This testing procedure was used before in our lab [14,15]. On Days 21–22, animals had access to the highest concentration (24%) during 24 h, a procedure also used before in our lab [21]. The position of the bottles was changed daily to minimize the effects of side preference.

2.4. Histology and astrocyte count

Rats were anaesthetized (sodium pentobarbital 5 mg diluted in 10 ml 0,9% physiologic serum; 0.1 ml/100 g animal weight) and perfused with 0.01 M phosphate-buffered saline (PBS; pH 7.4), and then with 300 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB). The brains were removed and then post-fixed for a further 4 h in the same fixative at room temperature. Samples were then cryoprotected by immersion overnight at 4 °C in 0.1 M PB containing 30% sucrose. After this, they were embedded in OCT (Optimal Cutting Temperature; Sakura, Alphen aan den Rijn,The Netherlands) and the brain was let solidify with Peltier system. Serial 40 µm coronal sections were prepared using a cryostat (Leica Microsystems CM1950, Barcelona, Spain) and stained with immunohistochemistry to label the astrocytic

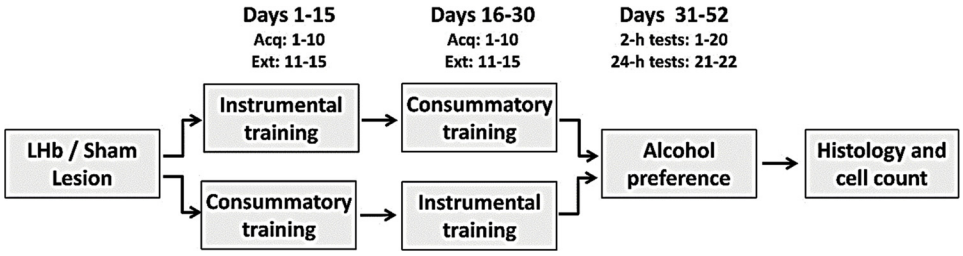

Fig. 1. A timeline of the procedures implemented in this experiment.

intermediate filaments protein Glial Fibrillary Acidic Protein (GFAP), thus analyzing the effect of quinolinic acid on the astrocytic population. Astrocyte proliferation and a dense, long-lasting astrogliosis have been found after quinolinic acid infusions [24].

Free-floating sections were treated as follows. The endogenous peroxidase was inhibited by a hydrogen peroxide (Panreac, Barcelona, Spain) by 0,3% (v/v) in PBS 001 M solution, during 30 min, at room temperature and in stirring. Sections were washed with PBS solution by 3 changes of 5 min at room temperature and in a slow agitation. Slides were incubated (4 h) in PBS containing 0.1% Triton X-100 (Panreac, Barcelona, Spain), and then in rabbit polyclonal anti-GFAP (Santa Cruz Biotechnology, Heidelberg, Germany), 1:500 overnight in PBS containing 0.2% Triton X-100 (Panreac, Barcelona, Spain). After 3 rinses in PBS for 5 min, sections were incubated with biotinylated goat anti-rabbit IgG, 1:100 (Standard Ultra-Sensitive ABC Staining Kit, Thermo Scientific, Walthan, MA). Sections were then washed in PBS 3 times for 5 min, processed by the avidin-biotin peroxidase complex (ABC) procedure (Pierce, Rockford, IL, USA) and washed 3 times for 5 min in acetate buffer pH 6. The peroxidase activity was demonstrated following the nickel-enhanced diaminobenzidine assay [25]. Sections were then mounted on slides, dehydrated, and covered using DPX.

The A/P position of sections was identified with the Paxinos and Watson atlas [23]. Brain slices located at -3.30 in the A/P axis were selected for astrocyte count, conducted by two observers blind to the surgical condition. A Ura Technic Professional microscope with a 100-square integrating graticule mounted in an eyepiece (Zuzi XSZ-I07BN, Barcelona, Spain) and a 10X objective was used to count astrocytes in the target area (700 μm^2) bilaterally.

2.5. Statistics

In the consummatory task, the mean fluid intake on extinction sessions 11–13 was divided by the fluid intake on acquisition session 10 and used as dependent variable. The mean response latency on extinction sessions 11–13 was divided by the mean latency on acquisition session 10 in the instrumental task. Alcohol consumption (ml/kg, g/kg), water consumption (ml/kg), and preference ratio (alcohol consumption/total consumption) were registered in preference tests. The number of astrocytes identified in the left plus the right hemisphere of the selected brain slices was used as the dependent variable for the histological analysis.

Analyses of variance were calculated for each dependent variable with an alpha value set at the 0.05 level. Partial eta square (η^2) was used to compute effect size for all significant effects. Where appropriate, pairwise comparisons were computed with the LSD test. Performance registered in the consummatory and the instrumental task, as well as astrocytes count values were subjected to a one-way analyses of variance, with Group (LHb vs Sham) as factor. In the preference task, alcohol consumption, water intake, and preference ratio registered in the 2-h tests were subjected to a three-factor, mixed-model analysis of variance, with Group (LHb vs Sham), Bottle (alcohol vs. water), and Concentration (2–24%) as factors, the last two with repeated measures. The values corresponding to the 24-h test were analyzed separately. Statistics were calculated with IBM SPSS Statistics 24.

3. Results

3.1. Histology

One brain was lost during the slicing procedure. In 5 animals the histological material was inadequate to compute astrocyte counting. In 5 brains the portion of the brain where LHb was located was not selected for immunolabelling. The remaining 17 brains (11 LHb, 6 Sham) were included in the histological analysis. Each animal contributed to the final computation with 1–6 slices corresponding to the A/P −3.30 coordinate. When the astrocyte count was assessed in more than one slice, a mean was calculated for the animal. Data were ranked and overlapped LHb (5) and Sham (2) brains (in terms of number of astrocytes) were eliminated. Fig. 2, top, shows the result of the remaining animals. Rats with LHb lesions had a significantly higher astrocyte count than rats with Sham lesions, $F(1, 8) = 12.585$, $p < 0.009$, $\eta^2 = 0.61$. Selected slices treated with GFAP immunolabelling and cresyl violet are shown in Fig. 2, bottom. As expected (e.g. [24]), quinolinic acid infusions produced astrogliosis.

3.2. Extinction tasks

There was no evidence that the LHb lesion affected appetitive acquisition. The overall mean (± SEM) fluid intake for sessions 1–10 in the consummatory task was 8.2 ml (± 0.8) for LHb rats and 8.4 ml (± 0.7) for Sham rats ($F < 1$). In the instrumental task, one Sham animal had long latencies during the initial sessions, but its latencies were similar to other animals in later acquisition sessions. The mean (± SEM) latency was 2.6 s (± 0.2) for LHb animals and 6.44 s (± 2.27) for Sham animals. Still, the difference was not significant, $F(1, 8) = 3.03$, $p > 0.11$.

Fig. 3, top, shows relative sucrose consumption registered during the extinction phase of the consummatory task. LHb animals showed higher levels of fluid intake than Sham animals. A one-way analysis revealed a significant difference, $F(1, 8) = 6.09$, $p < 0.04$, $\eta^2 = 0.43$. This difference was not due to differences in drinking behavior. Fig. 3, bottom, shows water intake during the four habituation sessions of the preference test ($F < 1$).

Fig. 4, top, shows relative latency data registered during the extinction phase of the instrumental task. LHb animals exhibited significantly lower response latencies than Sham animals, $F(1, 8) = 5.47$, $p < 0.05$, $\eta^2 = 0.41$. Fig. 4, bottom, shows that these differences were not attributable to body weight, which was similar in both groups ($F < 1$).

3.3. Preference test

Fig. 5, top, presents alcohol and water consumption for both groups. Across all concentrations and in both groups, animals preferred alcohol to water, although the preference diminished as alcohol concentration increased. A Lesion (LHb, Sham) by Bottle (Alcohol, Water) by Concentration (2–24%) analysis, with repeated measures for the last two factors, yielded the following results. There was a significant reduction in consumption across concentrations, $F(7, 56) = 10.24$, $p < 0.001$, η

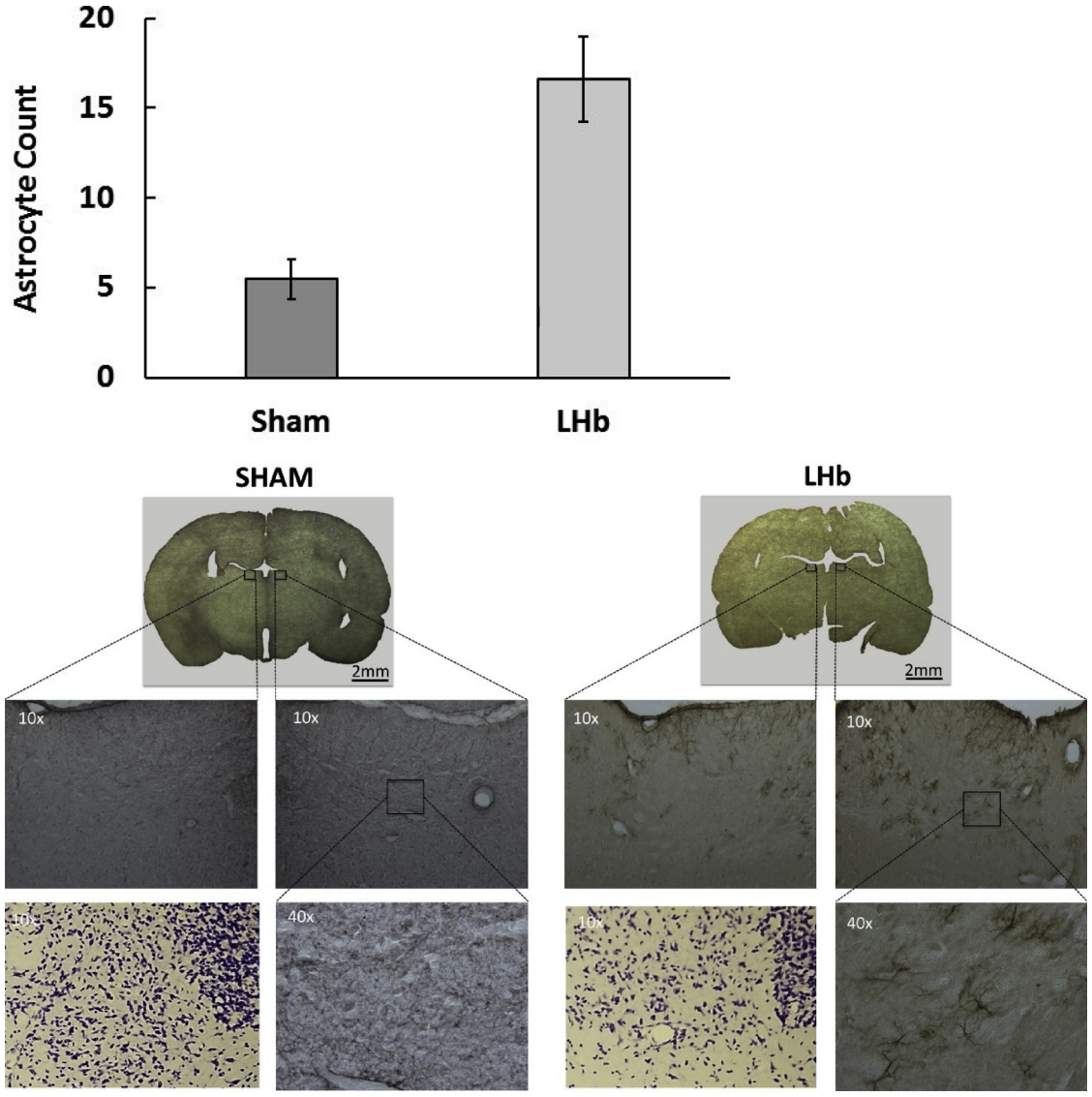

Fig. 2. Top: Mean (± SEM) number of astrocytes for groups LHb and Sham. Bottom: Representative brain slices (A/P: −3.30) showing reactivity for GFAP in the LHb after quinolinic acid (right panel) or vehicle infusions (left panel). Squares point to the region shown below in enlarged images (10X, 40X). For each group, the lower left image was obtained with cresyl violet staining.

^{2} = 0.56, and significantly more consumption of alcohol than water, F (1, 8) = 131.36, $p < 0.001$, $\eta^2 = 0.94$. The concentration by bottle interaction also reached a significant level, as alcohol intake was reduced with increasing concentrations, $F(7, 56) = 9.16$, $p < 0.001$, $\eta^2 = 0.53$. Despite this significant interaction, alcohol consumption was significantly higher than water consumption at all concentrations, as revealed by LSD tests, $ps < 0.002$. None of the factors involving Lesion was significant, $Fs < 1$.

Fig. 5, middle, shows the preference ratio (alcohol consumption/ total consumption). All ratios were above the 0.5 indifference level, thus showing preference for alcohol across all concentrations. A Lesion by Concentration analysis revealed a significant reduction in preference ratio across concentrations, $F(7, 67) = 3.62$, $p < 0.004$, $\eta^2 = 0.31$, but no lesion or lesion by concentration effects, $Fs < 1.54$, $ps > 0.16$.

Alcohol consumption in g/kg increased at about the same rate in LHb and Sham animals. A Lesion x Concentration analysis confirmed this conclusion. There was a significant increase in alcohol consumption across concentrations, $F(7, 56) = 20.11$, $p < 0.001$, $\eta^2 = 0.72$, but no lesion or lesion by concentration effects, $Fs < 1$.

The results of the 24-h preference test are presented in Fig. 6, top, showing that animals reversed their preference and consumed more water than alcohol, $F(1, 8) = 18.30$, $p < 0.004$, $\eta^2 = 0.70$. No Lesion effects were observed either in terms of preference or consumption (Fig. 6, middle and bottom), $Fs < 1.05$, $ps > 0.33$.

4. Discussion

The LHb has been shown to regulate behaviors driven by negative outcomes, including reward loss and drug-evoked aversive states [26]. In the present experiment LHb and Sham animals were exposed to two extinction tasks and, once concluded, to a preference test with increasing concentrations of ethanol. We expected that LHb lesions would disrupt extinction and enhance alcohol consumption, especially at high concentrations. These predictions were partially confirmed: LHb lesions significantly delayed extinction, as indicated by higher fluid consumption in the consummatory task and lower response latencies in the instrumental task. By contrast, the lesion did not affect alcohol intake and preference, regardless the concentration and length of the preference test.

Evidence suggests that the LHb is involved in processing reward events that include negative or aversive components. For example, LHb neurons are activated by unexpected small rewards or their signals, and

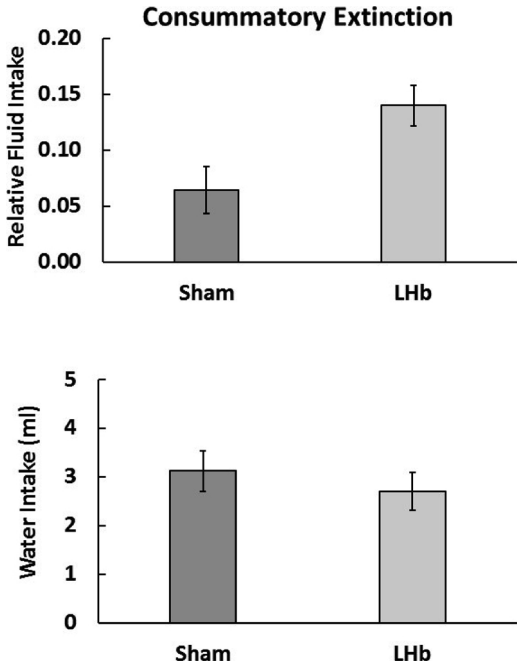

Fig. 3. Top: Mean (± SEMs) relative sucrose intake during consummatory extinction. Bottom: Water intake during habituation sessions prior to preference tests.

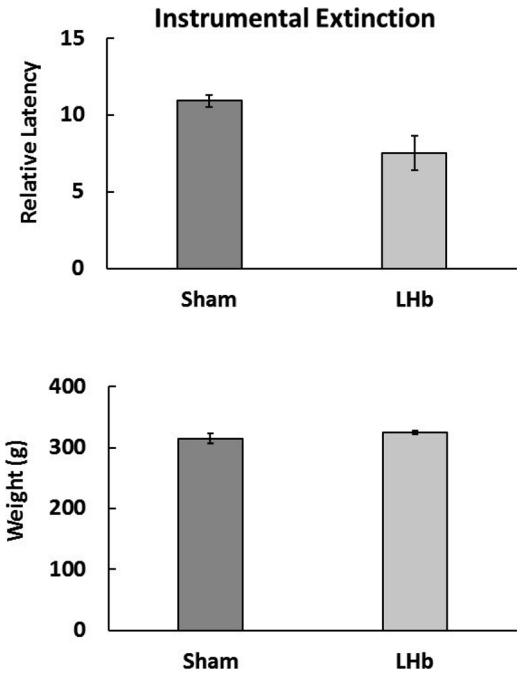

Fig. 4. Top: Mean (± SEMs) relative latency during instrumental extinction. Bottom: Body weight during habituation sessions prior to preference tests.

inhibited by surprising large rewards or their signals [6]. Interestingly, Genn, Ahn, and Phillips [27] found reduced dopamine efflux in the nucleus accumbens (NAc) during a 32–4% sucrose downshift task, relative to unshifted 4% sucrose controls. Genn et al. [27] hypothesized that dopamine efflux in the NAc reflects the current reward value of sucrose—lower in downshifted animals compared to unshifted controls. As discussed above, this function would be modulated by an inhibitory LHb-RMTg-VTA-NAc pathway [28]. In the present experiment, LHb lesions could have disrupted this pathway, interfering with the detection of reward omission and thus delaying response extinction.

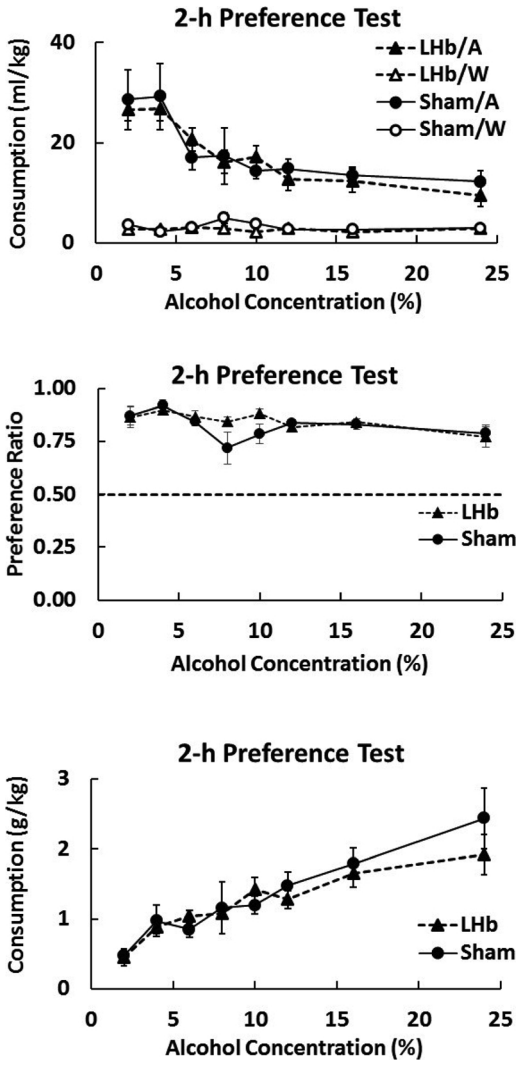

Fig. 5. Top: Mean (± SEM) alcohol and water consumption as a function of lesion and alcohol concentration. Middle: Mean (± SEM) preference ratio (alcohol consumption/total consumption) as a function of lesion and alcohol concentration. Dashed line: indifference; preference scores above 0.5 indicate preference for alcohol over water. Bottom: Mean (± SEM) alcohol consumption in g/kg as a function of lesion and alcohol concentration. Data from 2 h preference tests.

Comparable results were reported in operant self-administration tasks involving sucrose or cocaine [7,8]. LHb lesions did not affect sucrose or cocaine self-administration during acquisition training, a result comparable to the lack of a lesion effect found in the present experiment during acquisition. Therefore, rather than reducing reward value [7], stimulating motor behavior [29], or increasing distractability [30], LHb lesions impaired the detection of reward absence (extinction), therefore disrupting the ability to discriminate the transition from acquisition to extinction.

The LHb has been also involved in alcohol intake [20]. Alcohol exposure facilitated the pacemaker firing *in vitro* and increased c-fos protein expression *in vivo* in LHb neurons [31]. Moreover, LHb lesions attenuated a conditioned taste aversion induced by systemic administration of 0.7 g/kg of alcohol [17]. Since there is an inverse relationship between alcohol-induced conditioned taste aversion and voluntary alcohol consumption [32], these results suggested that LHb lesions interfered with the aversive properties of alcohol. Additionally, LHb lesions increased voluntary consumption of 20% alcohol in an intermittent-access procedure, an effect that was dependent on lateral

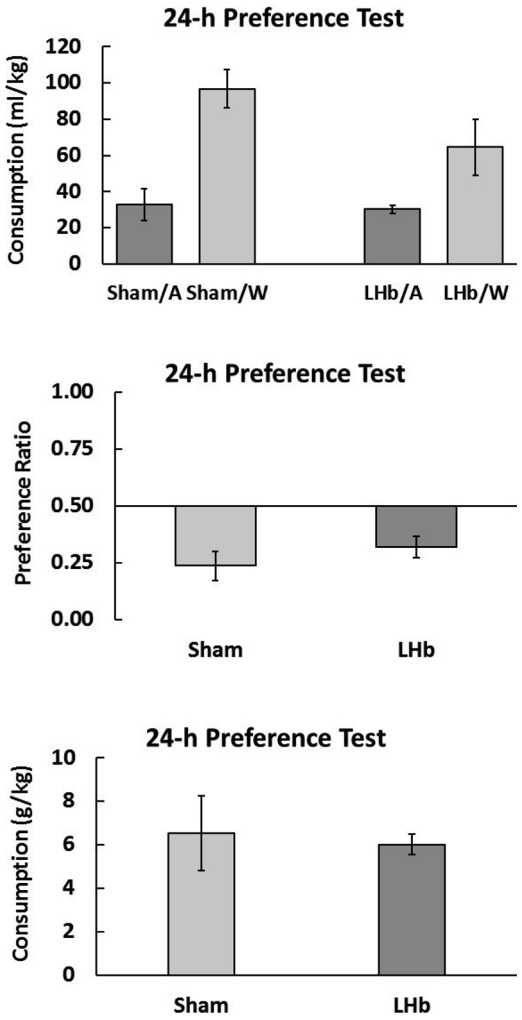

Fig. 6. Top: Mean (± SEM) alcohol and water consumption as a function of lesion. Middle: Mean (± SEM) preference ratio (alcohol consumption/total consumption) as a function of lesion. Line: indifference; preference scores below 0.5 indicate preference for water over alcohol. Bottom: Mean (± SEM) alcohol consumption in g/kg as a function of lesion. Data from 24 h preference tests.

hypothalamus input to the LHb [18].

However, there was no evidence of an effect of LHb lesions on alcohol consumption in the present study. Three reasons for the disparity between previous and present results are possible. First, prior exposure to sucrose and food in the appetitive tasks could have attenuated the aversive components of alcohol consumption, thus masking possible effects of the LHb lesion. Second, rats did not consume large amounts of alcohol in this testing procedure (see Figs. 5 and 6), a fact that could obscure effects of the LHb lesions. Third, perhaps gradually increasing the concentrations minimized the aversive value of alcohol preventing the effects of the LHb lesions to be detected. In previous studies [17–19], alcohol preference was below indifference in at least half of the sessions, consistent with an aversion to 20% alcohol relative to water in both LHb and Sham animals. By contrast, high levels of alcohol preference were found here (Fig. 5), suggesting that alcohol had appetitive value. However, when the highest concentration (24%) was presented for 24 h, animals exhibited a preference for water over alcohol (Fig. 6), a result resembling the aversion observed in the studies described above, but an effect of the LHb lesion was still not observed. The lack of effects of LHb lesions on alcohol intake under the 2-h test conditions, corresponding to the procedure used in ESM experiments [14,15,33], encourages a look at the role of the LHb on alcohol consumption induced by reward loss in future studies.

Reward loss recruits cognitive, motivational, and emotional processes [13,16,34]. It is tempting to attribute a pivotal role to the LHb in integrating these processes based on its connections with brain regions involved in the processing of absolute reward, relative reward, and emotion [9]. However, whether the LHb is involved in processing absolute reward, relative reward, expectancy violation, and/or negative emotion will require additional research.

Acknowledgments

Research supported by Grants (PSI-2013-44945-P and PSI2017-87340-P) to CT and by a Fulbright US Scholar Award to MRP. The authors thank A. Ibáñez and A. Chica for their assistance, and the University of Jaén for services provided (SCAI).

References

[1] I.H. Bianco, S.W. Wilson, The habenular nuclei: a conserved asymmetric relay station in the vertebrate brain, Philos. Trans. R. Soc. Lond., B, Biol. Sci. 364 (2009) 1005–1020.
[2] L. Lecourtier, P.H. Kelly, A conductor hidden in the orchestra? Role of the habenular complex in monoamine transmission and cognition, Neurosci. Biobehav. Rev. 31 (2007) 658–672.
[3] E.T. Rolls, The roles of the orbitofrontal cortex via the habenula in non-reward and depression, and in the responses of serotonin and dopamine neurons, Neurosci. Biobehav. Rev. 75 (2017) 331–334.
[4] D.S. Zahm, D.H. Root, Review of the cytology and connections of the lateral habenula, an avatar of adaptive behaving, Pharmacol. Biochem. Behav. 162 (2017) 3–21.
[5] O. Hikosaka, The habenula: from stress evasion to value-based decision-making, Nat. Rev. Neurosci. 11 (2010) 503–513.
[6] M. Matsumoto, O. Hikosaka, Lateral habenula as a source of negative reward signal on dopamine neurons, Nature 447 (2007) 1111–1115.
[7] A. Friedman, E. Lax, Y. Dikshtein, L. Abraham, Y. Flaumenhaft, E. Sudai, M. Ben-Tzion, L. Ami-Ad, R. Yaka, G. Yadid, Electrical stimulation of the lateral habenula produces enduring inhibitory effect on cocaine seeking behavior, Neuropharmacol. 59 (2010) 452–459.
[8] A. Friedman, E. Lax, Y. Dikshtein, L. Abraham, Y. Flaumenhaft, E. Sudai, M. Ben-Tzion, G. Yadid, Electrical stimulation of the lateral habenula produces an inhibitory effect on sucrose self-administration, Neuropharmacol. 60 (2011) 381–387.
[9] S. Ide, C.-S.R. Li, Error-related functional connectivity of the habenula in humans, Front. Hum. Neurosci. 5 (2011) 1–13.
[10] C.S. Li, P. Yan, H.H. Chao, R. Sinha, P. Paliwal, R.T. Constable, S. Zhang, T.W. Lee, Error-specific medial cortical and subcortical activity during the stop signal task: a functional magnetic resonance imaging study, Neurosci. 155 (2008) 1142–1151.
[11] R. Salas, P. Baldwin, M. De Biasi, R. Montague, BOLD responses to negative reward prediction errors in human habenula, Front. Hum. Neurosci. 4 (2010) 36.
[12] M. Ullsperger, D.Y. von Cramon, Error monitoring using external feedback: specific roles of the habenular complex, the reward system and the cingulate motor area revealed by functional magnetic resonance imaging, J. Neurosci. 23 (2003) 4308–4314.
[13] C. Torres, M.R. Papini, Emotional self-medication and addiction, in: V.R. Preedy (Ed.), The Neuropathology of Drug Addictions and Substance Misuse, vol. I, Elsevier Inc., 2016, pp. 71–81.
[14] L. Manzo, M.J. Gómez, J.E. Callejas-Aguilera, A. Fernández-Teruel, M.R. Papini, C. Torres, Anti-anxiety self-medication induced by incentive loss in rats, Physiol. Behav. 123 (2014) 86–92.
[15] L. Manzo, R. Donaire, M. Sabariego, M.R. Papini, C. Torres, Anti-anxiety self-medication in rats: oral consumption of chlordiazepoxide and ethanol after reward devaluation, Behav. Brain Res. 278 (2015) 90–97.
[16] M.R. Papini, P.M. Fuchs, C. Torres, Behavioral neuroscience of psychological pain, Neurosci. Biobehav. Rev. 48 (2015) 53–69.
[17] A.K. Haack, C. Sheth, A.L. Schwager, M.S. Sinclair, S.S. Tandon, A. Taha, Lesions of the lateral habenula increase voluntary ethanol consumption and operant self-administration, block yohimbine-induced reinstatement of ethanol seeking, and attenuate ethanol-induced conditioned taste aversion, PLoS One 9 (2014) e92701.
[18] C. Sheth, T.M. Furlong, K.A. Keefe, S.A. Taha, The lateral hypothalamus to lateral habenula projection, but not the ventral pallidum to lateral habenula projection, regulates voluntary ethanol consumption, Behav. Brain Res. 328 (2017) 195–208.
[19] J. Li, W. Zuo, R. Fu, G. Xie, A. Kaur, A. Bekker, J.H. Ye, High frequency electrical stimulation of lateral habenula reduces voluntary ethanol consumption in rats, Int. J. Neuropsychopharmacol. 19 (2016) 1–8.
[20] A. Shah, W. Zuo, S. Kang, J. Li, R. Fu, H. Zhang, A. Bekker, J.-H. Ye, The lateral habenula and alcohol: role of glutamate and M-type potassium channels, Pharmacol. Biochem. Behav. 162 (2017) 94–102.
[21] L. Manzo, M.J. Gómez, J.E. Callejas-Aguilera, A. Fernández-Teruel, M.R. Papini, C. Torres, Oral ethanol self-administration in inbred Roman high-and low-avoidance rats: gradual versus abrupt ethanol presentation, Physiol. Behav. 108

(2012) 1–5.
[22] A.E. Mustaca, M. Bentosela, E. Ruetti, G. Kamenetzky, L. Cuenya, N. Justel, F. Lopez Seal, S. Fosacheca, M.R. Papini, Similitudes y discrepancias en dos modelos animales de frustración, in: M.C. Richaud, E. Moreno (Eds.), Recientes Avances En Investigación En Ciencias Del Comportamiento, vol. 2, CIIPME-CONICET, Buenos Aires, Argentina, 2009, pp. 921–940.
[23] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, Academic press, New York, 2013.
[24] M. Dihné, F. Block, H. Korr, R. Töpper, Time course of glial proliferation and glial apoptosis following excitotoxic CNS injury, Brain Res. 902 (2001) 178–189.
[25] S.Y. Shu, G. Ju, L.Z. Fan, The glucose oxidase-DAB-nickel method in peroxidase histochemistry of the nervous system, Neurosci. Lett. 85 (1988) 169–171.
[26] S. Lecca, F.J. Meye, M. Mameli, The lateral habenula in addiction and depression: an anatomical, synaptic and behavioral overview, Eur. J. Neurosci. 39 (2014) 1170–1178.
[27] R.F. Genn, S. Ahn, A.G. Phillips, Attenuated dopamine efflux in the rat nucleus accumbens during successive negative contrast, Behav. Neurosci. 118 (2004) 869.
[28] S.G. Nair, N.S. Strand, J.F. Neumairer, DREADDing the lateral habenula: a review of methodological approaches for studying lateral habenula function, Brain Res. 1511 (2013) 93–101.
[29] E.H. Lee, S.L. Huang, Role of lateral habenula in the regulation of exploratory behavior and its relationship to stress in rats, Behav. Brain Res. 30 (1988) 265–271.
[30] L. Lecourtier, P.H. Kelly, Bilateral lesions of the habenula induce attentional disturbances in rats, Neuropsychopharmacol. 30 (2005) 484–496.
[31] W. Zuo, R. Fu, F.W. Hopf, G. Xie, K. Krnjević, J. Li, J.-H. Ye, Ethanol drives aversive conditioning through dopamine I receptor and glutamate receptor-mediated activation of lateral habenula neurons, Addict. Biol. 22 (2015) 103–116.
[32] A.S. Green, N.J. Grahame, Ethanol drinking in rodents: is free-choice drinking related to the reinforcing effects of ethanol? Alcohol 42 (2008) 1–11.
[33] R. Donaire, S.E. Conrad, J.B. Thompson, M.R. Papini, C. Torres, Augmented voluntary consumption of ethanol induced by reward downshift increases locomotor activity of male Wistar rats in the elevated plus maze, Behav. Proc. 150 (2018) 59–65.
[34] L.A. Ortega, J.L. Solano, C. Torres, M.R. Papini, Reward loss and addiction: opportunities for cross-pollination, Pharmacol. Biochem. Behav. 154 (2017) 39–52.