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Learned preferences induced by electrical stimulation of a food-related area of the parabrachial complex: Effects of naloxone

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Abstract

Electrical stimulation of the External Lateral Parabrachial Subnucleus (LPBe), a food-related area, induced behavioral preferences for associated stimuli in a taste discrimination learning task. Although this stimulation appeared to be ineffective to elicit standard lever press self-stimulation, it induced place preference for one of two training compartments of a rectangular maze in which animals (adult male Wistar rats) received concurrent electrical brain stimulation. In subjects that consistently showed a preference behavior in different trials, administration of the opioid antagonist naloxone (4 mg/ml/kg) blocked concurrent learning when the test was made in a new maze but not in the same maze in which animals had learned the task. These results are discussed in terms of the possible participation of the LPBe subnucleus in different natural and artificial brain reward systems.

Keywords: Electrical brain stimulation; Opioids; Parabrachial nucleus; Place preference; Reward; Taste preference

1. Introduction

Various studies have demonstrated involvement of the Parabrachial Complex in several "motivated behaviors" (Le Magnen, 1992; Ritter, Calingasan, Hutton, & Dinh, 1992b). Thus, the external lateral subnucleus (*LPBe*), found at the dorsolateral end of this anatomical complex (Fulwiler & Saper, 1984; Herbert & Bellintani-Guardia, 1995), is involved in both gustatory information, from the rostral nucleus of the solitary tract (NST), and visceral information, from the caudal NST and Area Postrema (AP) (De Lacalle & Saper, 2000; Halsell & Travers, 1997; Karimnamazi, Travers, & Travers, 2002; Papas & Ferguson, 1990).

Based on the study of the sensory information received by the LPBe, several authors have implicated this subnucleus in taste aversion learning, especially after the administration of copper sulfate, morphine, amphetamines or cocaine, among other drugs (Sakai & Yamamoto, 1997), and particularly in tasks requiring a neural processing of visceral information (Mediavilla, Molina, & Puerto, 2000a, 2005). However, the LPBe has also been related to reward mechanisms. Thus, duodenal loading with glucose (Wang, Cardin, Martinez, Tache, & Lloyd, 1999) and gastric loading with ethanol, lactose, or sucrose (Yamamoto & Sawa, 2000a, 2000b) elicited c-fos-like immunoreactivity in its lateral end. Conversely, lesions to this lateral end of the parabrachial area attenuated over-ingestion of highly palatable food produced by AP lesions (Edwards & Ritter, 1989) and blocked taste preferences induced by administration of rewarding foods (Zafra, Simon, Molina, & Puerto, 2002). Likewise, it has been proposed that the LPBe may be associated with the effects of various endogenous intake-related substances, such as cholecystokinin (CCK) (Li & Rowland, 1995; Trifunovic & Reilly, 2001), or leptin (Elias et al., 2000).

Finally, it has been shown that a number of drugs that are rewarding and/or related to food intake control may be processed via the LPBe, e.g., fenfluramine (Li &

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Rowland, 1995; Li, Spector, & Rowland, 1994; Simansky & Nicklous, 2002; Trifunovic & Reilly, 2001), amphetamines (Sakai & Yamamoto, 1997), and opiates (Chamberlin, Mansour, Watson, & Saper, 1999; Ding, Kaneko, Nomura, & Mizuno, 1996; Gutstein, Thome, Fine, Watson, & Akil, 1998). In fact, it has been shown that the latter receptors can be modulated by food restriction (Wolinsky, Carr, Hiller, & Simon, 1996). These studies suggest that, besides its known involvement in the aversive system, the LPBe may be involved in motivational systems related to the processing of positive, appetizing, or rewarding stimuli (Agüero, Arnedo, Gallo, & Puerto, 1993a, 1993b; Mediavilla et al., 2000a; Reilly, 1999; Sakai & Yamamoto, 1997, 1998; Swank & Bernstein, 1994; Yamamoto, Shimura, Sakai, & Ozaki, 1994).

Therefore, we hypothesized that intracerebral electrical stimulation, a technique that has proven to be an effective substitute for noxious or rewarding stimuli in taste discrimination tasks (Agüero, Arnedo, Gallo, & Puerto, 1993b; Cubero & Puerto, 2000; Gallo, Arnedo, Agüero, & Puerto, 1988), could also act in the LPBe as a rewarding stimulus in both taste discrimination tasks and in conditioned place preference tasks.

The question arises whether the reinforcing effect of LPBe electrical stimulation is specific to a taste discrimination task or might be extended to other types of task in which, for example, there is a predominance of place cues (Bardo & Bevins, 2000; Tzschentke, 1998), characteristic of Conditioned Place Preference (CPP) paradigms, which have proven an adequate procedure for research into brain reward systems (Schechter & Calcagnetti, 1998; Shippenberg & Elmer, 1998; Spiteri, Le Pape, & Agmo, 2000). In this context, opiates have been implicated in hedonic and rewarding aspects of natural (e.g., food intake) (Carr & Papadouka, 1994; Le Magnen, 1992; Papadouka & Carr, 1994) and artificial (e.g., drugs of abuse or brain self-stimulation) (Bielajew, Diotte, & Milairessis, 2003; De Vries & Shippenberg, 2002; Fernandez-Espejo, 2002; Shippenberg & Elmer, 1998; Spanagel, Herz, & Shippenberg, 1992) substances/procedures. Given the presence of opiate mechanisms in the LPBe (Carr, Aleman, Bak, & Simon, 1991; Chamberlin et al., 1999; Engström et al., 2001; Gutstein et al., 1998; Moufid-Bellancourt, Razafimanalina, & Velley, 1996; Wolinsky et al., 1996), the present study was designed to investigate the possibility of blocking the rewarding effects of LPBe electrical stimulation by administration of an antagonist of the opiate system, i.e., naloxone.

2. Materials and methods

2.1. Subjects and surgery

Male Wistar rats from the breeding colony at the University of Granada, weighing between 270 and 360 g at the time of surgery, were used in this study. Upon their arrival at the lab, animals were housed individually in $30 \times 15 \times 30$ cm cages. The room was maintained on a 12-h light/12-h dark cycle at 22–24 °C. All behavioral procedures and surgical or pharmacological techniques were conducted in agreement with the animal care guidelines established by the Spanish Royal Law, 223/1988.

Animals were implanted with a monopolar electrode (diameter of approximately 200 μm) in the LPBe [Coordinates: AP = −0.16; $V = 3.0$; $L = \pm 2.5$; Paxinos and Watson (1996)]. Different modalities of control groups were used in each experiment, with similar results.

-Experiment 1 used 14 animals with electrode implanted in the LPBe and 10 animals (controls) with electrode implanted 0.6 mm above the LPBe.

-Experiment 2 used 33 animals with electrode implanted in the LPBe and seven animals (controls) with electrode placed over the cranial surface and around four small jewelry screws without penetrating the brain.

-Experiment 3 used 36 animals with electrode implanted in the LPBe; 28 of these were used as stimulated group and eight as non-stimulated group (controls).

Surgery was carried out under general anesthesia with sodium pentothal (50 mg/kg B. Braun Medical S.A. Barcelona. Spain). Once anesthetized, the animals were placed in a stereotaxic device (Stoelting Co. Stereotaxic 51600, USA) and a small trephine hole was drilled to allow chronic implantation of active electrodes (Hawkins, Roll, Puerto, & Yeomans, 1983). Electrodes were lowered in the LPBe nucleus and fixed to the skull with acrylic dental resin (S.R. Denture Base, Quick 3/60, Ivoclar. Liechtenstein). Current return was by a stainless steel wire (0.9 mm) wrapped around four anchoring screws placed in the skull. In order to avoid risk of infection, subjects were given an intramuscular (i.m.) 0.1-cc. dose of penicillin (250,000 IU/ml Benzetacil 6-3-3, Antibióticos Farma S.A., Madrid, Spain) and an antiseptic solution was applied locally on the implant (Betadine, Asta Médica, Madrid, Spain).

After the surgery, animals were returned to their cages where they stayed for at least 7–10 days of recovery with water and food ad libitum (Laboratory Food, A-04 Rat-mouse maintenance, Panlab Diets S.L., Barcelona, Spain).

2.2. Apparatus

Electrical stimulation was supplied (Experiments 1 and 2) via an LI12100 stimulator (Letica, Barcelona, Spain) and CS-20 stimulator (Cibertec, Madrid, Spain) (Experiment 3) connected to an ISU isolation unit 165 (Cibertec, Madrid, Spain). Cathodal rectangular pulses (66.6 Hz, 0.1 ms) were applied to the LPBe at a current below the threshold for producing undesired behavioral effects (Gallistel & Karras, 1984). The stimulation process was monitored with a DM63 oscilloscope (Textronic Ltd, London, UK), which allowed constant visualization of the electrical pulses administered to animals during experimental sessions.

In Experiment 1, the same cages in which animals were housed on their arrival at the laboratory (home cages) were used as training chamber. The sides of the cages were black and opaque; the front and back panels were transparent. The front side had two 1.6 cm holes at the same distance from the center and edges and at the same height above the floor of the cage. Through those orifices, the animal had access to spouts attached to cylindrical graduated burettes for delivery of flavors and water (See Fig. 1 in Mediavilla, Molina, & Puerto, 2005).

An unbiased, counterbalanced concurrent CPP procedure was used for Experiments 2 and 3. Animals were concurrently stimulated in one of two distinct open compartments of a rectangular maze that differed in color, texture, and wall drawings. These training compartments were separated by a narrow neutral area on which the animal was placed at the start of each test session.

Two different models of maze were utilized:

Model 1: Rectangular maze ($50 \times 25 \times 30$ cm), in which the walls of the two lateral compartments were painted with black and white 1-cm wide stripes that were vertical in one compartment and horizontal in the other. In one compartment, the floor was synthetic cork painted with black and white stripes and in the other it was brown cork. The floor of the central area (8×25 cm^2) was white methacrylate, and the walls were a natural-wood color.

Model 2: Rectangular maze (70 × 15 × 15 cm.), in which the walls of the two lateral compartments were made of black methacrylate, with a round hole in one end-wall and a square hole in the other. The floor was made of cork with transverse or longitudinal incisions, respectively. The central area (10 × 15 cm^2) had a metal grille floor and the walls were white.

2.3. Behavioral procedures

2.3.1. Experiment 1: conditioned taste preference

After the recovery period, all animals (parabrachial and control groups) were subjected to a two-day pre-training period during which they had access to water for only 7 min per day. Water was offered via a burette placed alternatively in the left or right hole on the front panel of the cage. Once the water was withdrawn, the animals had access to 15 g of food.

The experimental phase consisted of a taste discriminating learning task (see Fig. 1) similar to one previously used by Cubero and Puerto (2000).

On Day 1, animals were presented with only one of two possible flavored solutions for 7 min: 0.5% Strawberry (S) or Coconut (C) extracts diluted in water (McCormick & Co. Inc. San Francisco, CA). Immediately after removal of the burette, electrodes in the animals were connected to the stimulator for 15 min, using leads of sufficient length to permit freedom of movement. Half of the animals of both parabrachial and control groups (the latter implanted with electrode at different vertical coordinate) were electrically stimulated (paired-condition) and the other half were connected for an identical period but without current administration (unpaired condition). Every day, animals had access to 15 g of food at the end of the experimental session.

On Day 2, 24 h later, the second flavored solution was presented and the procedure described above for day 1 was repeated. The sequence of experimental conditions was properly balanced in such a way that all animals experienced both flavored solutions but only one of the solutions had been paired with IC electrical stimulation (paired condition). The experimental conditions for Day 1 were repeated on Day 3, and the conditions for Day 2 were repeated on Day 4.

A two-bottle free choice test was conducted on day 5 by placing two burettes in the cage simultaneously, each containing one of the two flavored solutions previously used during the training sessions. During this phase, animals were allowed to freely drink the flavored solution for 7 min and the total amount ingested was recorded; they were connected to the stimulator throughout but no current was administered.

2.3.1.1. Brain self-stimulation test. A standard operant procedure described elsewhere (Cubero & Puerto, 2000; Garcia, Simon, & Puerto, 2002; Hawkins et al., 1983; Simon, 2003) was used to explore LPBe involvement in electrical self-stimulation. The self-stimulation tests were conducted in a Plexiglas chamber (50 × 55 × 60 cm) with a lever mounted on the front wall connected to a stimulator and a pulse counter (lever presses). Each bar press triggered a 250 ms train of cathodal rectangular pulses of 66.6 Hz and 0.1 ms duration at the same currents as used in the previous phase, below the threshold for producing undesired behavioral effects.

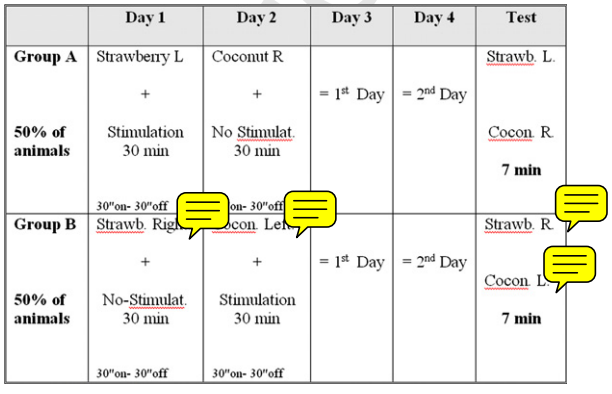

Fig. 1. Diagram showing the balanced experimental conditions used in Experiment 1.

2.3.2. Experiment 2: concurrent CPP (cCPP)

After the recovery period, an exploratory test was carried out in the home cages in order to establish optimal stimulation parameters. Current intensity ranged from 55 to 92 μA. After 48 h, the cCPP was started in Model 1 maze (described in the Section 2.2). Each animal was subjected to two 10-min sessions of cCPP on consecutive days. During these sessions, electrical stimulation of the LPBe was administered concurrently with the voluntary stay of the animal in one of the two lateral compartments of the maze, randomly selected prior to the session. The time that each animal stayed in the stimulated compartment was recorded for each session. The process was identical for the animals in the control group except that they did not receive brain stimulation.

Animals that remained in the compartment in which they were stimulated for more than 50% of the session time were assigned to a "positive group" and those remaining for less than 30% of the time were assigned to a "negative group". Finally, a "neutral group" was formed by animals that alternated between compartments during each session or showed no preference for the stimulated compartment (30–50% of session time).

After each session, animals were returned to their home cages and had ad libitum access to food and water.

2.3.3. Experiment 3: cCPP in different mazes: Effects of naloxone administration

After the recovery period, animals were subjected to an exploratory test to establish the current intensity to be used in each animal (similar to the test described in Experiment 2), obtaining a value of 70–150 μA. The 3-phase experiment was then performed.

2.3.3.1. First phase: cCPP in rectangular maze. Two experimental sessions were carried out using the 'Model 1' maze (see Section 2.2), following the same behavioral procedure as described in Experiment 2. In this case, however, animals showing no preference for either compartment after the two initial sessions (following the criteria of the previous experiment) were considered 'neutral' and formed a control group, receiving no further electrical stimulation in subsequent phases.

2.3.3.2. Second phase: naloxone injection and cCPP in Model 1 maze. All animals received a subcutaneous (s.c.) injection (4 mg/ml/kg) of naloxone (Naloxone Hydrochloride, Lab. Sigma, St. Louis, USA) at 48 h after the end of the previous phase. Then, after a 20-min interval, they all underwent a further CPP session.

2.3.3.3. Third phase: naloxone injection and cCPP in Model 2 maze. At 48 h after the end of the second phase, animals underwent a further cCPP session but in Model 2 maze (see Section 2.2) to examine the possible effect on learning of the previous phases of the experiment. Twenty minutes before the beginning of the sessions, animals received a new s.c. injection of naloxone (4 mg/ml/kg) in Model 2 maze. In this maze, both the internal sensory clues and the orientation of the rectangular maze were modified (from N–S to E–W).

2.4. Histology

At the end of each experiment, animals were deeply anesthetized with an overdose of sodium pentothal and intracardially perfused with isotonic saline and 4% formaldehyde. Correct placement of electrodes into the LPBe was verified by a small electrolytic lesion with 0.3 mA of cathodic current for 5 s. Brains were removed and stored in paraformaldehyde for at least 1 week before their subsequent lamination in 50-μ sections (1320M microtome-freezer, Leitz, Wetzlar, Germany; Vibroslice 752M vibratome, Campden Instruments, Loughborough, UK). Sections were mounted, stained with cresyl violet, and photographed (VMZ-4F stereoscopic magnifying glass and PM-6 camera, Olympus, Tokyo, Japan).

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Results of the histological study are depicted in Figs. 2A and B.

2.5. Statistical analysis

The Statistica 5.1 program (Statsoft Inc., OK) was used for the statistical analyses. In Experiment 1, intakes in the two-bottle test for the LPBe-stimulated and control groups were analyzed using repeated measures analysis of variance (ANOVA). In Experiment 2, the Pearson correlation coefficient was used for the time spent by animals in the 'stimulated compartment' during each of the two conditioning trials. In the first part of the third experiment, Pearson correlation was used to classify the animals as a function of behavioral effects (behavioral consistency with electrical stimulation), and one-way ANOVA was then used to analyze the effects of stimulation and naloxone administration on the different groups. Finally, two-way mixed ANOVA tests were used to compare the effects of learning retention and naloxone in different mazes, (Group × Substance in maze 1; Group × Maze—under effects of naloxone—and Group × Substance in maze 2). After each ANOVA, the Newman–Keuls Test was used for post-hoc comparisons.

3. Results

3.1. Experiment 1: taste discrimination learning

Two of the 14 animals in the LPBe-stimulated group were excluded from the statistic analysis because the implant became detached during the behavioral procedure.

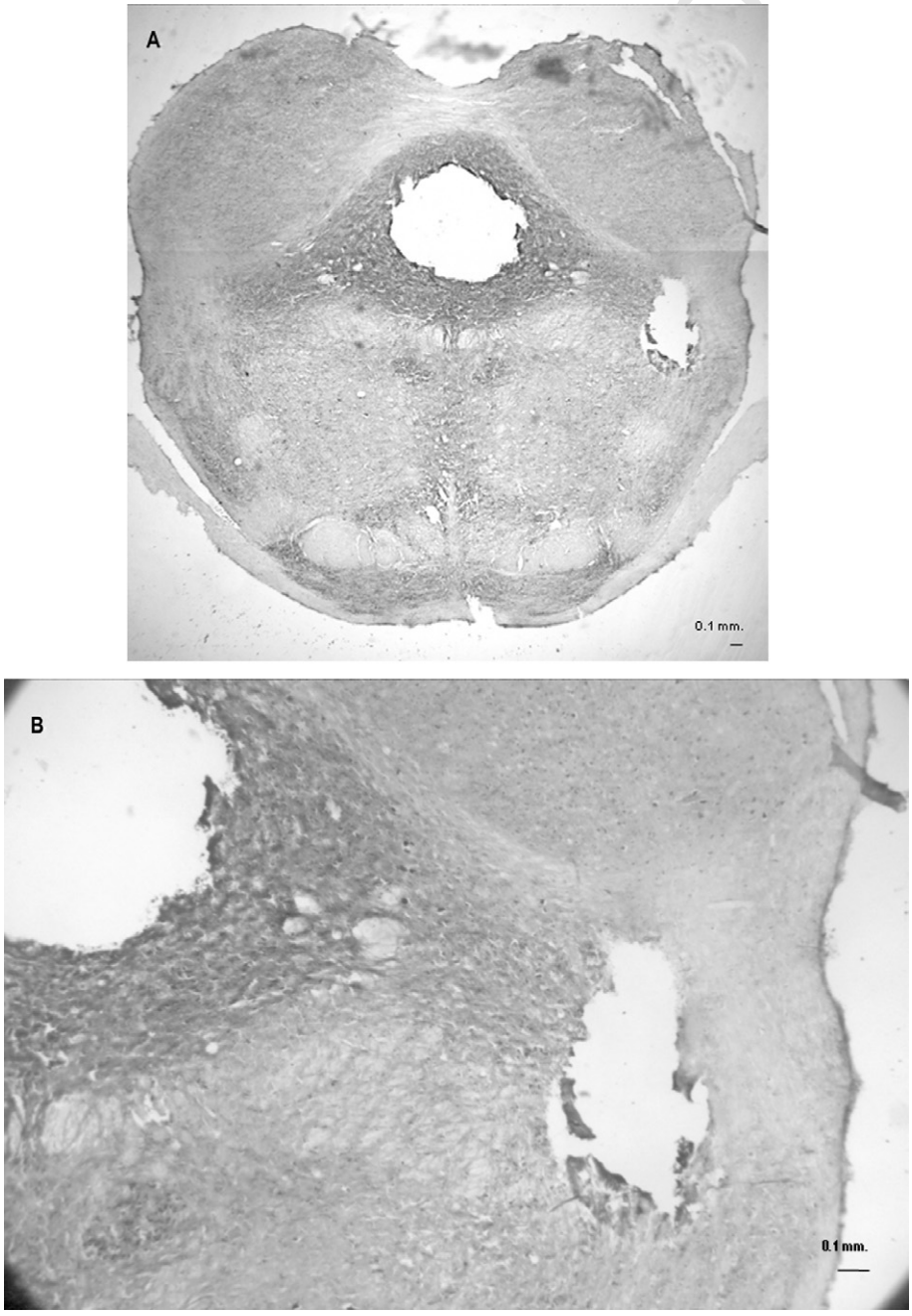

Fig. 2. (A) Localization of end of electrode in an Experimental Group animal (2X). (B) Magnification of the same area (4X).

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One-way ANOVA for stimulated group and control group results showed that LPBe-stimulated animals preferred the flavor related to the stimulation [$F(1,11) = 11.33$, $p < .006$] (see Fig. 3a), whereas the control group showed no significant differences in intake [$F(1,9) = 0.007$, $p < .935$] (Fig. 3b). See also Table 1.

3.1.1. Intracranial self-stimulation of the LPBe

The animals in this experiment and the following experiments failed to learn a lever press task to induce electrical self-stimulation, as ~~also~~ reported in other brain areas (Hawkins et al., 1983). In fact, the animals showed avoidance behaviors when the current parameters were similar in intensity to those usually applied in the stimulation of other brain areas, e.g., lateral hypothalamus (Simon, 2003). It is possible that this reward effect in the parabrachial area requires a greater effort or a different procedure from that habitually used and employed in this article (i.e., lever press self-stimulation).

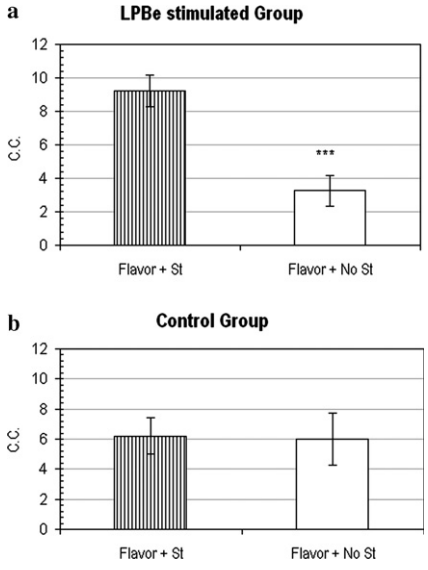

Fig. 3. (a) (upper) Mean intake in c.c. of flavors by experimental LPBe-stimulated animals with and without stimulation in Experiment 1 [$F(1,11) = 11.33$, $p < .006$**]. (b) (lower) Mean intake in c.c. of flavors by control animals with and without stimulation [$F(1,9) = 0.007$, $p < .935$] in the same experiment.

Table 1
Amount (in ~~c.c.~~) of the two taste stimuli, associated (Flavor + St column) and not associated (Flavor + Non St. column) with electrical stimulation of LPBe, ingested by the LPBE- stimulated group in Experiment 1

	~~IPBe~~ stimulated group	
Rat	~~Flavor + SL r.t~~	~~Flavor + Hon-SL r.t~~
1	15.5	1.3
2	8.0	5.2
3	5.7	8.1
4	9.7	8.7
5	8.9	6.7
6	12.3	0.2
7	5.0	8.7
8	9.9	0.4
9	10.5	1.9
10	12.5	1.0
11	9.8	1.0
12	8.0	1.0

3.2. Experiment 2: conditioned place preference

During the behavioral process, 6 out of the 33 animals in the LPBe-implanted group and 2 out of the 7 animals in the control group were excluded because of detachment of the implant. Their data were not included in the statistical analysis.

Comparison of the performance of each experimental animal between the two conditioning sessions showed a significant correlation between the two days [$r = .8710$, $p < .001$] (Fig. 4, upper), indicating consistent preference or rejection behavior for the stimulated compartment. In contrast, animals in the 'non-stimulated control' group alternated randomly between the two compartments of the maze [$r = -.2931$, $p < .632$] (Fig. 4, lower), showing no preference for either.

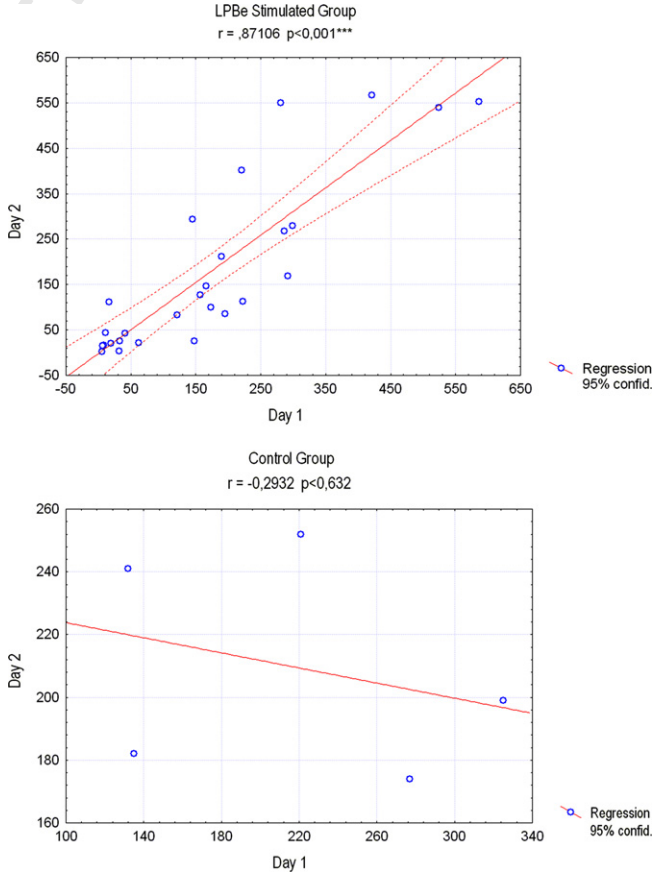

Fig. 4. ~~(a)~~ (upper) Correlation for the time spent by experimental animals in the stimulation compartment at each of the two conditioning sessions. ~~(b)~~ (lower) Correlation for the time spent by the control animals without stimulation in one of the two randomly selected compartments at each of the two conditioning sessions (Experiment 2).

According to the previously established behavioral criteria, out of the 27 LPBe-stimulated animals, 6 were assigned to the 'positive group', 12 to the 'negative group' and 9 to the 'neutral group'. The values obtained in the sham implant control group ($N = 5$), which showed random behavior, also fulfilled the criteria of the 'neutral group'.

K-Means clustering results reflected the groupings established according to the behavioral criteria, except for slight variations of 1–2 animals in the composition of the groups, and places the 'non-stimulated' Control group within cluster 2 of animals without a defined preference. The same results were obtained from the one-way ANOVA used to study the effect between implanted and control groups [$F(2,24) = 73.351$, $p < .001$] and from the planned comparisons (See Fig. 5):

$$[F(1,24)_{\text{Positive}\times\text{negative}} = 28.602 \quad p < .001;$$

$$F(1,24)_{\text{positive}\times\text{neutral}} = 140.121 \quad p < .001;$$

$$F(1,24)_{\text{negative}\times\text{neutral}} = 26.922 \quad p < .001].$$

After an interval, the animals in the experimental group were individually subjected to a standard lever-press electrical self-stimulation procedure similar to that described in Experiment 1. The results of this test were negative, as in the previous experiment.

3.3. Experiment 3: cCPP in different mazes. Effects of naloxone infusion and learning retention

3.3.1. First Phase: cCPP in Model 1 maze

In this experiment, performances of each animal in the two conditioning sessions were significantly correlated [$r = .8063$, $p < .001$] (See Fig. 6).

After two CPP sessions in the maze and applying the same behavioral criterion as in the previous experiment, three groups of animals were formed as a function of the time they stayed in the stimulated compartment: 'positive group' comprised 13 animals, 'negative group' 15 animals, and 'neutral group' 8 animals. Two animals in the 'negative' implanted group and one in the 'positive' group were excluded from the results analysis due to detachment of the implant. The average stay times (maximum of 10') in the stimulated area during the two conditioning sessions were: $X_{\text{positive}} = 414.42$ s., $X_{\text{negative}} = 81.05$ s. and $X_{\text{control}} = 286.75$ s.

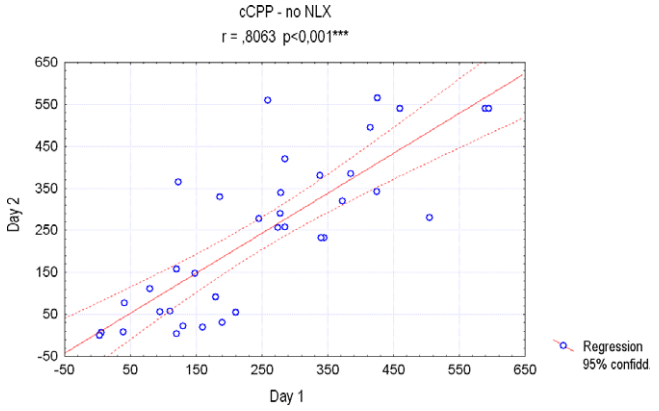

Fig. 6. Correlation for the time spent by animals of Experiment 3 in the stimulation compartment at each of the two conditioning sessions.

3.3.2. Second phase: cCPP in Model 1 maze. Effect of naloxone administration

As expected, the one-way ANOVA showed significant differences between the groups established according to behavioral criteria [$F(2,33) = 83.0808$, $p < .001$] (See Fig. 7), considering as learning index the average length of stay of animals in the compartment associated with stimulation in the two conditioning trials.

However, after administration of naloxone, the two-way mixed ANOVA (Group x substance) showed no main effect of substance [$F(1,33) = 2{,}9121$ $p < .0973$] or Group x substance interaction [$F(2,33) = 0{,}2131$ $p < .8091$], although the main group effect was significant [$F(2,33) = 54.3002$, $p < .001$] (See also Fig. 9, behavioral effects in Model 1 maze: No-Nx vs. Nx). Thus, under these circumstances, naloxone did not block the preferences/aversions induced by electrical stimulation of the LPBe.

Analysis of the main effect, group factor, by post-hoc comparisons showed significant differences among all groups (Newman–Keuls test, $p < .001$).

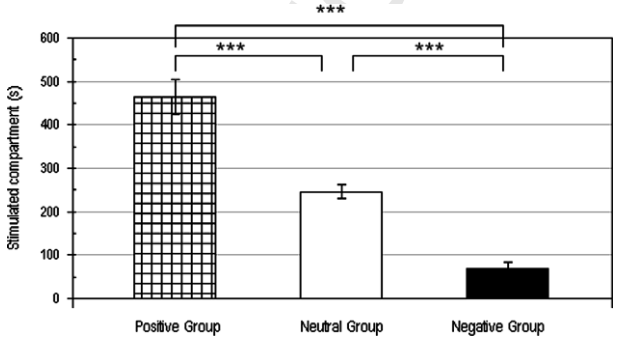

Fig. 5. Duration of stay (in seconds) by experimental groups of Experiment 2 in the compartment associated with electrical stimulation of the LPBe (mean of two spatial learning tests). The asterisks (***) indicate significant ($p < .001$) differences among groups.

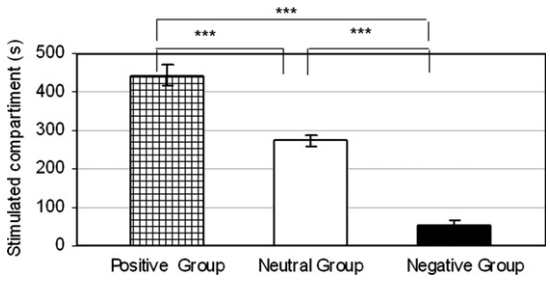

Fig. 7. Duration of stay (in seconds) by experimental groups of Experiment 3, following the behavioral criterion established in Experiment 2, in the compartment associated with electrical stimulation of the LPBe in Model 1 maze. The asterisks (***) indicate significant ($p < .001$) differences among groups.

3.3.3. Third Phase: cCPP in Model 2 maze. Effect of naloxone administration

One-way ANOVA analysis of the conditioning results in Model 2 maze confirmed (Fig. 8) that naloxone blocked differences among the groups [$F(2,33) = 1.0754$ $p < .3528$], a result that has been replicated in follow-up experiments undertaken in our laboratory regardless of the type of maze used (manuscript in preparation).

Comparison of the effects of naloxone administration between Model 2 and Model 1 mazes by means of a two-way mixed ANOVA (Group x Maze) showed a significant effect of the interaction [$F(2,33) = 7.6367$ $p < .0018$] (See also Fig. 9: effects of naloxone in Model 1 vs. Model 2 mazes).

Post-hoc comparisons showed significant differences in the positive group as a function of the maze used (Model 1 vs. Model 2, $p < .0459$), although no differences were observed in the remaining groups (negative group, $p < .2274$; control group $p < 0.8173$).

After an interval, all animals were individually subjected to a standard lever-press electrical self-stimulation procedure, using the current parameters established in phase 1. The results of this test were negative, as in the previous experiments.

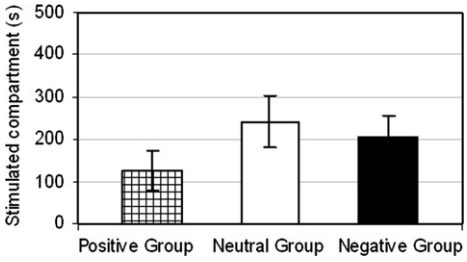

Fig. 8. Duration of stay (in seconds) by groups of Experiment 3 in the Model 2 maze compartment associated with electrical stimulation of LPBe after naloxone administration (no significant differences among groups, $p > .01$).

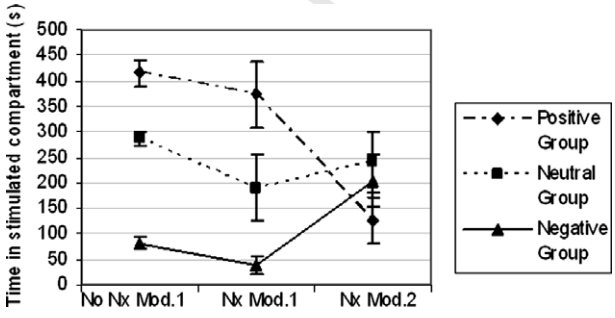

Fig. 9. Duration of stay (in seconds) by groups of Experiment 3 in the compartment associated with electrical stimulation of the LPBe before naloxone administration in Model 1 maze and after naloxone administration in Model 1 and Model 2 mazes.

4. Discussion

Results of these experiments show that LPBe stimulation can induce preference for an associated stimulus in both taste discrimination learning tasks and cCPP.

Thus, in Experiment 1, in an implicit taste discrimination task (Mediavilla et al., 2000a, 2005; Simon, 2003), animals preferred the flavor associated with LPBe electrical stimulation to the flavor that had not been associated with this stimulation. This effect appears to be specific to electrical stimulation of the LPBe, since it was not observed in control animals. However, the effect was not universal, since some of the LPBe-stimulated animals showed a preference for the alternative 'neutral' taste stimulus. This finding was confirmed in subsequent experiments using a different behavioral procedure, cCPP (see below).

The present results suggest the LPBe may be involved in reward processes and form part of some rewarding systems. This possibility is compatible with previous observations of c-fos immunoreactivity in the LPBe after intragastric administration of nutrients, e.g., lactose, sucrose, glucose, maltose or polycose (Wang et al., 1999; Yamamoto & Sawa, 2000a), or appetitive substances, e.g., saccharin (Yamamoto & Sawa, 2000b; Yamamoto et al., 1994). ~~Conversely, specific lesions of the LPBe or general lesions of the parabrachial area, including the external lateral subnucleus, eliminated preference for palatable food~~ (Edwards & Ritter, 1989) or taste stimuli associated with intragastric administration of rewarding predigested food (Zafra et al., 2002).

The involvement of the LPBe in reward processes could be related either to a reduction in states of need and/or to a specific modification in the hedonic value of the taste stimuli (Berridge, 2003; Le Magnen, 1992). In fact, the LPBe constitutes one of the main central relays in the processing of taste and visceral cues (De Lacalle & Saper, 2000; Fulwiler & Saper, 1984; Halsell & Travers, 1997; Karimnamazi et al., 2002). With regard to the former possibility, LPBe electrical stimulation may have acted as an adequate substitute for visceral stimuli and/or the consequences of their rewarding motivational effects (Cubero & Puerto, 2000). In fact, the LPBe is strategically placed to receive peripheral information related to intake (Calingasan & Ritter, 1993; Ritter, Dinh, & Friedman, 1994; Wang et al., 1999; Yamamoto & Sawa, 2000a, 2000b). It can therefore be hypothesized that the electrical stimulation might have generated preferences for the associated stimulus, similar to the rapid rewarding effect induced by the intragastric loading of some nutritive substances (Puerto, Deutsch, Molina, & Roll, 1976). In this regard, electrical stimulation of afferent branches of the vagus nerve has been shown to induce c-fos immunoreactivity at the lateral end of the LPBe (Gieroba & Blessing, 1994; Saleh & Cechetto, 1993). It has also been verified that the presence of nutrients in the intestine combined with the release of hormones such as CCK generates signals that are processed via the vagal pathway (Ritter, Brenner, & Yox, 1992a) as well as by the LPBe (Li & Rowland, 1995).

However, it could also be interpreted from the present data that intracerebral electrical stimulation might have specifically modified the hedonic-motivational value of the flavor stimulus intake. Thus, neurons sensitive to the motivational and orosensorial properties of gustatory stimuli have been identified in the LPBe (Halsell & Frank, 1992; Halsell & Travers, 1997; Karimnamazi et al., ~~1992~~; Sewards, 2004; Yamamoto et al., 1994). Moreover, general lesions of the lateral area of the parabrachial nucleus, which includes the LPBe, attenuated the over-ingestion of appetizing taste stimulus produced by removal of the Area Postrema (Edwards & Ritter, 1989). This suggests the LPBe may be an important modulating structure in the hedonic evaluation of certain 'innately preferred' taste stimuli. In this sense, drugs such as midazolam, which increase intake by modifying its hedonic component (Treit & Berridge, 1990; Berridge & Peciña, 1995), may specifically act on the lateral parabrachial area (Söderpalm & Berridge, 2000).

Experiment 2 in the present study shows that electrical stimulation of the LPBe can also induce preference behaviors when associated with environmental cues in a CPP paradigm, probably a classical conditioning procedure in which motivational consequences or a reinforcer (e.g., morphine) are associated with environmental cues (Bardo & Bevins, 2000; Tzschentke, 1998). In contrast, non-stimulated controls alternated between the two compartments of the maze, showing no preferences or consistent behaviors. At any rate, these data suggest that the rewarding effect obtained might not be specific to a single sensory modality, although the present experiments do not allow definitive conclusions to be drawn in this respect. Once again, it could be interpreted from these results that intracerebral electrical stimulation might have acted as an adequate substitute for visceral stimulus and/or their motivational consequences. In this regard, it has been shown that place preferences can be induced when water intake or intragastric infusion of sucrose or water occurs immediately before confinement of animals in a specific compartment of a T-maze (Arnold & Agmo, 1999). In relation to the parabrachial Area, some researchers found that lesions to the dorsolateral end (which includes the LPBe) blocked aversive spatial conditioning induced by peripheral administration of morphine (Bechara, Martin, Pridgar, & Vanderkooy, 1993). However, we have found no published data relating the LPBe to the rewarding effect of drugs of abuse. Furthermore, we cannot rule out the possibility that electrical stimulation of the LPBe affected specific sensorial and/or motivational cells of the gustatory system, causing them to have a rewarding taste experience in a specific area of the maze (Experiment 2).

Results obtained in Experiment 3 demonstrate that administration of the opiate antagonist naloxone blocked the rewarding consequences of stimulation when the acquisition process was carried out in a new maze, but not when the effects of this substance were evaluated in the same context in which the learning took place. These findings are compatible with those obtained after intervention in the vagal-LPBe-cerebellum axis in taste aversion learning tasks (Mediavilla, Molina, & Puerto, 2000b). This blocking effect of naloxone seems to be more powerful than that observed in self-stimulation induced in the ventral tegmental area (Bielajew et al., 2003). However, we have been unable to induce this behavior, at least not as readily as in other areas such as the lateral hypothalamus (Hawkins et al., 1983; Simon, 2003).

Several studies have shown that opiate substances may play an important role in intake, probably by potentiation of the hedonic value of nutrients or by reducing feelings of 'discomfort' produced by homeostatic imbalance (Carr, 2002; Le Magnen, 1992). Some authors have even pointed out that the hedonic value of food could be increased when it is associated with the elimination of homeostatic imbalance (Carr, 2002; Le Magnen, 1992). Concerning ~~the first possibility~~, the activation of μ and κ opiate receptors of this parabrachial area (Chamberlin et al., 1999; Gutstein et al., 1998; Mansour, Fox, Akil, & Watson, 1995) appear to mediate some of the effects related to modification of the hedonic value of gustatory stimuli (Carr et al., 1991; Moufid-Bellancourt et al., 1996; Wilson, Nicklous, Aloyo, & Simansky, 2003). These explanatory proposals suggest that LPBe electrical stimulation may have acted on an intake-related opiate mechanism (Carr et al., 1991; Carr & Papadouka, 1994; Papadouka & Carr, 1994) or even on a general rewarding mechanism that would critically include a brainstem opioid system, since some studies reported that c-fos immunoreactivity is elicited in the LPBe by certain opioid drugs, e.g., morphine, and by amphetamines, cocaine, or ethanol (Grabus, Glowa, & Riley, 2004; Sakai & Yamamoto, 1997; Yamamoto & Sawa, 2000a, 2000b). In fact, various authors have suggested that different rewarding modalities (homeostatic, abuse substances, electrical stimulation, etc.) may be neurobiologically related in some way (Berman, Devi, & Carr, 1994; Fernandez-Espejo, 2002; Kelley & Berridge, 2002; Wolinsky et al., 1996).

On the other hand, the association of flavors with metabolic benefits is not limited to the reduction of natural states of need, e.g., hunger or thirst, and can also be extended to unnatural states of discomfort (withdrawal syndrome) produced by the absence of drugs in addicts (Parker, Failor, & Weidman, 1973; Yeomans, 2000). In this context, it has been reported that this parabrachial region may be a relay area of the spinoparabrachial nociceptive pathway involved in the affective-emotional, autonomic and visceral component of pain (Bernard, Carroué, & Besson, 1991; Bernard, Dallel, Raboisson, Villanueva, & Le Bars, 1995; Bernard, Huang, & Besson, 1994; Bester, Matsumoto, Besson, & Bernard, 1997; Gauriau & Bernard, 2002; Huang, Besson, & Bernard, 1993; Jasmin, Burkey, Card, & Basbaum, 1997; Saper, 1995). This may explain the avoidance behavior of some of our animals, probably because the implanted electrodes may have affected the nociceptive neurons of this pathway in these animals. These results are also in agreement with recent evidence

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published by our laboratory that LPBe lesions block concurrent gustatory aversive learning, an implicit learning that requires rapid visceral processing (Mediavilla et al., 2000a, 2005).

Finally, the present results are compatible with the proposition that this same structure may be involved in both positive and negative motivational processes (Reynolds & Berridge, 2002; Salamone, 1994; Yamamoto et al., 1994). Thus, it has been demonstrated that there are opiate systems with opposed effects, mediated by the μ and κ receptors, respectively, which may regulate the action of the mesolimbic dopaminergic system (Spanagel et al., 1992). Furthermore, differential chemical stimulation of μ and κ receptors of the lateral parabrachial nucleus was shown to induce preferences and aversions as a function of the systems activated (Moufid-Bellancourt et al., 1996).

In conclusion, this experimental series showed that electrical stimulation of the LPBe generates preferences for stimuli with which it is contiguously or concurrently associated. This stimulation may act as a substitute of biological processes that have yet to be determined. These rewarding effects are blocked by naloxone administration when the tasks involve a new learning but not when they are carried out in the maze in which preferences were acquired. Therefore, the LPBe, which has been proposed as a region on which rewarding or food intake-related drugs may act, is also involved in the processing of natural food rewards, and, as demonstrated here, in the induction of artificial rewards induced by electrical brain stimulation via opioid neurochemical mechanisms.

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