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Rewarding effects of the electrical stimulation of the parabrachial complex: Taste or place preference?

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ABSTRACT

The lateral parabrachial complex has been related to various emotional-affective processes. It has been shown that electrical stimulation of the external Lateral Parabrachial (LPBe) nucleus can induce reinforcing effects in place preference and taste discrimination tasks but does not appear to support self-stimulation. This study examined the relative relevance of place and taste stimuli after electrical stimulation of the LPBe nucleus. A learning discrimination task was conducted that simultaneously included both sensory indexes (taste and place) in order to determine the preference of animals for one or the other. After a taste stimulus reversal task, the rewarding effect of stimulation was found to be preferentially associated with place. These results are discussed in the context of the rewarding action and biological constraints induced by different natural and artificial reinforcing agents.

1. Introduction

The Lateral Parabrachial (LPB) complex appears to participate in neurobiological systems related to the motivational or hedonic evaluation of rewarding natural products and other substances for which preference has been acquired by learning (Calingasan & Ritter, 1993; Edwards & Ritter, 1989; Yamamoto & Sawa, 2000a, 2000b; Yamamoto et al., 2009). Thus, it has been related to the aversive processing of lithium chloride (Sakai & Yamamoto, 1997; Yamamoto & Sawa, 2000a) and drugs of abuse, such as opiates (Bechara, Martin, Pridgar, & Van der Kooy, 1993; Nader, Bechara, & Van der Kooy, 1996), and in the processing of pain and its affective components (Bernard, Huang, & Besson, 1994; Bester, Menendez, Besson, & Bernard, 1995; Jasmin, Burkey, Card, & Basbaum, 1997).

The external Lateral Parabrachial (LPBe) nucleus is located in the ventral region of the lateral parabrachial complex (Fulwiler & Saper, 1984; Herbert & Bellintani-Guardia, 1995) and has been related to various homeostatic, sensory, and learning processes (De Lacalle & Saper, 2000; Edward & Ritter, 1989; Karimnamazi, Travers, & Travers, 2002; Mediavilla, Molina, & Puerto, 2000; Yamamoto, Shimura, Sakai, & Ozaki, 1994). More specifically, rewarding food (Zafra, Simon, Molina, & Puerto, 2002) and/or intake-related substances such as fenfluramine (Li & 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) may be processed *via* the LPBe, among other brain nuclei.

It has been demonstrated that electrical stimulation of the LPBe nucleus can induce aversion or preference for associated stimuli in learning tasks of taste discrimination and conditioning place preference, although it does not appear to support self-stimulation, or at least not as readily as can be achieved by stimulation of the lateral hypothalamus, for example (Simon, García, & Puerto, 2011, 2013; Simon, García, Zafra, Molina, & Puerto, 2007; Simon, Zafra, Molina, & Puerto, 2008). These tasks have proven useful to analyze specific preferences (Spiteri, Le Pape, & Agmo, 2000) generated by natural (food or water intake) (Schroeder & Packard, 2000; Stefurak & Van der Kooy, 1992; Zafra et al., 2002) or artificial (electrical stimulation, drugs of abuse) (Jaeger & van der Kooy, 1996; McBride, Murphy, & Ikemoto, 1999; Schecter & Calcagnetti, 1998; Simon et al., 2007; Tzschentke, 2007) reinforcing treatments. In the case of electrical stimulation, animals learn the task by relating the rewarding (or aversive) stimulation to simultaneously available place, space, proprioceptive, or sensory (taste/flavor) stimuli (Simon et al., 2007, 2008). Some treatments frequently induce an associative bias (biological constraint) towards specific related stimuli (Garcia, Hankins, & Rusiniak, 1974; Garcia & Koelling, 1966; Lett, 1985). Thus, there is a tendency to associate taste stimuli with states of internal malaise or sickness and to associate place/exteroceptive cues with the aversive effects induced by noxious exteroceptive stimuli (Garcia & Koelling, 1966; Garcia et al., 1974; Lett, 1985). Moreover, morphine and amphetamines, among

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other drugs of abuse, induce preferences for associated environmental cues, whereas aversive components of these drugs are more readily evidenced in taste discrimination tasks (Bechara et al., 1993; Parker, 2003; White, Nessier, & Carr, 1987). LPBe nucleus reinforcing effects may initially be associated to both types of stimuli, taste and place (Simon et al., 2007, 2013; Yamamoto et al., 1994; Zafra et al., 2002). However, the nature of the reinforcement induced by the electrical stimulation of the LPBe nucleus is not known and it would be relevant to determine any biological constraint or associative preference (e.g., for taste or place) that may help to define this rewarding effect. With this background, the objectives of this study were to examine the relative importance of taste and place sensory indexes simultaneously presented in a discriminative learning task induced by electrical stimulation of the LPBe nucleus. The initial hypothesized preference for a taste stimulus located in a (right or left) place was re-examined in a second test in which taste and place were dissociated (by reversing the place of the taste), with the aim of establishing the priority ranking assigned by animals to one or other type of stimulus.

2. Materials and methods

2.1. Subjects and surgery

Forty male Wistar rats from the breeding colony at the University of Granada, weighing 270–360 g at the time of surgery, were randomly assigned to an experimental group ($n = 27$) for implantation with intracerebral electrodes in LPBe nucleus or to a control group ($n = 13$) with the reference electrode on the skull surface. Animals were housed in individual methacrylate cages ($30 \times 15 \times 30$ cm) that also served as training chambers during the experiments, in which they remained for at least one week of habituation before the surgery, with water and food *ad libitum* (Panlab Diets S.L., Barcelona, Spain).

The laboratory was maintained at 20–24 °C with a 12:12 h light/dark cycle. Experimental procedures were conducted during light periods with white noise. All behavioral procedures and surgical techniques complied with Spanish legislation (Royal Law 1201/2005) and the European Community Council Directive (86/609/EEC).

Animals were implanted with a stainless steel grounded monopolar electrode (00) (Hawkins, Roll, Puerto, & Yeomans, 1983; Simon et al., 2007) in the LPBe nucleus [Coordinates: AP = −0.16; $V = +3.0$; $L = +2.5$, according to the atlas by Paxinos and Watson (1998)] using a stereotaxic unit (Stoelting Co., Wood Dale, IL) under general anesthesia (Sodium Pentathol, 50 mg/kg, B Braun Medical S.A. Barcelona, Spain). As prophylactic measures, 0.1 cc penicillin (Penilevel, (Laboratorio Level, S.A., Barcelona, Spain) was intramuscularly injected, and povidone-iodine (Betadine, Asta Médica, Madrid, Spain) was applied around the implant.

After the surgery, animals were returned to their cages, in which they remained for a recovery period of $\geqslant 10$ days with water and food *ad libitum*.

2.2. Apparatus

2.2.1. Concurrent place preference task

An unbiased, counterbalanced concurrent place preference procedure was used for trials 1 and 2. Animals were concurrently stimulated in one of two distinct compartments of a rectangular maze ($50 \times 25 \times 30$ cm), which differed in color, texture, and wall pattern. These lateral compartments were separated by a narrow area in which animals were placed at the start of each test. 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 (Simon et al., 2007).

2.2.2. Taste/place discrimination task

The taste/place discrimination test was conducted in the methacrylate home cages in which the animals were housed upon arrival at the laboratory (Mediavilla, Molina, & Puerto, 1998). The sides of the cages were black and opaque and 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 the delivery of flavors and water (Mediavilla et al., 1998; Simon et al., 2007).

2.2.3. Electrical brain stimulation

For the electrical stimulation, a continuous current range of 60–170 μA with rectangular cathodic pulses at 66.6 Hz and 0.1 ms pulse duration was supplied by a CS-20 stimulator (Cibertec, Madrid, Spain) connected to an ISU 165 isolation unit (Cibertec, Madrid, Spain) and HM 404-2 oscilloscope (HAMEG Instrument GMBH, Frankfurt, Germany). The current intensity was established individually for each animal, avoiding current levels that could generate involuntary movements, escape responses, or pain (Simon, Molina, & Puerto, 2009; Simon et al., 2007, 2008; Tehovnik, 1996).

2.3. Behavioral procedures

2.3.1. Concurrent place preference

At 48 h after establishing the optimal current intensity, animals underwent a concurrent place preference task. For the 10-min session-test, one of the two lateral compartments was randomly selected as the area of intracranial electric stimulation, the animal was placed in the center of the maze, and the voluntary stay of the animal in one of the two areas was accompanied concurrently by intracranial electrical stimulation (half of the animals received stimulation in one lateral compartment of the maze and the other half received it in the other lateral compartment). The time the animal stayed in each compartment was recorded. Control group animals bore stimulation connectors connected to the reference electrode but received no electrical stimulation. This procedure was repeated in a second session after a 24-h interval. After each session, the animal was returned to its cage with water and food available *ad libitum*.

Following the behavioral criteria established in previous studies (Simon et al., 2007, 2009), animals staying in the "stimulated" compartment for >50% of the total time were classified as "positive", those staying for <30% of total time as "negative", and those staying for 30–50% of total time each session or showing alternating behavior between sessions, as "neutral".

2.3.2. Experiment A: learning of taste/place preference

2.3.2.1. Pre-training. At 48 h after the concurrent place preference phase, a two-day pre-training period was initiated, during which water was available to the animals for only 10 min on day 1 and 7 min on day 2 from a burette placed alternately in the left or right hole on the front panel of the cage. After removing the water, the animals were supplied with 14 g of food.

2.3.2.2. Taste/place preference. Table 1 exhibits the discriminative learning procedure: In each of the four experimental sessions, animals were offered one of two flavored solutions [0.5% Strawberry

Table 1
Diagram showing the experimental procedure used in the learning discrimination task (L: Left; R: Right).

	Day 1	Day 2	Day 3	Day 4	Choice Test	Reversal Test
50% of animals	Strawberry L + Stimulation (10 min)	Coconut R + No Stimulation (10 min)	=Day 1	=Day 2	Strawberry L Coconut R (7 min)	Strawberry R Coconut L (7 min)
50% of animals	Strawberry L + No Stimulation (10 min)	Coconut R + Stimulation (10 min)	=Day 1	=Day 2	Strawberry L Coconut R (7 min)	Strawberry R Coconut L (7 min)

Abbreviations: L: Left; R: Right.

(S) or Coconut (C) extracts diluted in water (McCormick & Co. Inc. San Francisco, CA)] and, after 7 min, the LPBe nucleus was electrically stimulated for 10 min; liquid intakes during the first 7 min and during the stimulation period were recorded (total of 17 min). In each daily session, half of the LPBe nucleus-implanted animals were stimulated (paired-condition) and the other half were connected for the same period of time but were not stimulated (unpaired-condition). The same procedure was followed with the control group animals except that no electrical stimulation was applied. The sequence of experimental conditions was properly balanced in such a way that all animals experienced both flavored solutions, but only one solution was paired with LPBe nucleus electrical stimulation (paired condition); specifically, half of the animals were stimulated when drinking S and not when drinking C, whereas other half were stimulated when drinking C and not when drinking S. Animals had access to 14 g of food after the end of each experimental session.

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

At 6 h after the choice test, the animals were subjected to a reversal test, in which S or C was again available for 7 min from the two burettes, but these were now placed on the opposite side (right/left) to that experienced by the animal during training sessions; their intake of each solution was recorded.

2.3.3. Experiment B: learning of taste/place aversion

2.3.3.1. Pre-training.

The pre-training was the same as described for Experiment A.

2.3.3.2. Taste/place aversion. The procedure was the same as described for Experiment A (Table 1) except that the four-session cycle was repeated, giving a total of eight learning trials in addition to the choice tests.

As in Experiment A, a reversal test was conducted at 6 h after the second choice test.

2.4. Histology

At the end of the experiments, animals were deeply anesthetized with an overdose of sodium pentothal and intracardially perfused with isotonic saline and 10% formaldehyde. Correct placement of electrodes into the LPBe nucleus was verified by a small electrolytic lesion with 0.3 mA of cathodic current for 5 s. Brains were removed and stored in formaldehyde for at least 1 week before their subsequent lamination in 70 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). Fig. 1 depicts the results of the histological study.

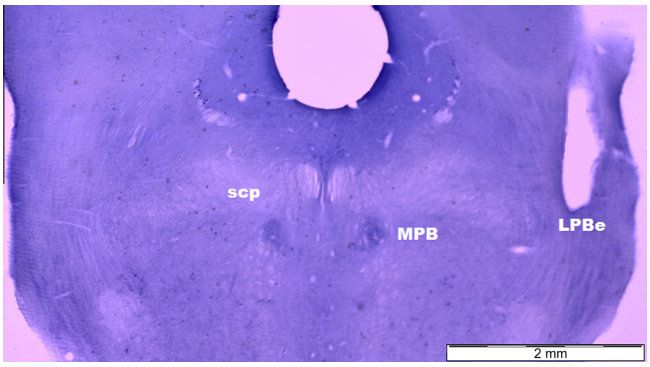

Fig. 1. Coronal slice of the brain of an animal from the "positive" group showing the localization of the electrode tract. Abbreviations: LPBe, External Lateral Parabrachial Nucleus; MPB, Medial Parabrachial Nucleus; scp, Superior Cerebellar Peduncle.

Three of the LPBe nucleus-implanted animals were excluded from the study because they showed circling behavior.

2.5. Statistical analysis

Statistica 6.0 program (Statsoft Inc., OK) was used for the statistical analyses. Pearsońs correlation coefficient for the time spent by animals in the 'stimulated compartment' was used to distribute the animals as a function of the behavioral effects of the electrical stimulation during the (two) concurrent place preference trials (Simon et al., 2007, 2009).

Preference proportions (Parker, Cyr, Santi, & Burton, 2002; Spiteri et al., 2000) were calculated as follows for intakes during choice-tests in Experiments A and B: [(intake in ml of the stimulated taste/(intake expressed in ml of the stimulated taste + intake expressed in ml of the non-stimulated taste)] × 100. A between-group one-factor ANOVA was then used to analyze these data.

3. Results

3.1. Concurrent place preference

Performances of each animal in the two conditioning sessions were significantly correlated in this experiment ($r = 0.7607$, $p < 0.001$) (see Fig. 2). After two concurrent place preference sessions in the maze, three groups of animals could be differentiated as a function of the time they spent in the stimulated compartment: 'positive group' ($n = 7$), 'negative group' ($n = 13$), and 'neutral group' ($n = 4$) (Simon et al., 2007, 2009). Mean stay times (out of a maximum of 600 s) in the stimulated area during both concurrent place preference sessions were: $X_{positive} = 471.857$ s.; $X_{negative} = 86.154$ s.; $X_{neutral}$: 269.625 s. The animals in the "neutral" group were then included in this study as control animals (2 in group A; 2 in group B) and did not receive electrical stimulation in any subsequent experimental procedure. The animals in the

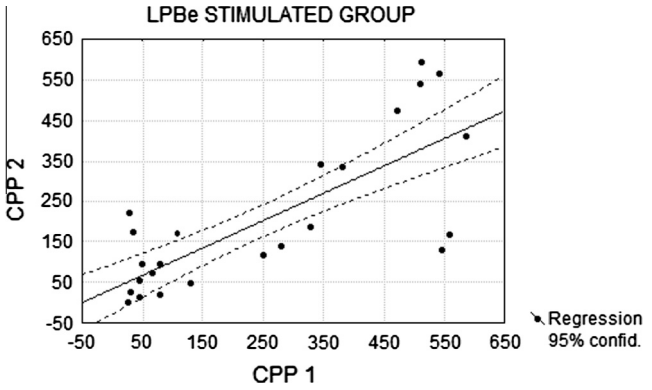

Fig. 2. Correlation of the time spent by LPBe-stimulated animals in the stimulation compartment in each of the two concurrent place preference sessions.

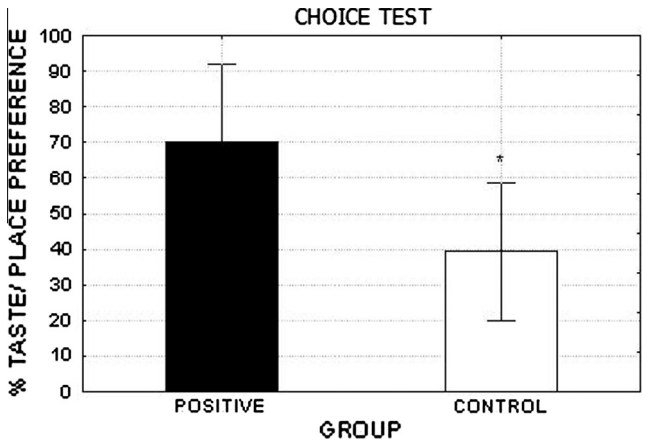

Fig. 3. Percentage of preference for the taste/place stimulus associated with the electrical stimulation of the LPBe nucleus shown by the animals in the "positive" group (black) and "control" group (white) in the first choice test.

"control group" had a mean stay in the stimulated area of 244.769 s and were randomly distributed into two groups, control groups A ($n = 7$) and B ($n = 6$).

3.2. Experiment A: learning of taste/place preference

The one-factor between-group ANOVA showed that electrical stimulation of the LPBe nucleus induced preference for the taste/place associated with stimulation, which was significantly higher in the "positive" group than in the "control" group [$F_{(1,14)} = 5.0166$, $p < 0.0418$] (see Fig. 3).

In the reversal test, there were significant differences in the preference proportion between the groups ($F_{(1,14)} = 13.249$ $p < 0.0027$) (see Fig. 4), with the electrically-stimulated animals developing a greater preference for the stimulus-associated place than taste, whereas the controls showed no preference.

3.3. Experiment B: learning of taste/place aversion

Results of the first choice test (after 4 sessions/2 learning trials) showed no intergroup differences in the preference of the animals for the place/taste stimulus associated with the stimulation ($F_{(1,19)} = 3.22$, $p < 0.0885$; ANOVA). In contrast, the results of the second choice test showed significant intergroup differences in the preference proportion ($F_{(1,19)} = 5.772$; $p < 0.0267$; ANOVA),

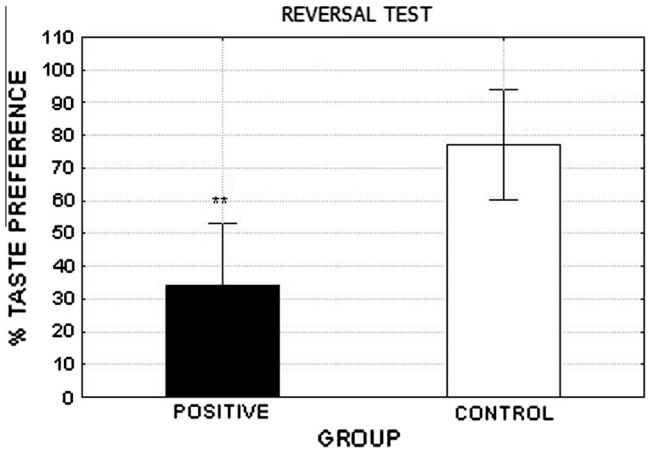

Fig. 4. Percentage of preference for the taste stimulus associated with electrical stimulation of the LPBe nucleus shown by the animals in the "positive" group (black) and "control" group (white) in the Reversal Test.

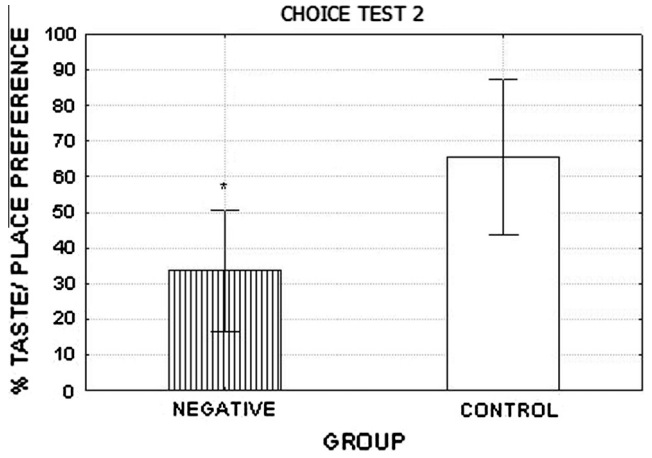

Fig. 5. Percentage of preference for the taste/place stimulus associated with electrical stimulation of the LPBe nucleus shown by the animals in the "negative" group (striped) and "control" group (white) in the second choice test.

with the "negative" group showing a lesser preference for the stimulus-associated taste/place (Fig. 5).

Significant intergroup differences were also found in the reversal test results ($F_{(1,19)} = 6.7316$; $p < 0.0178$), with the stimulated animals now showing a preference for the place not associated with the stimulation (Fig. 6).

4. Discussion

This study examined the relative relevance of place and taste stimuli after electrical stimulation of the LPBe subnucleus. Electrical stimulation of this brain area induced consistent individual aversions or preferences for the stimuli with which it was associated in concurrent place preference and taste/place discrimination tasks. However, it is not known whether the animals develop a preference or aversion behavior for a taste stimulus or for the place at which it is simultaneously available. The present results suggest that the animals may establish a preferential association with place and/or proprioceptive indexes. Indeed, our finding in the reversal tests confirm that a priority choice can be established towards place and/or proprioceptive indexes (Arnold & Agmo, 1999), because the animals chose to ingest the taste stimulus located in the position (place) previously associated with the reinforcing

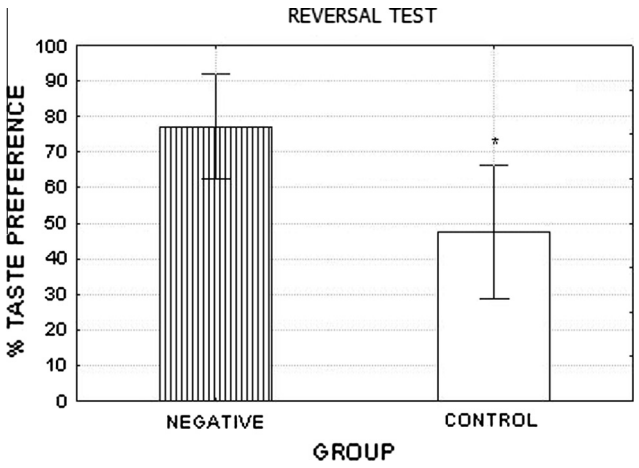

Fig. 6. Percentage of preference for the taste stimulus associated with electrical stimulation of the LPBe nucleus shown by the animals in the "negative" group (striped) and "control" group (white) in the Reversal Test.

stimulation of the LPBe nucleus but not the taste stimulus that had been preferred in the choice test.

The fact that electrical stimulation of the IC from the same stereotaxic coordinates generates either preferences or aversions suggests that the systems processing rewarding and aversive motivational information may be anatomically very close together (Hoebel, 1976; O'Doherty et al., 2001; Salamone, 1994). The stainless steel 00 electrodes used for electrical brain stimulation in our study can activate cell bodies, initial axon segments, and Ranvier nodules within a small spherical field of electrical influence (Ranck, 1975; Yeomans, 1990). Dissociation among different functional systems that are anatomically very close to the electrode tip (Yeomans, 1990) depends on the specific placement of the electrode within the subnucleus and may also be achieved by modification of the current parameters to activate some or other systems (e.g. stimulus-bound eating and self-stimulation) (Hawkins et al., 1983). Specifically, electrical stimulation of the LPBe nucleus seems to be involved in opposite behavioral processes (Mediavilla et al., 2000; Zafra et al., 2002), as observed with stimulation of other brain areas, such as the lateral hypothalamus (e.g., eating, drinking, self-stimulation, or aversion, etc.) (Gratton & Wise, 1983; Hawkins et al., 1983) or the periaqueductal gray matter (pain or analgesia) (Mayer, Wolfle, Akil, Carder, & Liebeskind, 1971; Prado & Roberts, 1985). Presumably, therefore, electrical stimulation in the "neutral" animals may have simultaneously activated cells that process appetitive and aversive information from neighboring neuronal populations, as observed in other brain regions (Moufid-Bellancourt, Razafimanalina, & Velley, 1996; Yamamoto, Matsuo, Kiyomitsu, & Kitamura, 1989; O'Doherty et al., 2001).

The nature of the reinforcement induced by electrical stimulation of the LPBe nucleus has not been elucidated but may involve opioid mechanisms (Simon et al., 2007, 2011). It is well known that taste and place preferences can be induced by liquids or foods (Puerto, Deutsch, Molina, & Roll, 1976; Schroeder & Packard, 2000; Spiteri et al., 2000; Stefurak & Van der Kooy, 1992; White et al., 1987; Zafra et al., 2002). Thus, the LPBe nucleus may participate in the processing of information related to hedonic and regulatory aspects of food (Horn & Friedman, 1998; Li et al., 1994; Wang, Cardin, Martinez, Tache, & Lloyd, 1999; Yamamoto & Sawa, 2000a, 2000b; Yamamoto et al., 1994), and wide lesions of the LPB (which may include the external lateral subnucleus) impaired preferences for rewarding nutritive substances (Reilly & Trifunovic, 2000a, 2000b) and palatable food (Edwards & Ritter, 1989). Moreover, specific lesions of the LPBe nucleus blocked preferences for the taste stimuli associated with the administration of rewarding nutrients (Zafra et al., 2002).

Likewise, electrical stimulation of the LPBe nucleus may have activated an opioid brain region (Simon et al., 2007, 2011) involved in incentive attribution. In fact, the parabrachial complex has been implicated in positive hedonic-affective processes, among others (Edwards & Ritter, 1989; Sewards, 2004; Yamamoto & Sawa, 2000a, 2000b; Yamamoto et al., 2009); therefore, activation of μ and κ opioid receptors of the parabrachial complex may participate in the hedonic assessment of different stimuli (Carr, Aleman, Bak, & Simon, 1991; Moufid-Bellancourt et al., 1996; Simon et al., 2007; Wilson, Nicklous, Aloyo, & Simansky, 2003). In contrast, the blockage of opioid receptors in the ventrolateral PB region eliminates preferences for appetizing products (Edwards & Ritter, 1989; Moufid-Bellancourt et al., 1996), whereas chronic food restriction modifies the activity of μ and κ receptors in the LPBe and external medial PB nuclei (Carr, Park, & Stone, 1998; Wolinsky, Carr, Hiller, & Simon, 1996). Furthermore, administration of a naloxone opioid antagonist was found to block place preferences induced by reinforcing electrical stimulation of the LPBe nucleus (Simon et al., 2007, 2011).

All of these studies suggest that the LBPe nucleus may act as a part of a general rewarding system and the administration of various drugs of abuse is known to elicit c-fos immunoreactivity in the LPBe nucleus (Grabus, Glowa, & Riley, 2004; Gutstein et al., 1998; Li et al., 1994; Sakai & Yamamoto, 1997; Yamamoto & Sawa, 2000b). It is well documented that these substances can induce positive 'affective states' that may explain the facility of animals to establish associative learning (Ikemoto, 2010; Spiteri et al., 2000; Tzschentke, 1998; White et al., 1987), as shown by approach behaviors towards or lengths of stay in contact with stimuli (tactile, visual) present during learning (Spiteri et al., 2000; Vezina & Stewart, 1987; White et al., 1987). In fact, these rewarding effects might preferentially be associated with environmental stimuli rather than taste stimuli (White et al., 1987). This preferential behavior is very similar to that observed during the choice test and the reversal test in the present study. In fact, most of the substances that generate addiction in humans also induce CPP (Carr, Phillips, & Fibiger, 1989; McBride et al., 1999; Mucha, Van Der Kooy, ÓShaughnessy, & Bucenieks, 1982; Tzschentke, 1998), although they do not all generate conditioned taste preference (Bechara et al., 1993; Mackey, Keller, & Van der Kooy, 1986; Mucha & Herz, 1985; White et al., 1987).

In the reversal test, the animals in the negative group maintained their preference for the previous "safe" place and not for the previous "safe" flavor, increasing their intake of the taste stimulus previously associated with stimulation, i.e., showing a preference for the place not associated with stimulation. Previous studies demonstrated that the reduction in the taste stimuli consumption generated by drugs such as amphetamines, nicotine, morphine, cocaine, is not accompanied by conditioned disgust [in the taste reactivity (RT) test] (Parker, 1991, 1993, 1995), but there would have been an avoidance learning of the location of the taste stimulus in the experiments (Parker, 2003). Conversely, many treatments that produce reduced consumption or rejection of a taste (RT test) do not generate place preference in a CPP task; therefore, the reduction in intake may result from the development of conditioned taste aversion (Parker, 2003). In this context, it was reported to be difficult to develop concurrent taste aversion learning in neurologically intact animals with the use of spatial/proprioceptive stimuli but not with taste/olfactory stimuli (Mediavilla, Molina, & Puerto, 2001). In brief, the results obtained in this study suggest that the reinforcing stimulation of the LPBe nucleus may impose a biological constraint that initially directs the animal towards the location of the stimulus, although it is also possible to develop specific taste preferences using a larger number of trials (Simon et al., 2013).

The environment is known to be important in developing dependence and/or tolerance associated with the repeated administration of drugs of abuse (Ghitza, Fabbricatore, Prokopenko, Pawlak, & West, 2003; See, 2002; Siegel, 1999). Thus, the mere presence of a stimulus associated with drug administration may produce the onset of abstinence syndrome symptoms (Siegel, 1999; Siegel & Ramos, 2002). In fact, various studies have implicated the lateral region of the PB complex in the opioid abstinence syndrome (Hamlin, Buller, Day, & Osborne, 2001; Nader et al., 1996), and chronic opiate administration alters μ receptors in the Medial and Lateral Parabrachial Complex, among other brain regions (Sim, Selley, Dworkin, & Childers, 1996; Sim-Selley, Selley, Vogt, Childers, & Martin, 2000). In addition, wide lesions of the LPB Complex were found to block conditioned place aversions induced by opiate withdrawal syndrome (Nader et al., 1996) or after morphine administration (Bechara et al., 1993). In this context, the LPBe is one of the nuclei involved in the aversive processing of noxious substances such as hypertonic NaCl, copper sulfate, lithium chloride (Mediavilla et al., 2000; Sakai & Yamamoto, 1997), and drugs of abuse, including morphine, cocaine, and methamphetamines (Bechara et al., 1993; Grabus et al., 2004; Yamamoto & Sawa, 2000a, 2000b; Sakai & Yamamoto, 1997). Thus, the intra-parabrachial administration of morphine modified preferences for a sweet solution, reducing its consumption (Moufid-Bellancourt et al., 1996), whereas specific LPBe lesions (Mediavilla et al., 2000) and wide LPB lesions that would have included the LPBe subnucleus (Bechara et al., 1993) interrupted the aversive learning induced by hypertonic NaCl and morphine, respectively.

It is therefore possible that electrical stimulation of the LPBe nucleus activated cells that codify negative (aversive) hedonic information (Bernard et al., 1994; Sewards, 2004; Yamamoto et al., 1994), comparable to the malaise observed after an aversive treatment (Bechara et al., 1993; Bernard et al., 1994; Mediavilla et al., 2000) or even after a painful treatment, given that this subnucleus is also part of the circuit involved in the affective processing of nociceptive information (Bernard et al., 1994; Bester et al., 1995; Gauriau & Bernard, 2001; Jasmin et al., 1997), so that associated stimuli (place or taste) are now aversive for the animal.

The present results demonstrate that electrical stimulation of the LPBe nucleus may induce rewarding (or aversive) effects that appear to impose biological constraints preferentially related to place (exteroceptive, proprioceptive...) rather than taste/olfactory stimuli, as in some rewarding treatments.

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