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ScienceDirect
Neurobiology of Learning and Memory 87 (2007) 332–342
Neurobiology of Learning and Memory
www.elsevier.com/locate/ynlme

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

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Received 11 July 2006; revised 22 September 2006; accepted 22 September 2006
Available online 3 November 2006

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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1074-7427/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.nlm.2006.09.009

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.