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Research report

Differential effects of naloxone on rewarding electrical stimulation of the central nucleus of the amygdala and parabrachial complex in a place preference study

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ABSTRACT

The central nucleus of the amygdala (CeA) is considered to be involved in different affective, sensory, regulatory, and acquisition processes. This study analyzed whether electrical stimulation of the PB-CeA system induces preferences in a concurrent place preference (cPP) task, as observed after stimulation of the parabrachial-insular cortex (PB-IC) axis. It also examined whether the rewarding effects are naloxone-dependent. The results show that electrical stimulation of the CeA and external lateral parabrachial subnucleus (LPBe) induces consistent preference behaviors in a cPP task. However, subcutaneous administration of an opiate antagonist (naloxone; 4 mg/ml/kg) blocked the rewarding effect of the parabrachial stimulation but not that of the amygdala stimulation. These results are interpreted in the context of multiple brain reward systems that appear to differ both anatomically and neurochemically, notably with respect to the opiate system.

1. Introduction

Intracranial electrical stimulation is one of the experimental procedures used to study brain mechanisms of reinforcement (Olds and Milner, 1954; Yeomans, 1990; for review, see Ikemoto, 2010). These studies habitually use lever-press tests, although place preference tasks have also been employed (Zimmermann et al., 1999; for review, see Koob and Le Moal, 2008). These latter tasks have been used to study the rewarding effects of food and water intake (Spiteri et al., 2000) but have mainly been employed to investigate the rewarding/aversive effects of drugs of abuse (Spiteri et al., 2000; for review, see Tzschentke, 2007).

Different regions involved in the brain reward system have been identified by means of intracranial electrical stimulation, most frequently in relation to the medial forebrain bundle (Deutsch, 1963; Yeomans, 1990; for review, see Ikemoto, 2010) but also to other more caudal and rostral regions, extending from the brainstem to the cortex (Cubero and Puerto, 2000; Simón et al., 2007; García and Simón, 2013; for review, see Ikemoto, 2010). Thus, authors have demonstrated the rewarding effects of electrically stimulating regions specifically related to visceral-gustatory information processing (Fulwiler and Saper, 1984; De Lacalle and Saper, 2000; Contreras et al., 2007). These include the external lateral parabrachial nucleus (LPBe) and its anatomical projection to the insular cortex (IC), whose activation was found to induce place and taste preferences (Cubero and Puerto, 2000; Simón et al., 2007; García and Simón, 2013) and to be subject to tolerance (Hurtado and Puerto, submitted; García and Zafra, 2015; Hurtado et al., 2016), consistent with the hedonic impact attributed to the parabrachial complex (Söderpalm and Berridge, 2000). It was observed that place preference behaviors induced by activation of the LPBe and IC can be blocked by the administration of naloxone (Nx), an opiate antagonist (Simón et al., 2007; García and Simón, 2013), suggesting the possible involvement of the opiate system in the rewarding effect induced by their electrical stimulation. In fact, c-fos studies revealed the involvement of the LPBe, alongside other structures, in the effects of nutrients, food-related drugs, and drugs of abuse (Sakai and Yamamoto, 1997; Yamamoto and Sawa, 2000).

The LPBe not only connects anatomically to the IC (Fulwiler and Saper 1984; Dobolyi et al., 2005) but also projects towards the central nucleus of the amygdala (CeA) (Norgren 1976; Fulwiler and Saper, 1984; Bernard et al., 1993; Jia et al., 1994; for review, see Cassell et al., 1999). However, involvement of the opiate system has been investigated in the parabrachial-insular (PB-IC) axis but not in the parabrachial-amygdala (PB-CeA) system.

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The CeA receives sensory-visceral and exteroceptive information (Ottersen, 1982; Bernard et al., 1993; Yamamoto et al., 1997; Michl et al., 2001) and has been related to the rewarding effect of natural reinforcers, such as food (Nishijo et al., 2000) or sexual activity (Kling and Brothers, 1992), as well as to the acquisition of flavor-conditioned preferences (Touzani et al., 2009) and aversions (Agüera and Puerto, 2015). Various amygdala subnuclei, including the CeA, sustain rewarding electrical self-stimulation (Touzani and Velley, 1998), while electrical stimulation of reward areas such as the lateral hypothalamus is known to increase their cellular activity (Touzani and Velley, 1998; Nakahara et al., 1999).

The CeA has also been associated with the rewarding effect of some drugs of abuse (e.g. Criado and Morales, 2000; Roberto et al., 2003; Chen et al., 2013; Herman and Roberto, 2014; for review, see Koob and Le Moal, 2008), notably opiate substances (Criado and Morales, 2000; Roberto et al., 2003; Bajo et al., 2014; for review, see Koob and Le Moal, 2008). The CeA has also been implicated in the negative affective states caused by Nx administration in both naïve (Gestreau et al., 2000; Pomonis et al., 2000) and morphine-dependent subjects (e.g. Heinrichs et al., 1995; Le Guen et al., 2001; Watanabe et al., 2003; Nakagawa et al., 2005; Bajo et al., 2014).

With this background, and as observed in the PB-IC axis (Simón et al., 2007; García and Simón, 2013), the objective of the present study was to establish whether place preference behaviors can be induced by electrical stimulation of the PB-CeA system and whether the induced reward is Nx-dependent. Two experiments were conducted: in experiment 1, preference behaviors were induced by electrical stimulation of the CeA in a concurrent place preference (cPP) task; in experiment 2, preference behaviors were induced by electrical stimulation of the LPBe in an identical task. Studies were then performed to examine the effect of Nx on the rewarding action of CeA and LPBe electrical stimulation.

2. Materials and methods

2.1. Subjects and surgical procedure

The study used 44 Wistar rats from the University of Granada weighing 270–320 g at baseline. They were housed in cages with food (Panlab, Barcelona, Spain) and water ad libitum before surgery. The laboratory was maintained at 22°–24° with a 12:12 h light/dark cycle. All experimental procedures were conducted during the light period with white noise. All behavioral, pharmacological, and surgical procedures complied with the animal care guidelines established by the Spanish Royal Law (1201/2005) and European Community Council Directive (86/609/EEC).

Animals were implanted under general anesthesia (sodium pentothal, 50 mg/kg, Laboratorios Abbot S.A., Madrid) with a stainless steel monopolar electrode (00) (Hawkins et al., 1983; Simón et al., 2011) using a stereotaxic device (Stoelting Co. Stereotaxic 51600, USA). As a prophylactic measure, 0.1 cc penicillin (Penilevel Retard. Lab., Level, S.A., Barcelona, Spain) was intramuscularly injected and an antiseptic solution was applied around the implant (Betadine. Povidone Iodine. Asta Médica, Madrid). After surgery, animals were returned to their individual cages for a recovery period of at least one week with food and water ad libitum. The animals were then randomly assigned to experiment 1 or experiment 2 (27 in each group).

In experiment 1, 16 animals were randomly selected for implantation of a monopolar electrode in the left CeA (AP = +6.7; L = +4.0; V = +2.0; Paxinos and Watson, 1998) (CeA-S group), while a reference electrode was placed on the skull surface of the remaining 7 animals (Yeomans, 1990), which served as neurologically intact control (CeA-C) group.

In experiment 2, 15 animals were randomly selected for implantation of a monopolar electrode in the left LPBe (AP = −0.16; L = +2.5; V = +3.0; Paxinos and Watson, 1998) (LPBe-S group), while a reference electrode was placed on the skull surface of the remaining 6 animals, which served as neurologically intact control (LPBe-C) group.

2.2. Equipment

For the monopolar electrical stimulation, cathodal rectangular constant-current pulses of 66.6 Hz and 0.1 ms pulse duration were 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).

As a procedure to control for progressive error effects (Myers and Hansen 1993), two different rectangular mazes were employed for the cPP phases. In phase 1, we used Model A rectangular maze (50 × 25 × 30 cm), oriented East-West (E-W), with three differentiated areas: a central area (8 × 25 cm^2), in which the floor and walls were white methacrylate; and two lateral compartments with walls of methacrylate with white 2-cm wide stripes that were vertical in one compartment and horizontal in the other. The floor was brown cork with longitudinal (8 × 1 cm) or circular (1.5 cm) incisions, respectively. In phase 2, we used model B rectangular maze (50 × 25 × 30 cm), oriented North-South (N-S), in which the wooden 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 natural wood color (Simón et al., 2007; García and Simón, 2013).

2.3. Behavioral procedures

During the postoperative recovery period (≥7 days), four animals in the CeA-S group (experiment 1) and five animals in the LPBe-S group (experiment 2) were excluded from the study because of circling behavior.

2.3.1. Phase 1: electrical stimulation-induced cPP in maze A

As in previous studies in our laboratory (Simón et al., 2007; García and Simón, 2013), the appropriate current intensity was individually established for each animal by applying progressive increments of 10 μA and observing in detail the preference behavior of the animal after each increase. The intensity level selected for future experimental phases was immediately below that at which behavioral preferences were accompanied by signs of nervousness, e.g., involuntary movements, escape responses, or vocal reactions (Tehovnik, 1996; Simón et al., 2007; García and Simón, 2013).

Current values ranged between 90 and 200 μA (mean: 165.62 μA) for the CeA-S group (experiment 1) and between 60 and 170 μA (mean: 94 μA) for the LPBe group (experiment 2). At 48 h after establishing the optimal current intensity for each animal, they underwent a 10-min cPP session (cPP1 and cPP2) on two consecutive days. The animal was placed in the center of maze A (oriented E-W), and its voluntary stay in one of the lateral compartments, previously randomly selected and the same for both sessions, was accompanied by the corresponding concurrent intracranial electrical stimulation of the CeA (CeA-S group in experiment 1) or LPBe (LPBe-S group in experiment 2), recording the stay time in each area. The animals in the control groups of each experiment (CeA-C and LPBe-C) underwent the same procedure but received no electrical stimulation.

After each session, animals were returned to their home cages with water and food ad libitum. Animals were classified accord-