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Naloxone blocks the aversive effects of electrical stimulation of the parabrachial complex in a place discrimination task

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ABSTRACT

The parabrachial complex is known to participate in various rewarding and aversive processes, including those related to the learning of taste or place discrimination and the motivational effects of drugs of abuse, such as morphine. This study shows that electrical stimulation of the external lateral parabrachial (LPBe) subnucleus induces consistent place avoidance or place preference in three-compartment rectangular mazes. Administration of naloxone, an opiate antagonist, blocks both motivational effects induced by the intracranial electrical stimulation. Subsequent re-administration of the electrical stimulation was found to recover its aversive but not its rewarding effects after vehicle administration. These results are discussed in relation to different natural and artificial agents involved in the induction of avoidance and preference motivational processes, especially with regard to the opioid system.

1. Introduction

The activation of certain brain areas can induce various negative behaviors, including escape, action aimed at stopping the stimulation, or avoidance of specific areas in place aversion tasks (Carter, Han, & Palmiter, 2015; Diotte, Bielajew, Miguelez, & Miliaressis, 2001; Gomita, Ichimaru, Moriyama, Araki, & Futagami, 2003; Gomita, Moriyama, Ichimaru, & Araki, 1991; Simón, Zafra, Molina, & Puerto, 2008). However, brain stimulation procedures can also induce behavioral preferences (Ettenberg, 1979; Ettenberg & White, 1978; Ettenberg & White, 1981; Olds & Milner, 1954).

In this regard, while the LPBe has been related to place and taste preferences (García, Simón, & Puerto, 2013; García, Simón, & Puerto, 2014; Simón, García, Zafra, Molina, & Puerto, 2007; Simón, Molina, & Puerto, 2009), a role in aversion processing has also been described (Hurtado, García, & Puerto, 2014; Simón et al., 2008). The LPBe, among other regions, has also been reported to be involved in flavor aversion learning (Sakai & Yamamoto, 1997; Spencer, Eckel, Nardos, & Houpt, 2012; Yamamoto, Shimura, Sakai, & Ozaki, 1994; Yamamoto, Shimura, Sako, Yasoshima, & Sakai, 1994) which was found to be impaired by lesions of this subnucleus (Mediavilla, Molina, & Puerto, 2000; Mediavilla, Molina, & Puerto, 2005). The LPBe subnucleus appears to be part of an anatomical axis that includes related nervous structures such as the vagus nerve or nucleus of the solitary tract (Fulwiler & Saper, 1984) and is considered essential in this learning modality (Agüero, Gallo, Arnedo, Molina, & Puerto, 1997; Mediavilla et al., 2000, 2005; Simón et al., 2007, 2008, 2009; Zafra, Simón, Molina, & Puerto, 2002).

Various studies have demonstrated the presence of high concentrations of opioid receptors in the parabrachial complex (Mansour, Fox, Akil, & Watson, 1995; Quirion, Zajac, Morgat, & Roques, 1983), compatible with the rewarding effect induced from this pons region, which is subject to tolerance, as is the insular cortex, with which it is connected (Hurtado and Puerto, 2016; García, Zafra, & Puerto, 2015; Hurtado, García, & Puerto, 2016). In addition, this effect can be blocked by the administration of naloxone, an opiate receptor antagonist (Simón et al., 2007).

However, naloxone is known to generate taste aversion when its administration is associated with a flavor (Desko, Cobuzzi, & Riley, 2012; Mucha & Walker, 1987) and place aversion when administered in a specific context (Cagniard & Murphy, 2013; Mucha & Walker, 1987; Solecki, Turek, Kubik, & Przewlocki, 2009), and it was even found to produce a severe withdrawal syndrome in animals pretreated with morphine (Martínez-Laorden et al., 2014; Radke, Holtz, Gewirtz, & Carroll, 2013).

Given the above data, the question arises as to whether the negative effect of naloxone administration would add to the aversive effect induced by electrical PBLe stimulation or would interrupt it by blocking opiate systems. In fact, the involvement of the opiate

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system in the aversive effects induced by PBLe activation has not yet been established. Various authors have proposed that it may constitute one aversive neurochemical component that interacts with GABA mechanisms (Johnson & North, 1992), followed by involvement of the dopamine system (Dacher & Nugent, 2010; David et al., 2008; Hurtado et al., 2014; Zhang, Zhang, Jin, Zhang, & Zhen, 2008).

We hypothesized that naloxone administration may interfere with the aversive effect induced by electrical LPBe stimulation, as in the case of the rewarding effect generated from this brain region, which would suggest the likely participation of opiate mechanisms in both cases.

2. Method

2.1. Subjects and surgical procedure

This study used 43 male Wistar rats from the breeding colony at the University of Granada, weighing 280–350 g at surgery. They were randomly distributed into two groups, one implanted with intracranial electrodes in the LPBe subnucleus (26 animals) and a neurologically intact control group (17 animals). Animals were housed in methacrylate cages, with water and food *ad libitum* (A-04, Panlab Diets S.L., Barcelona, Spain). The laboratory was maintained at 20–24 °C with a 12:12 h light/dark cycle. All experimental procedures were conducted during light periods with white noise.

The animals remained under these conditions for an adaptation period of at least 7 days before surgery. All behavioral procedures and surgical techniques complied with the Spanish regulations (Royal Law 23/1988) and the European Community Council Directive (86/609/EEC).

Animals were implanted with a stainless steel monopolar electrode (00) in the LPBe subnucleus [Coordinates: AP = −0.16; V = 3.0; L = ±2.5, according to the atlas by Paxinos and Watson (1998)] using a stereotaxic apparatus (Stoelting Co. Stereotaxic 511.600, USA) under general anesthesia (sodium thiopental, 50 mg/kg, B. Braun Medical S.A. Barcelona, Spain). As prophylactic measures, 0.1 cc penicillin (Penilevel, Level Laboratory, S.A., Barcelona, Spain) was intramuscularly injected and an antiseptic solution was applied around the implant (Betadine. Povidone-Iodine. Asta Médica, Madrid, Spain). There was a post-surgery recovery period of at least 7 days.

2.2. Equipment

For the monopolar electrical stimulation, a rectangular cathodal constant-current of 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). As in previous studies in our laboratory, the appropriate current intensity was individually established for each of the 26 animals (between 80 and 260 μA in this study) by applying progressive increments of 10 μA and observing in detail the behavior of the animal after each increase, selecting for future experimental phases the intensity level immediately below that at which behavioral signs of nervousness were observed, e.g., unmotivated motor activity or vocalizations (Tehovnik, 1996).

Three different three-compartment rectangular mazes and orientations were used to avoid transference effects (carry over):

Model 1: Rectangular maze (50 × 25 × 30 cm) oriented East-West, 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) was white methacrylate, and the walls were a natural wood color.

Model 2: Rectangular maze (70 × 15 × 15 cm.) oriented North-South, 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) had a metal grill floor and the walls were white.

Model 3: Rectangular maze (50 × 25 × 30 cm) oriented Northeast-Southwest, in which the walls of the two lateral compartments were painted with wider black and white wide stripes that were vertical in one compartment and horizontal in the other. In both compartments, the floor was brown cork with transverse incisions in one compartment and vertical incisions in the other. The floor and walls of the central area (8 × 25 cm) were white methacrylate.

2.3. Behavioral procedure

2.3.1. Phase 1: Animal distribution

The concurrent place discrimination task in the model 1 maze commenced at 48 h after establishing the individual optimal electrical current (see "equipment"). The maze area associated with stimulation was selected in a random and counterbalanced manner such that half of the animals were stimulated in one area and the remaining animals in the other. After placing each animal in the center of the maze, the voluntary stay of the animal in the stimulation-associated area was accompanied by the corresponding intracranial electrical stimulation in the LPBe subnucleus, and the stay time in each area was recorded, with each session lasting for 10 min. The stimulation was activated immediately when the animal entered the stimulation-associated area (heading into the compartment and with both forepaws already inside it) and was deactivated immediately when the animal left it (heading out of the compartment with both forepaws already outside it). The neurologically intact animals underwent the same procedure without stimulation.

This process was conducted in two sessions on consecutive days, but results on the second day alone were considered as aversion or preference index.

The surgically intervened animals were distributed into three groups as a function of their behavior in the test, applying behavioral criteria established in previous studies (García et al., 2013, 2014, 2015; Simón et al., 2007, 2008, 2009): (a) "positive" animals, which consistently preferred the stimulated maze compartment and stayed for >50% of the time in this area; (b) "negative" animals, which consistently avoided the stimulated compartment, staying in it for <30% of the time; and (c) "neutral" animals, which evidenced no consistent preference or aversive behavior and stayed for 30–50% of the time in the stimulated compartment. Only the six animals that showed avoidance behaviors were selected for experiment 1, along with the seven neurologically intact animals, which formed a control group. Twelve animals showing preference behavior were used as stimulated group in experiment 2, in which 10 neurologically intact animals served as control group.

2.3.2. Phase 2: Baseline

At 48 h after ending phase 1, two more place aversion/preference sessions were conducted, identical to those reported above and in the same maze, with the aim of establishing baseline values.

2.3.3. Phase 3: Effect of naloxone administration

After a further 48-h interval, we conducted two more place conditioning sessions but in the model 2 maze in order to avoid learning transferences. The same procedure was followed as in phases 1