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Research report

Tolerance to repeated rewarding electrical stimulation of the parabrachial complex

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HIGHLIGHTS

- Stimulation of the parabrachial area induces naloxone-related behavioral preferences.
- The external lateral subnucleus (LPBe) was repeatedly stimulated in this study.
- Animals received daily or alternate sessions of rewarding electrical stimulation.
- As in the insula, only the daily-stimulated group showed decay in place preferences.
- These results suggest that LPBe-induced reward might also be subject to tolerance.

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ABSTRACT

The parabrachial complex has been related to various rewarding behavioral processes. As previously shown, electrical stimulation of the lateral parabrachial external (LPBe) subnucleus induces opiate-dependent concurrent place preference. In this study, two groups of animals (and their respective controls) were subjected to sessions of rewarding brain stimulation daily or on alternate days. The rats stimulated every other day maintained a consistent preference for the place associated with the brain stimulation. However, as also found in the Insular Cortex, there was a progressive decay in the initial place preference of animals receiving daily stimulation. These data suggest that the rewarding effects induced by electrical stimulation of LPBe subnucleus may be subject to tolerance. These findings are discussed with respect to other anatomical areas showing reward decay and to the reinforcing effects induced by various electrical and chemical rewarding agents.

1. Introduction

The lateral parabrachial complex has been related to different rewarding and aversive behavioral processes [1,35,37], including the effects of some drugs of abuse [2,23].

In particular, the external lateral parabrachial (LPBe) subnucleus has been related to taste aversion learning [45,21,37] and to the processing of affective components of pain [3,13]. However, this region has also been activated after intraoral and gastrointestinal (appetitive) infusions [41,43,44]. Furthermore, lesions of this area block taste preferences induced by the administration of rewarding nutrients [47].

Electrical stimulation of the LPBe subnucleus generates preferences for the associated taste or place in discrimination learning tasks [35,11]. In place discrimination tasks, the rewarding effect of LPBe stimulation was blocked by naloxone administration, indicating that opiate mechanisms are likely involved [35]. This result is compatible with experimental evidence that this parabrachial subnucleus is one of various brain regions that participate in the processing of rewarding substances of abuse, e.g., amphetamines [31] or opiates [15,5].

Results analogous to those found in the LPBe subnucleus have been observed after electrical stimulation of the insular cortex (IC) [7].

In this context, recent studies in our laboratory revealed a decay in the effectiveness of rewarding IC electrical stimulation after repeated successive tests [18].

With this background, the objective of the present experiment was to determine whether repeated electrical stimulation of the LPBe subnucleus decreases its rewarding effect in a place preference task, i.e., whether it produces the behavioral tolerance

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observed with repeated rewarding electrical stimulation of the IC [18], given the anatomical connection between these regions [10,8].

2. Material and methods

2.1. Subjects and surgical procedure

Thirty male Wistar rats weighing 310–410 g at baseline were used in this study. They were randomly distributed into two groups, one implanted with intracranial electrodes in the LPBe subnucleus (22 animals) and a neurologically intact control group (8 animals). Animals were housed in individual methacrylate cages (30 × 15 × 30 cm) with ad libitum water and food (Food, 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, using white noise to cancel out possible fortuitous sounds.

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 Spanish regulations (Royal Law 23/1988) and the European Community Council Directive (86/609/EEC), and were approved by the Ethical Committee for Animal Experimentation of the University of Granada.

Animals were implanted with an insulated (except at the lowest part) stainless steel monopolar electrode (00) in the LPBe subnucleus [Coordinates: AP = −0.16; V = 3.0; L = ±2.5, according to the atlas of Paxinos and Watson [28]] using a stereotaxic apparatus (Stereotaxic 511.600, Stoelting Co., Wood Dale, IL) under general anesthesia (50 mg/kg sodium thiopental, B. Braun Medical S.A. Barcelona, Spain). As prophylactic measures, povidone-iodine (Betadine, AstaMédica, Madrid, Spain) was applied around the implant, and 0.1 cc penicillin (Penilevel, Level Laboratory, S.A., Barcelona, Spain) was intramuscularly injected. There was a post-surgery recovery period of at least 10 days.

2.2. Equipment

For the monopolar electrical stimulation, cathodal constant-current rectangular pulses of 66.6 Hz with 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). Current intensity was individually established for each animal (between 50 and 190 μA in this study), avoiding levels that could generate involuntary movements, escape responses, or pain manifestations [38]. For this purpose, current intensity was increased in steps of 10 μA, observing the behavior of the animal in response to each increase. When the current intensity generated some initial manifestation of negative behaviors, the current was reduced until it was verified that behavioral activation was produced but not escape or pain responses.

A three-chamber rectangular maze (50 × 25 × 30 cm) oriented North-South was used, 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 [35,34,11].

2.3. Behavioral procedure

2.3.1. Phase 1: animal classification and distribution

The concurrent place preference (cPP) task comprised two 10-min sessions on consecutive days. After placing each animal in the center of the maze, they were allowed to wander freely in and among compartments. The animal received intracranial electrical stimulation when it was in the stimulation-associated compartment, which was randomly established in a counterbalanced manner, but not when in any other area of the maze. The neurologically intact animals underwent the same procedure but without stimulation.

The stimulated animals were classified into three groups according to the time spent in the stimulation-associated maze compartment, following criteria previously established in our laboratory [35,34,17,11,12]: (a) “positive” animals, which stayed in the compartment for >50% of the time; (b) “aversive” animals, which stayed in it for <30% of the time; and (c) “neutral” animals, which evidenced no consistent preference or aversive behavior, staying in the compartment for 30–50% of the time.

2.3.2. Phase 2: baseline

At 72 h after ending phase 1, another place preference session was conducted, identical to the sessions reported above and in the same maze, in order to establish baseline values.

2.3.3. Phase 3: repeated electrical stimulation of the LPBe subnucleus

This phase included a stimulation protocol in which the “positive” animals underwent four cPP sessions. These sessions were alternated with four sessions in which animals in the Positive Group 1 (but not Positive Group 2) received stimulation while confined in the maze area stimulation-associated compartment:

At 24 h after Phase 2, all animals underwent a 10-min cPP session during which they were confined in the stimulation-associated compartment. Only the animals in Positive Group 1 received electrical stimulation during their confinement in the compartment.

At 24 h after the confinement session, all animals underwent another cPP session. Both Positive 1 and Positive 2 Groups received electrical stimulation of the LPBe subnucleus during their stay in the stimulation-associated compartment, but the control groups did not.

This sequence was repeated four times, alternating four confinement sessions with four concurrent stimulation sessions (see Table 1).

2.4. Histology

After the behavioral tests, the animals were anesthetized, and a small electrolytic lesion was made (0.3 mA/5 s) to localize the position of the electrode in each animal, followed by intracardiac perfusion of isotonic saline and 40% formaldehyde solution. Brains were extracted and kept in 10% paraformaldehyde until sectioned in 60-μm coronal slices. These were stained with Cresyl Violet, examined under a stereoscopic magnifying glass (VMZ-4F, Olympus, Tokyo, Japan), and photographed with a PM-6 camera (Olympus, Tokyo, Japan) (see Fig. 1).

2.5. Statistical analysis

Statistica 6.0 (Statsoft Inc.; Tulsa, OK) was used for the statistical analysis. Intra-group univariate analysis of variance (ANOVA) was performed in each group, followed by planned comparisons, and a two-way ANOVA was performed to compare the two stimulated groups at baseline and after the differential treatment. $P < 0.05$ was considered significant in all tests.