Neurobiology of Learning and Memory 118 (2015) 64–73

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Rewarding effects of electrical stimulation of the insular cortex: Decayed effectiveness after repeated tests and subsequent increase in vertical behavioral activity and conditioned place aversion after naloxone administration

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ARTICLE INFO

Article history:
Received 5 July 2014
Revised 21 October 2014
Accepted 7 November 2014
Available online 22 November 2014

Keywords:
Insular cortex
Electrical brain stimulation
Place preference
Flavor preference
Naloxone

ABSTRACT

The insular cortex has been associated with various aversive and rewarding sensory, regulatory, and learning processes. The objective of this study was to examine the characteristics of the reinforcement induced by electrical stimulation of this brain area in rats. Results obtained confirm that electrical stimulation of the insular cortex may induce conditioned place and flavor preferences but the learning acquired is not transferred in a reversal test. Unexpectedly, they also demonstrate that this rewarding effect diminishes after repeated tests. In follow-up experiments, locomotor activity tests revealed an increased number of rearings (a sensitization index) in stimulated animals. Furthermore, in these same animals, administration of low doses of naloxone, an opiate antagonist, developed place aversion toward the maze compartment for which the animals had previously shown preference. These results are interpreted in relation to the effects induced by the repeated administration of natural and artificial rewarding stimuli.

1. Introduction

The insular cortex (IC) is a brain region that participates in the processing of interoceptive and exteroceptive sensory information (Cechetto & Saper, 1987; Hanamori, Kunitake, Kato, & Kannan, 1998; Ito, 1998; Yamamoto, Matsuo, Kiyomitsu, & Kitamura, 1989) and in various homeostatic, motivational, and learning processes (Cechetto & Saper, 1987; Contreras, Ceric, & Torrealba, 2007; Cubero & Puerto, 2000; Sewards, 2004). In this regard, the IC has been found to include neurons that are sensitive to the motivational aspects of gustatory stimuli (Maffei, Haley, & Fontanini, 2012; Yamamoto et al., 1989) and to innocuous and noxious somatosensory information (Ogawa & Wang, 2002). It has also been reported that lesions of the IC alter the changes in hedonic value usually observed in taste aversion learning tasks (Kiefer & Orr, 1992) and impair taste aversive learning (Cubero, Thiele, & Bernstein, 1999). It has also been suggested that the aversive properties of morphine may be processed through the IC (Mackey, Keller, & van der Kooy, 1986).

A role in reward learning has also been proposed for the IC, and lesions of this area affect recall of the magnitude of the rewarding value of food (De Couteau, Kesner, & Williams, 1997; Balleine & Dickinson, 2000; Ragozzino & Kesner, 1999). Furthermore, insular activity has also been reported in contexts associated with the administration of psychoactive substances (Schroeder, Binzak, & Kelley, 2001; Schroeder & Kelley, 2002), in the response to sexual stimuli (Garavan et al., 2000; Gizewski et al., 2006; Safron et al., 2007), and in anticipation of a future reward (or punishment) (Elliott, Friston, & Dolan, 2000; Kesner & Gilbert, 2007; Kirsch et al., 2003; Schoenbaum, Chiba, & Gallagher, 1998). The IC has been related to appetitive feelings, such as hunger (Hinton et al., 2004), thirst (Egan et al., 2003), and drug cravings (Garavan, 2010; Sell et al., 2000; Wang et al., 1999), and in general to natural (Olausson et al., 2002; Small, Zatorre, Dagher, Evans, & Jones-Gotman, 2001; Wang et al., 2004) and artificial rewarding stimuli, e.g., verbal or economic reinforcement (Elliott et al., 2000; O'Doherty et al., 2003; Ullsperger & von Cramon, 2003).

Electrical stimulation of the IC induces preferences for associated stimuli in flavor discrimination and conditioned place preference (CPP) tasks (Cubero & Puerto, 2000; García, Simón, & Puerto, 2013), as also observed for electrical stimulation of the external lateral parabrachial nucleus (LPBe) (Simón, García,

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http://dx.doi.org/10.1016/j.nlm.2014.11.008
1074-7427/

Zafra, Molina, & Puerto, 2007; Simón, Zafra, Molina, & Puerto, 2008), with which the IC establishes anatomic connections (Dobolyi, Irwin, Makara, Usdin, & Palkovits, 2005; Fulwiler & Saper, 1984). CPP induced by IC stimulation can be blocked by the administration of naloxone, an opiate antagonist (García et al., 2013). In fact, the IC has a high density of opiate receptors (Izenwasser, Staley, Cohn, & Mash, 1999; Mansour, Fox, Thompson, Akil, & Watson, 1994; Svingos, Cheng, Clarke, & Pickel, 1995) and has been implicated in the processing of drugs of abuse, such as morphine (Burkey, Carstens, Wenniger, Tang, & Jasmin, 1996; Mackey et al., 1986), psychostimulants (Bonson et al., 2002; Porrino & Lyons, 2000), and marihuana (Mathew, Wilson, Coleman, Tyrkington, & DeGrado, 1997). In this regard, interoceptive effects derived from the repeated consumption of substances of abuse (e.g., nicotine or amphetamines) may be processed through the IC (Contreras et al., 2007; Naqvi, Rudrauf, Damasio, & Bechara, 2007).

In this context, previous investigations have demonstrated that different reinforcing agents can favor the establishment of different learning modalities. Thus, artificial agents, such as drugs of abuse (e.g. alcohol, morphine, cocaine, or amphetamine) or stress, appear to favor implicit learning (Dickinson, Wood, & Smith, 2002; Miles, Everitt, & Dickinson, 2003; Nelson & Killcross, 2006; Schwabe & Wolf, 2009), whereas natural agents such as food may support the acquisition of delayed learning (Garcia, Ervin, & Koelling, 1966; Holman, 1975; Zafra, Simón, Molina, & Puerto, 2002, 2005) and flexible goal-directed behaviors (Dickinson et al., 2002; Miles et al., 2003). These two learning modalities involve different functional and anatomical systems (Mediavilla, Molina, & Puerto, 2001, 2005; Petri & Mishkin, 1994; Reber, Knowlton, & Squire, 1996). Thus, implicit learning requires inter-stimulus contiguity, a larger number of trials and is characterized by rigid learning and retention processes (Mediavilla, Molina, & Puerto, 2000; Mediavilla et al., 2005; Zafra et al., 2002), and the information is stored in such a manner that it can only be accessed if the experimental conditions are similar to those established during acquisition of the learning. In contrast, explicit learning can achieve delayed learning with a smaller number of acquisition trials and is flexible, allowing transfer of the learning to new situations in changed experimental conditions, e.g., in a reversal test (Mediavilla et al., 2001, 2005; Reber et al., 1996).

Hence, the objective of this study was to characterize the rewarding effect induced by electrical stimulation of the IC in a conditioned flavor task, examining whether it permits the development of flexible (explicit) behaviors (Mediavilla et al., 2001, 2005) or, on the contrary, only allows the acquisition of implicit learning. For this purpose, the rewarding effect induced by the electrical stimulation of the IC was associated with one of two flavor stimuli (properly balanced) in a learning discrimination test (phase 1), which was then repeated with the position of the stimuli inverted to test the flexibility of the acquired learning (phase 2, reversal test). Furthermore, given that the IC has been associated with the processing of different drugs of abuse (Burkey et al., 1996; Contreras et al., 2007; Mackey et al., 1986; Naqvi et al., 2007) and that rewarding electrical stimulation is naloxone-dependent (García et al., 2013), it is of interest to determine whether IC activation can generate similar behaviors to those induced by drugs of abuse, e.g., reward-related increases in locomotor activity (behavioral sensitization) (Anagnostaras & Robinson, 1996; Berke & Hyman, 2000; Robinson & Berridge, 2003) and the place aversion to a previously preferred maze compartment precipitated by low doses of naloxone (Blokhina, Sukhotina, & Bespalov, 2000; Hand, Koob, Stinus, & Le Moal, 1988; Kawasaki et al., 2005; McDonald, Parker, & Siegel, 1997; Parker, Cyr, Santi, & Burton, 2002; Stinus, Caille, & Koob, 2000).

2. Materials and methods

2.1. Subjects

Thirty-five male Wistar rats from the breeding colony of the University of Granada, weighing 270–330 g at baseline, were housed in individual methacrylate cages (30 × 15 × 30 cm), where they remained for at least one week before surgery, with water and food available *ad libitum* (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 (beginning at 09:30) with white noise. All behavioral procedures and surgical techniques complied with the relevant Spanish legislation (Royal Law (1201/2005) and the Animal Care and Use Guidelines established by European Community Council Directive (86/609/CEE), and approved by the Ethical Committee for Animal Experimentation of the University of Granada.

2.2. Surgical procedure

The animals were randomly assigned to an implanted group ($n = 24$) or a control group ($n = 11$). In the former, a stainless steel grounded monopolar electrode (00) was implanted in the IC of the right hemisphere [Coordinates: AP = +8.16; L = +5.9; V = +2.4; (Paxinos & Watson, 1998)] using a stereotaxic apparatus (Stoelting Co. Stereotaxic 51600, USA) under general anesthesia (Sodium Pentothal, 50 mg/kg., B. Braun Medical S.A. Barcelona, Spain). As prophylactic measures, povidone iodine (Betadine, Asta Médica, Madrid, Spain) was applied around the implant, and 0.1 cc intramuscular penicillin (Penilevel, Laboratorio Level, S.A., Barcelona, Spain) was administered. In the control group animals, a reference electrode was placed on their cranial surface (Yeomans, 1990). All animals had a post-surgery recovery period of at least 10 days, with water and food available *ad libitum*.

2.3. Equipment

Electrical stimulation was supplied by a CS-20 stimulator (Cibertec, Madrid, Spain) connected to an isolation unit (ISU 165, Cibertec, Madrid, Spain) and HM 404-2 oscilloscope (HAMEG Instrument GMBH, Frankfurt, Germany). A continuous electric current of 66.6 Hz and 0.1 ms pulse duration was used and current intensity was individually established for each animal (between 60 and 150 μA), always below levels that could generate involuntary movements, escape responses, or vocal reactions (García et al., 2013; Simón et al., 2007; Tehovnik, 1996). These current levels were further reduced by 25% during the behavioral procedure to avoid any potential undesirable effects and to reduce the magnitude of the reinforcing effect (García et al., 2013), allowing the study of other possible effects induced by electrical stimulation of this region (e.g., the effects of increasing currents or a ceiling effect).

Two different rectangular mazes were used for the CPP trials. Model A rectangular maze (50 × 25 × 30 cm), oriented East–West, had 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 made of brown cork with longitudinal (8 × 1 cm) or circular (1.5 cm) incisions, respectively.

In model B rectangular maze (50 × 25 × 30 cm), oriented North–South, 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