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RESEARCH ARTICLE

Conditioned place preference induced by electrical stimulation of the insular cortex: effects of naloxone

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Abstract The insular cortex has been related to various sensory, regulatory, and learning processes, which frequently include affective-emotional components. The objective of this study was to investigate the possibility of inducing reinforcing effects by electrical stimulation of this cortical region in Wistar rats. Concurrent conditioned place preference tasks were conducted for this purpose, using two rectangular mazes that differed in dimensions, texture, and spatial orientation. A significant correlation was found in the preferences induced by insular cortex electrical stimulation between the two mazes. Animals showed consistent preference or avoidance behaviors associated with simultaneous insular cortex stimulation. No electrical self-stimulation was achieved. In a second experiment, animals that showed consistent place preference after the simultaneous insular cortex electrical stimulation were administered with 4 mg/ml/kg of naloxone. The results revealed that this opiate antagonist blocked concurrent place preference learning when the task was conducted in a new maze but not when it was conducted in the same maze as that in which the animals had learned the task. These results are discussed in terms of the participation of the insular cortex in various reward and aversion modalities.

Keywords Insular cortex · Electrical stimulation · Reward · Aversion · Wistar rats · Naloxone

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Introduction

The insular cortex (IC) is a heterogeneous brain area that receives visceral sensory and exteroceptive information and has been related to various behavioral, sensory, regulatory, and adaptive functions (Cechetto and Saper 1987; Cubero and Puerto 2000; Sewards 2004; Contreras et al. 2007). For example, it participates in the processing (Hanamori et al. 1998; Ito 1998; Inui et al. 2003) and control (Burkey et al. 1996; Duncan et al. 1998) of innocuous and nociceptive somatosensory stimuli and the affective components that accompany them (Peyron et al. 2000; Jasmin et al. 2004).

The IC is also involved in learning related to the reward value of food (De Couteau et al. 1997; Ragozzino and Kesner 1999; Balleine and Dickinson 2000) and in the innate and acquired hedonic evaluation of gustatory stimuli (Yamamoto et al. 1989; Kiefer and Orr 1992; Sewards 2004). Previous studies by our group demonstrated that electrical stimulation of the posterior IC induces preference for associated gustatory stimuli in discriminative learning tasks (Cubero and Puerto 2000).

The IC is interconnected with the parabrachial complex, an important relay in the processing of visceral sensory information (Fulwiler and Saper 1984; De Lacalle and Saper 2000). Electrical stimulation of the external lateral parabrachial subnucleus (LPBe) induces preferences for associated stimuli in taste discrimination learning and conditioned place preference (CPP) tasks (Simón et al. 2007, 2008). These place preferences can be blocked by administering naloxone, an opioid antagonist (Simón et al. 2007, 2011). Numerous opioid receptors have been identified in the IC (Mansour et al. 1994; Svingos et al. 1995; Izenwasser et al. 1999), and this region has been related to the processing of drugs of abuse, such as morphine (Mackey et al. 1986), amphetamines (Porrino and Lyons 2000; Contreras

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et al. 2007), cocaine (Wang et al. 1999; Bonson et al. 2002), and marihuana (Mathew et al. 1997).

With this background, the aim of this study was to determine whether the reinforcing effect of electrical stimulation of the posterior IC is specific to gustatory stimuli, given its visceral character (Cubero and Puerto 2000), or can also be obtained with other types of stimulus or task (Spiteri et al. 2000). For this purpose, we used concurrent conditioned place preference (cCPP) tasks in two mazes that differed in dimensions, texture, and spatial orientation. The hypothesis was that the rewarding effect of this electrical stimulation would remain consistent in the different experimental settings.

Furthermore, given the presence of opioid systems in the IC (Mansour et al. 1994; Svingos et al. 1995; Izenwasser et al. 1999), a second experiment was designed to verify whether the reinforcing effect of stimulation can be mediated by opioid mechanisms and, as in the parabrachial complex, can be blocked by administering naloxone, an opioid antagonist.

Methods

Subjects and surgical procedure

Male Wistar rats from the breeding colony at the University of Granada, weighing 280–360 g at baseline, were used in this study. Animals were housed in individual methacrylate cages with water and food ad libitum (Food, A-04, Panlab Diets SL, 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 the surgery. All behavioral procedures and surgical techniques complied with Spanish legislation [Royal Law (1201/2005)] and European Community Council Directive (86/609/EEC).

Animals were implanted with a stainless steel grounded monopolar electrode (00) (as in Hawkins et al. 1983; Simón et al. 2011) in the IC [coordinates: AP = +8.16; L = +5.9; V = +2.4; (Paxinos and Watson 1998)] using a stereotaxic apparatus (Stoelting Co. Stereotaxic 51600, USA) under general anesthesia (Sodium Pentothal, 50 mg/kg, B. Braun Medical SA Barcelona, Spain). As prophylactic measures, povidone iodine (Betadine, Asta Médica, Madrid, Spain) was applied around the implant and 0.1 cc penicillin (Penilevel, Laboratorio Level, S.A., Barcelona, Spain) was intramuscularly injected. There was a post-surgery recovery period of at least 10 days.

Experiment 1 used 29 male Wistar rats that were randomly assigned to an implanted group for intracranial electrode stimulation in posterior IC ($n = 19$) or an intact control group ($n = 10$). Experiment 2 used 19 male Wistar rats randomly assigned to a stimulated group ($n = 14$) or non-stimulated control group ($n = 5$).

Equipment

For the electrical stimulation, a continuous 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). In order to avoid reaching aversive thresholds, an optimal current intensity was established for each animal (between 100 and 600 μA). The current was increased until a behaviorally observable level of response was achieved without producing escape behaviors, involuntary movements, or vocal reactions (Tehovnik 1996). These levels were further reduced by 25 % during the behavioral procedure to avoid any potential undesirable effect.

The following two mazes were used (Simón et al. 2007):

Model 1: Rectangular maze (50 × 25 × 30 cm) oriented North–South, 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.

Model 2: Rectangular maze (70 × 15 × 15 cm) oriented East–West, 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^2) had a metal grill floor and the walls were white.

Behavioral procedures

Experiment 1: Concurrent CPP in two different mazes

Phase 1: cCPP in model 1 maze

The cCPP task commenced at 48 h after establishing the individual optimal electrical current. After placing each animal in the center of the maze, the voluntary stay of the animal in one of the two compartments was accompanied by the corresponding intracranial electrical stimulation (half of the animals received stimulation in one side of the maze and the rest in the other), and the stay time in each area was recorded. The place in which the animals received stimulation was distributed at random. Each session lasted

for 10 min. 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 for the learning and preference index.

Phase 2: cCPP in model 2 maze

In this phase, we repeated the same conditioning process but used a second maze with a new spatial orientation. As in the previous phase, the place in which the animals received stimulation was distributed at random.

Phase 3: Induction of intracranial electrical self-stimulation

Animals implanted in posterior IC underwent a standard experimental procedure of intracranial electrical self-stimulation (Hawkins et al. 1983; Simón et al. 2009, 2011) in a transparent Plexiglas cage (50 × 55 × 60 cm) with a lever press on the front wall, which acted as stimulator switch and was also connected to a pulse counter and corresponding oscilloscope. Pressing of this lever triggered a train of cathodic rectangular pulses of 250 ms, with a frequency of 66.6 Hz and pulse duration of 0.1 ms. Current intensities used were always below the threshold at which detectable motor or behavioral alterations might appear.

Experiment 2: Concurrent CPP in different mazes: effects of naloxone

Phase 1: cCPP in model 1 maze

Following the same behavioral procedure as in the first phase of experiment 1, the animals were now subjected to two cCPP sessions in model 1 maze (Simón et al. 2007). In this case, and applying the behavioral criteria (see Results) established in previous experiments (Simón et al. 2007, 2009, 2011), the animals that showed no preference for either compartment after the two initial sessions were considered as the "neutral" control group and received no further electrical stimulation in subsequent phases. The place in which the animals received stimulation was established at random.

Phase 2: Naloxone administration and cCPP in model 1 maze

At 48 h after ending phase 1, all animals received a subcutaneous (sc) injection of naloxone (4 mg/ml/kg) (Naloxone Hydrochloride, Lab Sigma, St Louis, USA) at 20 min before undergoing a new cCPP session.

Phase 3: Naloxone administration and cCPP in model 2 maze

At 48 h after ending phase 2, all animals received a new sc injection of naloxone (4 mg/ml/kg) at 20 min before a new cCPP session but in Model 2 maze, which has a different space orientation (from N–S to E–W), in order to examine the possible effect on learning of the previous phases of the experiment. In this phase, as in phase 1, the place in which the animals received stimulation was established at random.

Phase 4: cCpp in model 2 maze

At 48 h after ending phase 3, the animals underwent a further cCPP session in Model 2 maze but without naloxone administration.

Phase 5: Induction of intracranial electrical self-stimulation

At 48 h after ending phase 4, the IC-implanted animals underwent a standard operant procedure of intracranial electrical self-stimulation similar to that reported in experiment 1 (phase 3).

Histology

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

Statistical analysis

Statistical version 6.0 (Statsoft Inc, OK) was used for the statistical analyses. Pearson's correlation was used to analyze differential data from experiment 1 (difference between stay time in electrical stimulated compartment and stay time in non-stimulated compartment, expressed in seconds) obtained on the second cCPP day in Model 1 and Model 2 mazes. For experiment 2 data, Pearson's correlation was used to classify the animals as a function of their behavioral effects (behavioral consistency of the electrical stimulation), and one-way ANOVA was then used to analyze the effects of stimulation and naloxone administration in the different groups and mazes. Finally, we used a two-factor mixed ANOVA (group × substance), with group (positive, negative, control) as between-group factor and substance

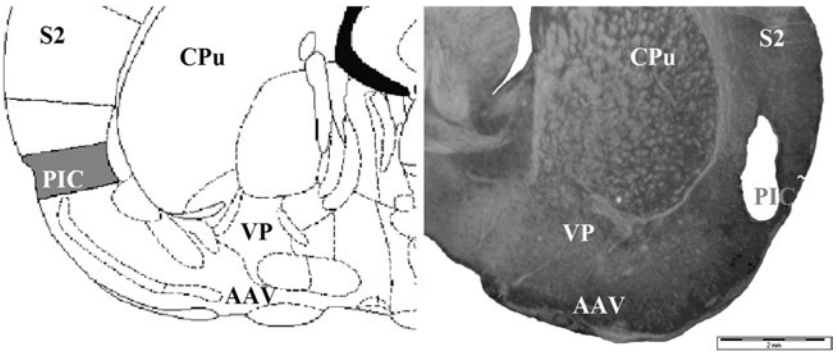

Fig. 1 Coronal section of the brain of a representative animal from the "positive" group, confirming the localization of the electrode in the posterior insular cortex. *AAV* anterior amygdaloid area, ventral part, *PIC* posterior insular cortex, *CPu* Caudate-Putamen, *S2* secondary somatosensory cortex, *VP* ventral pallidum

(naloxone vs. no naloxone) as repeated measure in order to reduce the number of animals required for this experiment. Specifically, two-way mixed ANOVAs were used to compare the effects of learning retention and naloxone in each maze [group × substance in maze 1; group × maze (under effects of naloxone) and group × substance in maze 2]. After each ANOVA, the Newman–Keuls test was applied for post hoc comparisons.

Results

Experiment 1: Concurrent conditioned place preference

In the IC-implanted group, a positive correlation was found between the concurrent conditioned place preference in the first and second mazes ($r = 0.531$; $p < .019$) (see Fig. 2 left). In the intact control group, an alternating or indifferent behavior was observed, with no significant correlation between the data obtained in the two mazes ($r = -0.619$; $p < .6408$) (see Fig. 2 right).

As shown at other brain sites and following the behavioral criteria established in previous studies (Simón et al. 2007, 2009, 2011), animals remaining >50 % of total time in the stimulated compartment were classified as "positive" (7 animals), those remaining there for <30 % of total time as "negative" (6 animals) and those remaining for 30–50 % of total time or showing an alternating behavior between sessions as "neutral" (6 animals). Mean stay times in the stimulated area (over a maximum of 10 min) for these groups during both learning sessions in both mazes were 480.786 s for the positive group; 57.5 s for the negative group, and 271.333 s for the neutral group.

Intracranial electrical self-stimulation of posterior IC

The intracranial electrical self-stimulation test in both experiments showed that IC-electrically stimulated animals fail to learn the lever-press task (Cubero and Puerto 2000), unlike animals stimulated in some other brain regions, such as the lateral hypothalamus (Hawkins et al. 1983; Simón et al. 2009, 2011). It is possible that the induction of IC

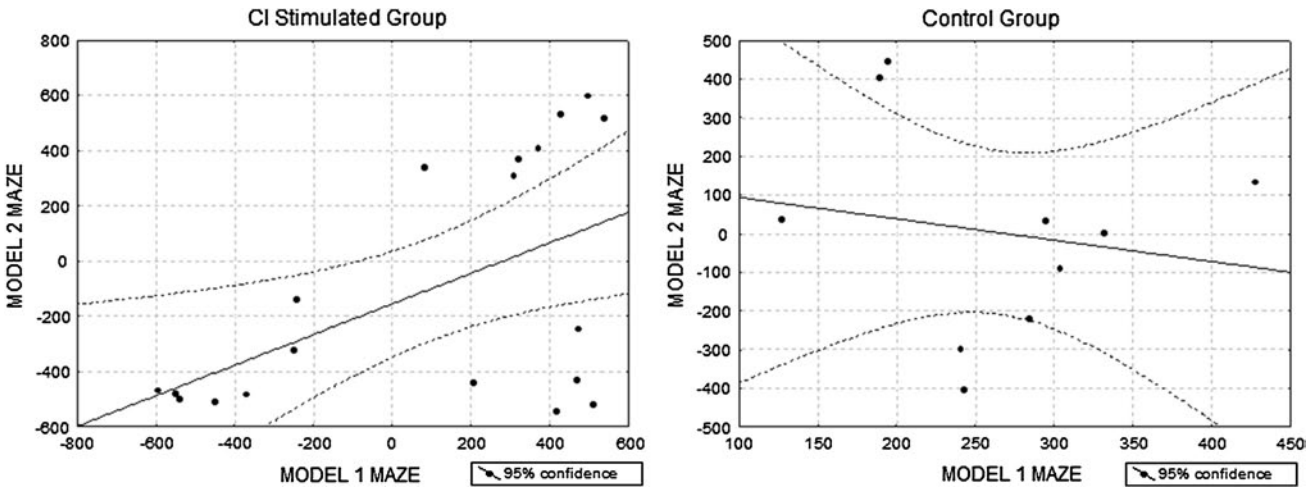

Fig. 2 Correlation of differential values (time in compartment associated with electrical stimulation of the posterior insular cortex—time in the non-stimulated compartment, expressed in seconds) obtained in the IC-stimulated animals (*left graph*) and in the non-stimulated intact control group (*right graph*) on the second day of concurrent conditioned place preference in Model 1 and Model 2 mazes

self-stimulation may require greater experimental effort or different rewarding brain self-stimulation procedures.

Experiment 2: cCPP in different mazes: effects of naloxone administration and learning retention

Phase 1: cCPP in Model 1 maze

The performance of the electrically stimulated animals during the two conditioning sessions showed a significant correlation between them ($r = 0.871$, $p < .001$). Applying the above-reported behavioral criteria (Simón et al. 2007, 2009, 2011) to the data obtained in the cCPP sessions, three animal groups were formed according to their mean stay time in the stimulated compartment: positive group ($n = 7$) 539.35 s; negative group ($n = 7$) 91.86 s; and neutral group ($n = 5$) 193.3 s. As shown in Fig. 3, the mean length of stay in the stimulation-associated compartment in the two CPP sessions (learning index) showed significant differences among the three groups ($F_{(2, 16)} = 151.46$, one-way ANOVA; $p < .001$).

Phase 2: Effect of naloxone on cCPP in model 1 maze

After naloxone administration, two-way mixed ANOVA showed no main effect of substance ($F_{(1, 16)} = 0.072$, $p < .792$) or of group x substance interaction ($F_{(2, 16)} = 1.381$, $p < .2796$), while the main group effect was significant ($F_{(2, 16)} = 35.68$, $p < .001$). Post hoc comparative analysis of the main effect, group factor, showed significant differences among the groups (Newman–Keuls test, $p < .05$).

Phase 3: Effect of naloxone on cCPP in the model 2 maze

After naloxone administration, one-way ANOVA showed no difference among groups ($F_{(2, 16)} = 1.119$, $p < .35$). Comparison between these results and those obtained after naloxone administration in the Model 1 maze, using a two-way mixed ANOVA (group × maze), showed a significant effect of the interaction ($F_{(2, 16)} = 7.667$, $p < .0046$) (see Fig. 4). Post hoc comparisons showed significant differences in the positive group as a function of the maze used (Model 1 vs. Model 2, $p = .015$), although no such differences were observed in the other groups (negative group, $p = .2823$; neutral group, $p = .6204$).

Phase 4: cCPP in model 2 maze

One-way ANOVA showed intergroup differences after IC stimulations ($F_{(2, 16)} = 18.683$, $p < .001$), as in phase 1. Two-way mixed ANOVA (group × substance) results for the effect of naloxone administration vs. no naloxone on

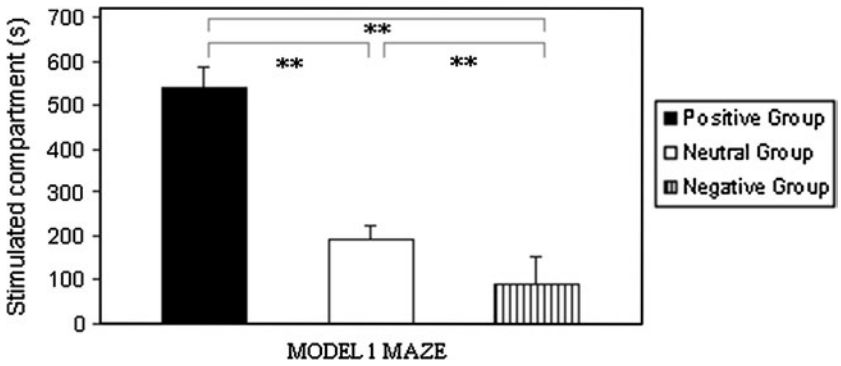

Fig. 3 Duration of stay (in seconds) by experimental groups of experiment 2, in the compartment associated with electrical stimulation of the insular cortex (mean of two spatial learning tests) in Model 1 maze, following the behavioral criteria established in experiment 1. *Asterisks* indicate significant ($p < .01$) differences among groups

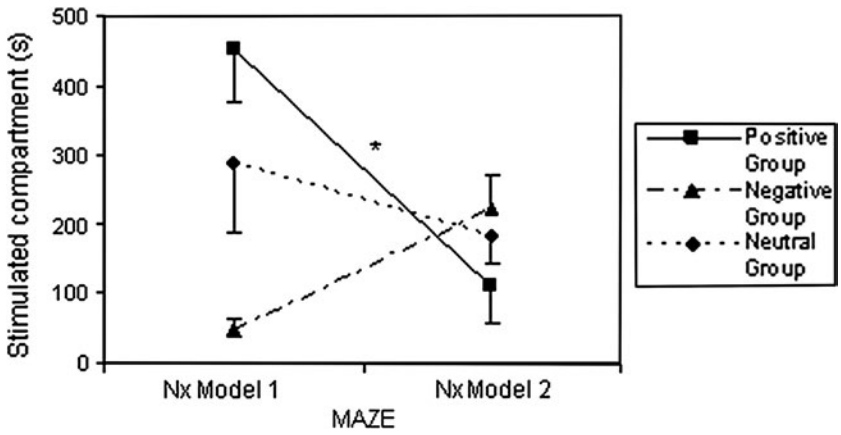

Fig. 4 Duration of stay (in seconds) by groups of experiment 2 in Model 1 and Model 2 maze compartment associated with electrical stimulation of the insular cortex after naloxone administration

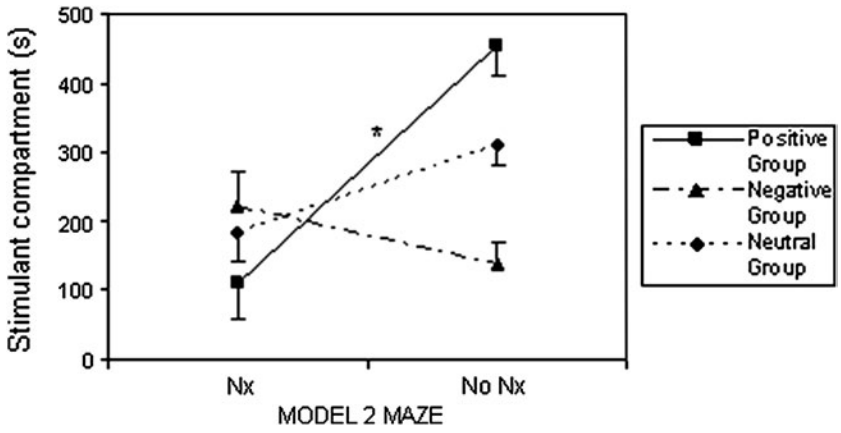

Fig. 5 Effect of naloxone administration (Nx vs. No Nx) on the duration of stay (in seconds) by groups of experiment 2 in the Model 2 maze compartment associated with electrical stimulation of the insular cortex

the cCPP in Model 2 maze showed no main group effect ($F_{(2, 16)} = 2.467$, $p < .116$) but a significant main effect of substance ($F_{(1, 16)} = 11.463$, $p < .01$) and a group × substance interaction ($F_{(2, 16)} = 11.846$, $p < .001$) (see Fig. 5). Post hoc comparisons showed significant differences in the positive group as a function of naloxone administration (Nx vs. No Nx, $p = .00065$), although no such differences were observed in the other groups (negative group, $p = .3958$; neutral group, $p = .228$).

Discussion

In this study, electrical stimulation of the posterior IC induced place-associated reinforcing effects in different animals that were consistently rewarding ("positive" group) or aversive ("negative group") or were inconsistent ("neutral group)" and led to alternating place preference behavior, as previously reported for the parabrachial complex (Simón et al. 2007, 2008, 2009).

These effects appear to be produced by the electrical stimulation of this cortical region, given that the neurologically intact animal groups showed no consistent preference behaviors for any area of the mazes. Moreover, the place preference effect of IC activation appears to be independent of specific internal (different mazes) or external (different spatial orientations) cues of the task and independent of the sensory qualities of the associated stimulus, that is, gustatory (Cubero and Puerto 2000) or environmental (present data).

The hedonic representation of different somatosensory and gustatory stimuli has been localized in the posterior IC (Sewards and Sewards 2001; Sewards, 2004), where it is likely to converge with visceral information (Cechetto and Saper 1987; Hanamori et al. 1998; Ogawa and Wang 2002) that may be processed through its interconnections with the parabrachial complex (Fulwiler and Saper 1984; De Lacalle and Saper 2000; Jasmin et al. 2004). Thus, electrical stimulation of the vagus nerve (Ito 1998) or esophagus (Hecht et al. 1999) produces activation of the IC, and conversely, electrical stimulation of the IC induces changes in contraction and gastric tone amplitude (Aleksandrov et al. 1996). Stimulation of the posterior IC may activate a rewarding visceral system, developing preference behaviors similar to those produced by the association of a gustatory stimulus with the metabolic benefits of a food product (Puerto et al. 1976). In other words, electrical stimulation of the LPBe nucleus and IC may act as an effective substitute for a visceral stimulus and/or its motivational consequences in reinforcing gustatory or place discrimination learning tests (Cubero and Puerto 2000; Simón et al. 2007, 2008).

Alternatively, the preference behaviors observed may have been due to activation by the electrical stimulation of some of the neural circuits involved in incentive attribution processes. The IC is involved not only in processing information on the reward value of food (De Couteau et al. 1997; Ragozzino and Kesner 1999; Balleine and Dickinson 2000), but also in tasks related to the anticipation of future reward (Schoenbaum et al. 1998; Kirsch et al. 2003) and to expected changes in its magnitude (Gallagher et al. 1999; Gottfried et al. 2003; Kirsch et al. 2003).

The above theoretical proposals may also be applicable to our "negative" experimental group, which tended to avoid areas associated with electrical stimulation of the IC. Electrical stimulation may act as a substitute for a noxious visceral stimulus (Gallo et al. 1988; Agüero et al. 1993), for example, the administration of a toxic/aversive agent (Contreras et al. 2007). In fact, the IC is known to participate in the processing of nociceptive (Hanamori et al. 1998; Ito 1998; Peyron et al. 2000) and aversive information induced by the visceral administration of lithium chloride, morphine (Mackey et al. 1986; Contreras et al. 2007), and other substances of abuse (Wang et al. 1999; Sell et al. 2000; Bonson et al. 2002). The electrical stimulation may have produced a change in incentive attribution, making the associated stimuli aversive for the animal and explaining the behaviors of avoidance

or aversion to the stimulation area. In fact, the IC has been implicated in various negative motivational-affective processes (Jasmin et al. 2004), including: the facial expression of fear (Wright et al. 2003) or disgust (Phillips et al. 1997), the recall of situations that generate anxiety (Liotti et al. 2000) or sadness (Lane et al. 1997), and the innate or learned hedonic evaluation of gustatory stimuli (Yamamoto et al. 1989; Kiefer and Orr 1992; Sewards 2004).

The fact that electrical stimulation of the IC from the same stereotaxic coordinates generates either preferences or aversions suggests that the systems processing rewarding and aversive motivational information may be anatomically very close together (Hoebel 1976; Salamone 1994; O'Doherty et al. 2001). In this way, electrical stimulation in the "neutral" animals may have simultaneously activated cells of neighboring neuronal populations that, respectively, process appetitive or aversive information, as observed in other brain regions (Yamamoto et al. 1989; Moufid-Bellancourt et al. 1996; O'Doherty et al. 2001; Ogawa and Wang 2002). For example, stimulation of the lateral hypothalamus (LH) can induce self-stimulation or aversion and increased water or food intake (Hawkins et al. 1983; Gratton and Wise 1983), and periaqueductal gray matter stimulation induced pain or analgesia (Mayer et al. 1971; Prado and Roberts 1985).

However, we have not yet been able to develop intracranial self-stimulation behaviors by activation of the IC (Cubero and Puerto 2000), which has been achieved by the activation of prefrontal regions, among many others (Phillips and Fibiger 1989; McGregor and Atrens 1991). Nevertheless, our results on the effects of IC electrical brain stimulation are compatible with the dissociation obtained with drugs such as lysergic acid diethylamide (LSD), buspirone, and pentylenetetrazole, which can induce place preferences but not self-administration behaviors, and conversely, with drugs such as pentobarbital or phencyclidine, which sustain self-administration behaviors but do not induce CPP (Bardo and Bevins 2000 for a review). Our finding is also consistent with immunohistochemical evidence that intracranial self-stimulation of the LH does not induce activation of the IC (Arvanitogiannis et al. 1997; Flores et al. 1997), despite the anatomical connections between them (Öngür et al. 1998). The results obtained in this and other studies (Cubero and Puerto 2000) suggest that electrical stimulation of the posterior IC may not be able to elicit self-stimulation behaviors or at least not to the degree observed after LH stimulation (Hawkins et al. 1983; Simón et al. 2009, 2011). Hence, we may be acting on different reward systems (Robertson 1989; Waraczynsky 2006) with distinct physiological and even neurochemical characteristics. Thus, our group demonstrated that the administration of opiate antagonists does not interfere with the CPP induced by LH electrical stimulation but blocks the CPP induced by stimulation of the LPBe nucleus, which is anatomically connected to the IC, in which self-stimulation has also not yet been achieved (Simón et al. 2011). Nevertheless, we cannot rule out the induction of alternative rewarding behaviors, such as a low rate of lever-press self-stimulation (Vale-Martinez et al. 1999).

In our second experiment, administration of the opiate antagonist naloxone blocked the rewarding effect of posterior IC electrical stimulation when the learning task was conducted in a new maze but not when it was conducted in the same maze as used for the initial learning. Analogous results have been obtained by administering drugs of abuse such as heroin or cocaine (McFarland and Ettenberg 1998; Mueller and Stewart 2000). Our finding cannot be attributed to an extinction of the stimulation effect because, in the absence of naloxone, the preference of animals in the "positive" group for a place associated with IC stimulation did not differ between the two mazes. It also does not appear to result from a general deficiency in the capacity to associate stimuli as an aversive or motor effect of naloxone, because no significant behavioral changes between the mazes were observed in the "negative" or "neutral" groups.

Using different experimental procedures, the IC has been shown to be involved in: learning processes (Nerad et al. 1996; Paredes et al. 2000; Contreras et al. 2007); the association between contexts and natural or artificial reinforcers (Schroeder et al. 2001; Volkow et al. 2006); and the effects of drugs of abuse such as cocaine, morphine, heroin, nicotine, or marihuana (Mathew et al. 1997; Sell et al. 2000; Bonson et al. 2002; Naqvi et al. 2007). The present findings demonstrate that the physiological electrical stimulation of this cortical region, which has a high density of κ, δ, and μ opioid receptors (Mansour et al. 1994; Svingos et al. 1995; Izenwasser et al. 1999), can activate some of the brain rewarding/aversion processes mediated by opioid systems and that this activation can be associated with somatosensory or contextual stimuli.

The IC has also been implicated in some other effects of opiates/opioids (Mackey et al. 1986; Burkey et al. 1996). Thus, behavioral and pharmacological studies have related the IC, among other brain regions, to the analgesic effect induced by morphine and other μ-opioid antagonists (Burkey et al. 1996; Casey et al. 2000; Wise et al. 2002). Likewise, both opioid-exogenous and opioid-endogenous substances have been considered as rewarding mechanisms that can induce CPP (Herz and Spanagel 1995; Paredes et al. 2000; Bodnar and Hadjimarkou 2003; Gerrits et al. 2003) and self-administration behavior in laboratory animals (Gerrits et al. 2003). Finally, the results obtained in the "positive" groups (Cubero and Puerto 2000; and this paper) are compatible with those reported after kindling seizure disorders of the granular IC (Paredes et al. 2000) or after morphine administration (Blokhina et al. 2000; Parker et al.

2002; Kawasaki et al. 2005). In short, it is possible that the rewarding effect of the electrical stimulation of this region mobilizes opioid systems that could be blocked by naloxone administration.

Naloxone administration, in a different maze (model 2), blocked the rewarding effect of stimulation ("positive" animals in phase 3) and generated avoidance or aversion behaviors toward the area associated with stimulation, as also found after the administration of low doses of naloxone to animals pre-treated with opiates (McDonald et al. 1997; Blokhina et al. 2000; Parker et al. 2002). In fact, the IC (along with regions such as the lateral parabrachial complex) has been implicated in the processing of aversive properties of morphine and other substances of abuse (Bechara et al. 1993; Mackey et al. 1986) and in the withdrawal syndrome (Nader et al. 1996; Georges et al. 2000; Lowe et al. 2002). Thus, damage in the IC has been reported to inhibit the craving of smokers and the need for tobacco (Naqvi et al. 2007), and conversely, IC activation has been observed during craving periods in subjects addicted to drugs of abuse (Wang et al. 1999; Sell et al. 2000; Bonson et al. 2002) and even during food craving episodes (Small et al. 2001; Pelchat et al. 2004).

Finally, the IC includes κ receptors among its opioid mechanisms (Izenwasser et al. 1999), and agonists of these receptors were not able to develop self-administration behaviors (Mansour et al. 1995) but could generate taste and place aversions in rodents (Mucha and Herz 1985; Herz and Spanagel 1995). Activation of this opioid system may explain the tendency observed in the "negative" group for naloxone to reduce the aversive effect of stimulation, although significance was not reached, possibly due to the small sample size.

In summary, the present results demonstrate that electrical stimulation of the posterior IC induces consistent preference or aversive behaviors in concurrent conditioned place preference tests, apparently acting as a substitute for biological processes that have yet to be determined. According to the behavioral criteria established in this and previous parabrachial complex experiments (Simón et al. 2007, 2009), electrical stimulation habitually generates three animal groups: "positive" animals, which consistently prefer the stimulated area in a rectangular maze; "negative" animals, which avoid the stimulated area; and "neutral" animals, which alternate between the different areas of the maze and show no consistent behavior. Naloxone administration impairs the rewarding effect of stimulation, suggesting the possible participation of opioid mechanisms. However, this learning blockage is only shown when the task is conducted in a novel context and not when it is carried out in the same maze as that in which the initial preferences were acquired, where the animals appear to retain the rewarding effects of the stimulation.

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