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Differential rewarding effect of electrical stimulation of the lateral hypothalamus and parabrachial complex: functional characterization and the relevance of opioid systems and dopamine

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Abstract:	Background: Since the discovery of rewarding intracranial self-stimulation by Olds and Milner, extensive data have been published on the biological basis of reward. Although participation of the mesolimbic dopaminergic system is well documented, its precise role has not been fully elucidated, and some authors have proposed the involvement of other neural systems in processing specific aspects of reinforced behavior. Aims and methods: We reviewed published data, including our own findings, on the rewarding effects induced by electrical stimulation of the lateral hypothalamus (LH) and of the external lateral parabrachial area (LPBe) -a brainstem region involved in processing the rewarding properties of natural and artificial substances-, and compared its functional characteristics as observed in operant and non-operant behavioral procedures. Results: Brain circuits involved in the induction of preferences for stimuli associated with electrical stimulation of the LBPe appear to functionally and neurochemically differ from those activated by electrical stimulation of the LH. Interpretation: We discuss the possible involvement of the LPBe in processing emotional-affective aspects of the brain reward system. Conflict of interest: None

Drs. D. J. Nutt/P. Blier

Editors

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Dear Professor Nutt,

We are pleased to submit a revised version of our paper entitled "**Differential rewarding effects of electrical stimulation of the lateral hypothalamus and parabrachial complex: a functional characterization and the relevance of opioid systems and dopamine**" **(JOP-2018-3666)**, as requested. We also enclose on separate pages our responses to the comments of your reviewers with an account of the corresponding modifications to the text.

We are grateful to reviewers for their insights and recommendations, which have allowed us to improve the quality and clarity of our manuscript.

If there are any further questions regarding our submittal, please do not hesitate to contact us at the address shown on the title page. We look forward to your comments and decision on our paper.

With thanks,

Sincerely yours,

Dr María Jose Simon

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TITLE: **Differential rewarding effects of electrical stimulation of the lateral hypothalamus and parabrachial complex: functional characterization and the relevance of opioid systems and dopamine**

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†In memoriam.

ABSTRACT:

Background: *Since the discovery of rewarding intracranial self-stimulation by Olds and Milner, extensive data have been published on the biological basis of reward. Although participation of the mesolimbic dopaminergic system is well documented, its precise role has not been fully elucidated, and some authors have proposed the involvement of other neural systems in processing specific aspects of reinforced behavior.*

Aims and methods: *We reviewed published data, including our own findings, on the rewarding effects induced by electrical stimulation of the lateral hypothalamus (LH) and of the external lateral parabrachial area (LPBe) -a brainstem region involved in processing the rewarding properties of natural and artificial substances-, and compared its functional characteristics as observed in operant and non-operant behavioral procedures.*

Results: *Brain circuits involved in the induction of preferences for stimuli associated with electrical stimulation of the LBPe appear to functionally and neurochemically differ from those activated by electrical stimulation of the LH.*

Interpretation: *We discuss the possible involvement of the LPBe in processing emotional-affective aspects of the brain reward system.*

Conflict of interest: None

Keywords: Motivation, Reward, lateral hypothalamus (LH), external lateral parabrachial nucleus (LPBe), opioids, dopamine.

1- Introduction

Organisms have evolved neurobiological mechanisms capable of detecting, processing, and evaluating the presence of natural stimuli essential for individual and/or species survival, generating rewarding reactions in their presence and triggering responses for their acquisition (Shizgal et al., 2001; Berthoud and Münzberg, 2011).

Affective reactions to reinforcing stimuli can give rise to the acquisition of new learning, which tends to identify cues of its availability and thereby increase the possibility of access to them (Bindra, 1974; Dayan & Balleine, 2002; Berridge, 2018). These motivational processes appear to be driven by complex mechanisms that can include various components with specific neural branches of a network involving common elements and likely interactions among them (White and Milner, 1992; Waraczynski, 2006; Berthoud and Munzberg, 2011; Salamone et al., 2016).

2-The brain reward system and dopamine

In 1954, James Olds and Peter Milner made the landmark discovery of rewarding brain stimulation (or intracranial self-stimulation, ICSS), which has proven to be a powerful tool for understanding the neurobiological bases of reward (Olds and Milner, 1954; De Haan, 2010). Anatomical regions supporting ICSS were first located around the medial forebrain bundle (MFB) (Gallistel et al., 1981; Wise and Rompré, 1989; Phillips and Fibiger, 1989), and it was subsequently found that this operant behavior can be elicited by electrodes located in many other areas, from the olfactory bulb to the nucleus of the solitary tract (NST) and cerebellum (Gallistel et al., 1981; Wise and Rompré, 1989; Phillips and Fibiger, 1989; Ikemoto, 2010; Vlachou and Markou, 2011 -for a review) (**Figure 1**).

Although ICSS can stimulate neurons containing different neurotransmitters (Stein & Wise, 1969; Yeomans et al., 1993; Ikemoto, 2010; Vlachou and Markou,

2011), trans-synaptic (indirect) activation of the mesoaccumbal dopaminergic rewarding system has been confirmed by classical lesion and/or stimulation experiments in combination with recording procedures (e.g., collision of pulses or voltammetry) (Wise and Rompré, 1989; Shizgal, 1989; Yeomans et al., 1993; Gallistel et al., 1996; Ikemoto, 2010; Berridge and Kringelbach, 2015).

Brain areas activated by self-stimulation of the LH have been examined using immunohistochemistry [C-Fos] (Flores et al., 1997; Arvanitogiannis et al., 1997; 2000; Hunt and McGregor, 1998), glycogen phosphorylase histochemistry (Konkle et al., 1999), 2DG autoradiography (Gallistel et al., 1985), and functional neuroimaging (Kolodziej et al., 2014) confirming an indirect activation of the mesoaccumbal dopamine. In addition, a quantitative autoradiographic study observed that ICSS of the lateral hypothalamus (LH) induces plastic changes in dopaminergic neurons of different brain areas, especially in D1 dopaminergic receptors (Simon et al., 2016) [See **Table 1** and **Figure 2**].

Reward is currently considered as a complex functional process with many dissociable components [e.g., hedonic impact, learning, incentive motivation, seeking, or goal-directed related behaviors…] that may simultaneously or successively intervene in the behavioral reward cycle (Waraczynski, 2006; Berridge and Kringelbach, 2015; Castro et al., 2015). The specific role of dopamine in relation to this process remains a controversial issue and warrants further research (Waraczynski, 2006; Hernandez et al., 2007; Ikemoto, 2010; Smith et al., 2011; Salamone and Correa, 2012; Berridge and Kringelbach, 2015; Morales and Margolis, 2017).

It has been suggested that the dopaminergic mesolimbic system may not be related to the specific encoding of the rewarding or hedonic value *per se* but rather to other aspects, such as: a) the novelty signal associated with the anticipation of reward

(Schultz et al., 1997), b) behavioral arousal and/or seeking mechanisms (Berridge and Robinson, 1998; Salamone, 1994; Salamone et al., 2016); and/or c) the incentive component, which would integrate space-time signals and the subjective effort that leads individuals to perform goal-directed behaviors (Hernandez et al, 2006; 2007;2012; Berridge and Kringelbach, 2015; Castro et al., 2015, among others).

In accordance with this hypothesis, some studies showed that electrical stimulation of the LH elicited food intake but did not enhance pleasure reactions (Berridge and Valenstein, 1991). Moreover, genetically engineered mice lacking dopamine had difficulties in carrying out goal-directed behaviors, although seeking behaviors were restored after the local administration of dopamine (Robinson et al., 2005; 2006). In addition, mice with a genetic disruption of dopamine transporter [DAT] and a consequent increase in synaptic DA not only required fewer trials to learn an incentive runway task but also ran faster to the goal and were better at avoiding distractions (Peciña et al., 2003).

It has also been reported that pharmacological dopamine blockade or even complete destruction of the DA mesolimbic system did not diminish facial expressions of hedonic impact (positive affective reactions), measured in a "taste reactivity test", a procedure that allows the recording of orofacial reactions to innately and learned gustative stimuli in human infants and animals (Grill and Norgren, 1978; Peciña et al., 1997; Berridge and Robinson, 1998)..

In addition, knock-out mice unable to synthesize the enzyme tyrosine hydroxylase were capable of experiencing affective/hedonic reactions to taste stimuli such as sucrose and/or saccharine, even in the absence of dopamine (Cannon and Palmiter, 2003). In a related study, dopamine-deficient and therefore severely hypoactive and hypophagic animals developed a strong contextual preference for

morphine when administered with caffeine or a dopaminergic precursor during the testing phase (Hnasko et al. 2005; Cannon and Bseikri, 2004).

Taken together, the above findings suggest that dopaminergic activity may not be essential for processing positive hedonic reactions in animals showing self stimulation of the LH or other sites (Peciña et al., 1997; Maldonado et al., 1997; Cannon and Palmiter, 2003; Hnasko et al., 2005), as initially assumed (Wise, 1982; Wise and Rompré, 1989). Indeed, some authors more recently affirmed that '*pleasure may not be a necessary correlate of dopamine elevations*' (Wise, 2008).

3- Reward induced by non-operant procedures

In their initial observations, Olds and Milner reported that animals not only showed no sign of rejection but also repeatedly returned to the corner where they had received the electrical stimulation (Olds and Milner, 1954; De Haan, 2010). This result was replicated in other experiments in which groups of rats were placed in a T-shaped maze and stimulated upon entry into a previously selected arm of the maze, for which they developed a clear preference (Olds, 1956).

Accordingly, the rewarding effect of electrical stimulation can be induced not only by the learning of an operant behavior but also by administration of electrical stimulation in association with a particular location or context (Olds, 1956). This second procedure, later known as Conditioned Place Preference (CPP), can be induced through association of the rewarding properties of a stimulus, treatment, drug, or substance with specific environmental cues, which are initially neutral (Bardo and Bevins, 2000; Tzschentke, 2007). In another non-operant procedure, called Conditioned Taste Preference [CTP], the stimulation can be associated with one of two gustatory stimuli (Cubero and Puerto, 2000; Simon et al., 2007; 2008). In all of these rate-free learning procedures, a recording is made of the time spent by the animal in the stimulated

compartment or the amount of liquid consumed after the associative learning, and this measure appears to be more closely related to 'consummatory' or 'pleasant' reactions than to 'preparatory' or 'seeking' behaviors (Tzschentke, 2007; Dayan and Berridge, 2014).

Some authors have employed CTP procedures to assess the rewarding nature of ICSS of the LH, which was associated with one of two flavors (Ettenberg, 1980). In another study involving CPP in combination with optogenetic stimulation of the CeA, two lever-presses were simultaneously available to the animals: one associated with obtaining sucrose + optogenetic stimulation of the CeA and the other with obtaining sucrose alone (Robinson et al., 2014). Although animals preferred the former option, they failed to establish any self-stimulation behavior not associated with an external source of reward, suggesting that the stimulation may have enhanced the motivation to obtain sucrose and implying its involvement in processing a component other than the hedonic (Robinson et al., 2014).

Taken together, these experiments demonstrated that CPP and CTP procedures can be useful to discriminate specific components of rewarding electrical stimulation in different areas of the brain (Tzschentke, 2007; Dayan and Berridge, 2014). In fact, these procedures have been widely used to study preferences for drugs of abuse in animals (Jaeger and Van der Kooy, 1993; Nader et al., 1996; McBride et al., 1999; Tzschentke, 2007), for natural stimuli such as food and drinks (Spiteri et al., 2000), for social and sexual interactions (Garcia-Horsman et al., 2008), and for electrical stimulation (Ettenberg, 1980; Cubero and Puerto, 2000; Simon et al., 2007; 2008; 2009; 2011; 2013; Garcia et al., 2013) and may contribute to further research on this issue (Dayan and Berridge, 2014).

4-Opiates and brain stimulation reward

In parallel to the above-cited studies centered on the dopaminergic mesolimbic system, researchers began to focus on other neurotransmitter systems that could participate in processing these affective aspects of reward (Peciña and Smith, 2010; Berthoud and Münzberg, 2011; Castro et al., 2015; Berridge and Kringelbach, 2015; Fields and Margolis, 2015; Morales and Margolis, 2017; Darcq and Kiefer, 2018).

Drugs of abuse that cause addiction in humans (e.g., cocaine, amphetamine, heroin, nicotine, etc. can be self-administered by laboratory animals in operant procedures and modulate ICSS behavior by changing rate/frequency curves and brain stimulation thresholds (Carlezon and Chartoff, 2007; Vlachou and Markou, 2011; Negus and Miller, 2014). Opiates are among these highly addictive substances and have potentially serious health consequences (Bodnar, 2017 -for a review-). Their action on opioid receptors can induce reinforcing effects by increasing the likelihood of behavioral responses associated with them (Negus and Miller, 2014; Fields and Margolis, 2015; Darcq and Kiefer, 2018).

With respect to the specific relationship between these opioid substances and ICSS, initial studies only observed changes in the lever-press rate when high doses of opioid substances were administered (Schaefer, 1988). Cazala et al. also investigated the effect of different doses of the opiate antagonist naloxone (0.5, 2, and 10 mg/Kg) on operant approach-escape behaviors in a shuttle box after LH or periaqueductal gray (PAG) stimulation (Cazala and Davis, 1991). They found that intermediate doses blocked escape responses alone, whereas very high doses blocked both approach and escape behaviors (Cazala and Davis, 1991). Likewise, the administration of 10 and 20 mg/kg naloxone caused a dose-dependent shift in the rate-frequency curve of VTA self-stimulation but did not completely block the operant behavior (Bielajew et al., 2003).

Similar results were obtained in a study using specific kappa receptor ligands (Todtenkopf et al., 2004).

In another study, Easterling and Holtzman demonstrated that acute morphine administration produced a small decrease in the titration point for ICSS behavior (the lowest stimulation frequency needed to maintain this operant behavior), reporting that this effect progressively diminished over time (Easterling and Holzman, 1997). In addition, they found that cumulative doses of naltrexone (opioid antagonist) during the course of ICSS only generate minimal dose-independent increases in the titration point, observing that this effect also decreased with longer time (Easterling and Holtzman, 1997; 2004). These results suggest a weak and non-determinant role of opiates in ICSS of the LH, that disappear over time and that opiate antagonists do not completely block this-behavior, even at high doses (Schaefer, 1988; Cazala & Davis, 1991; Easterling and Holzman, 1997; 2004; Bielajew et al., 2003; Wiebelhaus et al., 2016).

In adition, recent studies combining LH ICSS with quantitative autoradiography of specific D1, D2, or mu receptors have again raised questions about the relevance of the opioid systems in electrical self-stimulation of the LH (Simon et al., 2016). After ICSS of the LH, administration of the opiate agonist ^{3}H-DAMGO showed no significant differences in the concentration of mu receptors between self-stimulated and control animals across a wide range of brain sections from the whole rostrocaudal axis; however, significant differences were observed after administration of the specific D1-receptor antagonist ^{3}H-SCH-23390 in the NAC shell, caudate-putamen, ventral pallidum, and medial globus pallidus (Simon et al., 2016) *(*See **Figure 3***)*. These data are compatible with observations of few modifications in the activity of mu receptors in two groups of animals from related breeds that differed in operant response rates (ICSS

of LH) (Gross-Isserof et al., 1992). Thus, differences were only significant in the NAC (Gross-Isserof et al., 1992).

Furthermore, we employed a CPP procedure to assess the importance of the opioid system in rewarding electrical stimulation of the LH (administered by the experimenter) and showed that animals preferred the compartment associated with electrical stimulation of the LH (Simón et al., 2011**).** However, this effect was not blocked by naloxone, even at elevated doses of 10 mg/kg (Simón et al., 2011**) (Figure 4).**

Taken together, these data on the involvement of opioids in self-stimulation of the LH might be compatible with a dual action on dopamine-dependent and dopamine-independent mechanisms of reward (Wassum et al., 2009; Fields and Margolis, 2015; Ide et al., 2017) that cannot be completely blocked by the effect of antagonists in this region.

5-Involvement of opioids in rewarding homeostatic mechanisms

The hypothalamus is considered to be a critical region for homeostatic behaviors and rewards (Shizgal et al., 2001; Castro et al., 2015; Stuber and Wise, 2016). Initial studies in this research area showed that electrolytic or chemical lesions of the LH suppressed food and water intake, whereas its electrical stimulation could induce feeding and/or drinking behaviors in satiated animals (Hoebel and Teitelbaum, 1962; Stuber and Wise, 2016 -for a review-).

The involvement of opioids in the regulation of natural rewards, such as food intake, has been well documented for more than 30 years (Gosnell and Levine, 2009; Peciña and Smith, 2010 -for a review-). Various studies have attributed opiates present in the LH with an intake-activating role, generating the overconsumption of palatable foods that might become 'potentially addictive' and contributing to maintain the

consumption once initiated (Papadouka and Carr, 1994; Carr and Papadouka, 1994; Gosnell and Levine, 2009; Ikeda et al., 2015). These effects appear to be related to their action on dopamine-dependent circuits involved in deficit and/or motivational seeking processes (Gosnell and Levine, 2009; Ikeda et al., 2015). In fact, sucrose consumption produces plastic changes, including the upregulation of mu and D1 dopamine receptors (Colantuoni et al., 2001; Olson et al., 2007), and the release of dopamine during instrumental (operant) behaviors for food (Salamone et al, 1994; Sokolowski et al., 1998).

In this line, the manipulation of motivational mechanisms, such as chronic food restriction, has been found to activate opioid receptors in an opioid dopaminergic-dependent system, which in turn produces changes in dopaminergic D1 and glutamergic receptors of the NAC (Haberny & Carr, 2005; Ouyang et al., 2017). In fact, this effect can be blocked by the administration of both general (naltrexone) and selective agonists (for mu and kappa receptors) (Berman et al., 1995; Carr and Papadouka, 1994; Carr, 2002) and may correspond to the generation of adverse neuroadaptations and locomotor-activating effects in striatal dopaminergic neurons (Carr, 2011).

In summary, various studies have demonstrated that not only the electrical stimulation of certain brain regions such as the LH but also natural reinforcers (food) and drugs of abuse can share the capacity to induce increases in DA release in the NAC (Salamone, 1994; Sokolowski et al., 1998; Cameron et al., 2014). Their differential release pattern may be more or less transient according to the activation of microcircuits of dopaminergic neurons that appear to be related to the motivational or seeking component of reward (Spanagel et al., 1992; Olson et al., 2007; Cameron et al., 2014; Fields and Margolis, 2015).

In this regard, Carelli et al. described a functional dissociation in the NAC between neural microsystems involved in processing natural rewards (food and water) and those involved in processing artificial rewards (cocaine) (Carelli et al., 2000; Carelli, 2002; Cameron et al., 2014). Using the same operant response (lever pressing) to obtain food or self-administer cocaine, they recorded the trigger patterns of NAC cells and observed that they were determined by the nature of the reward and by its associated environmental cues (Carelli, 2002; Cameron et al., 2014). Moreover, although natural reinforcers and drugs of abuse appear to share the capacity to generate dopamine release in the NAC shell, the response induced by the former progressively decreases, while the DA response induced by drugs remains robust after every administration (Pontieri et al., 1995). These results led to the consideration of addiction as a special case of "overlearning" (Hyman et al., 2006).

This dissociation has also been behaviorally verified in analyses of the effects of food or morphine preference in a CPP paradigm, in which experimental animals showed a preference for the compartment associated with the drug and also for the natural reinforcer (Spiteri et al., 2000). However, while animals remained in close contact with the environmental setting in which they had experienced physiological reactions associated with morphine administration, their behavior was different in relation to natural rewards, with frequent entry into the reward-associated compartment of the maze and numerous exploratory (rearing, sniffing) and approach behaviors (Spiteri, et al., 2000).

In conclusion, studies on the role of opioids in homeostatic LH-related mechanisms indicate their possible relationship with activation of a dopamine-related system, possibly connected to goal-directed behaviors. However, as already noted, some authors have also observed the presence of opiate hedonic hotspots (NAC shell, ventral

pallidum) embedded in this mesolimbic system, which may generate and/or increase affective reactions to rewarding taste or smell stimuli from food (Wassum et al., 2009; Peciña and Smith, 2010; Smith et al., 2011).

6- Involvement of the Vagal-Parabrachial system in rewarding processes

Nutritional behavior allows organisms to recover the continuous energy expenditure produced by the metabolism of body cells and requires systems specialized in the detection and analysis of substances reaching the digestive system (Shizgal et al., 2001; Castro et al., 2015).

Information from the gastrointestinal tract can be transmitted to the brain *via* two complementary substrates: a rapid neural system and a slower humoral pathway, which make some of their first synaptic contacts in brainstem regions of the NST and Area Postrema (AP), respectively (Fulwiler and Saper, 1984;De Lacalle & Saper, 2000). The information then passes to the next relay, the parabrachial complex, which receives relevant gustatory and visceral information for different motivational and/ or rewarding aspects of intake behavior (Fulwiler and Saper, 1984; Halsell and Travers, 1997; De Lacalle and Saper, 2000; Baird et al., 2001; Karimnamazi et al., 2002) (**Figure 5**).

The differential involvement of these two systems in nutritional processes appears related to the type of substance and the experimental situation (Mediavilla et al., 2005). In this regard, "taste preference tests", which require the association of non-nutritive and innocuous taste stimuli (generally flavored water) with the intragastric or intra-intestinal administration of a visceral stimulus, allow the aversive or rewarding nature of viscerally administered substances to be analyzed (Mediavilla et al., 2005).

Taste learning can be induced by using *sequential* or *concurrent* procedures. In *sequential* learning, the taste stimulus is associated with intragastric administrations on alternating days/sessions (Mediavilla et al. 2000; 2005; Zafra et al., 2002; 2007b). In the

concurrent modality, these stimuli are presented at the same time, pairing the intake of tastes with the simultaneous intragastric administration of either the visceral stimulus or an innocuous substance, e.g., physiological saline (Puerto et al., 1976; Mediavilla et al., 2000; Zafra et al., 2007a). Concurrent learning permits a rapid detection of biologically relevant substances in the upper gastrointestinal tract, allowing individuals to efficiently select food without waiting for the long-term benefits that result from its absorption (Puerto et al., 1976). Consequently, the neural pathway formed by vagal and spinal afferent fibers is essential when the task imposes important time demands and requires the rapid detection of the stimuli present in the upper gastrointestinal tract, although spinal fibers appear to be less important (Furness et al., 1999; Raybould, 2010; Zafra et al., 2016).

The vagal system comprises nerve fibers connected to mechano-, chemo-, and osmo-receptors that can receive and calibrate the sensory components (pH or osmolality) of food as well as its micro- and macro-chemical nature (Furness et al., 1999; Raybould, 2010). These are mainly bipolar neurons with soma in the nodose ganglion, a peripheral branch, and a central branch that terminates in the NST (Andrews and Sanger, 2002). Glutamate, GABA, noradrenalin, and serotonin, among other neurotransmitters, have been identified in NST endings alongside opiate receptors (mu and, to a lesser extent, delta and kappa receptors (Mansour et al., 1995; Ozaki et al., 2000; Andrews and Sanger, 2002; Bogdanova et al., 2015) and receptors for cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), glutamate, substance P, prostaglandins, histamine, Y and YY neuropeptides, cannabinoids (CB1 and CB2), and leptin, among others (Andrews and Sanger, 2002; Fromentin et al., 2012).

With respect to opiate receptors, their density has been found to decrease after vagal deafferentation or ganglionectomy (Dashwood et al., 1988), suggesting a

presynaptic localization on vagal afferents, although they have also been identified at postsynaptic level. In fact, the presence of opiate peptides (enkephalins, β-endorphin) has been demonstrated in second-order neurons in the intermediate-caudal region of the NST (Velley et al., 1991; Ozaki et al., 2000). The utilization of complex retrograde labeling techniques revealed that some of these enkephalinergic neurons project to the parabrachial complex (Maley and Panneton, 1988).

At the most lateral end of this pontine region, surrounding the upper cerebellar peduncle, is the **external lateral parabrachial subnucleus** [LPBe] **(Figure 6)** (Fulwiller and Saper, 1984; Bernard et al.,1996; De Lacalle and Saper, 2000; Karimnamazi et al., 2002), whose activity can be modulated by gastric distension and/or vagus nerve stimulation (Suemori et al., 1994; Saleh and Cechetto, 1996). Conversely, LPBe activity is significantly attenuated by vagus nerve lesions (Yamamoto and Sawa, 2000a).

The LPBe is known to be involved in processing a wide range of stimuli, most of which may have affective value. It participates in analysis of the sensory and hedonic characteristics of taste stimuli (Yamamoto et al., 1994; Halsell and Travers, 1997; Sewards, 2004) and of different nutrients, such as intraduodenally administered glucose (Wang et al., 1999), and intragastrically administered lactose and sucrose (Yamamoto and Sawa 2000a; 2000b). Some hormones involved in regulating intake and nutritional metabolism, such as CCK, galanin, Y and YY neuropeptides, and leptin also appear to act *via* the LPBe (Li and Rowland, 1995; Trifunovich and Reilly, 2001; Elias et al., 2000; Alhadeff et al., 2015), as do antimetabolic products such as mercaptoacetate (Calingasan and Ritter, 1993). Finally, it has been observed that various brain areas, including the LPBe, can be activated by the administration of drugs with a potential intake-modulating role, including benzodiazepines (Söderpalm and Berridge, 2000),

fenfluramine (Li and Rowland, 1995; Simansky and Niclous, 2002), cannabinoids (DiPatricio and Simansky, 2008), and opiates (Chamberlin et al., 1999; Chaijale et al., 2013).

7- Functional characterization of the LPBe

The aforementioned reports on the involvement of the LPBe in processing a wide range of hedonic stimuli led our group to use Electrical Stimulation to activate this region and analyze its possible participation in rewarding brain mechanisms (Simon et al., 2007; 2008; 2011; 2013; García et al., 2014). Results of these and other related experiments revealed that most of animals showed a preference for **taste** stimuli associated with this stimulation in concurrent tasks (Simon et al., 2007; 2008; 2013; García et al., 2014). These data agree with the findings obtained by Grill and Norgren (1978) using taste reactivity tests, in which decerebrated animals displayed appetitive reactions at brainstem level when food was directly introduced into the oral cavity but did not exhibit seeking behaviors (Grill and Norgren, 1978). Data obtained by this procedure led various authors to consider these reactions as reflecting the hedonic impact of taste rather than merely sensory reflexes (Grill and Norgren, 1978; Castro et al., 2015).

Our results are also compatible with observations that lesions of the lateral end of the parabrachial nucleus, including the LPBe, attenuated the overconsumption of highly palatable food induced by previous AP lesions (Edwards and Ritter, 1989) and blocked taste preferences induced by the administration of rewarding meals (Zafra et al., 2002). Furthermore, recording techniques at cell level identified neurons in the LPBe that can specifically process the sensory and/or hedonic properties of taste stimuli (Yamamoto et al., 1994; Halsell and Travers, 1997; Karimnamazi et al., 2002; Sewards, 2004). In this sense, the rewarding effect of LPBe electrical stimulation (Simón et al.,

2007; 2008; 2013) might be related to the modulation of taste-perception mechanisms in this area through changes in palatability (Parker et al., 1992).

Alternatively, the rewarding effect of LPBe electrical stimulation may act as a substitute of visceral stimuli and/or its affective consequences (Simon et al., 2007; 2008) through the reception of visceral information from the NST (Fulwiller and Saper, 1984; De Lacalle and Saper, 2000). Indeed, an intact LPBe appears to be essential for rapid adjustments in neural systems related to short-term intake (Zafra et al., 2016) and for processing intragastrically administered rewarding nutrients (Zafra et al., 2002). As suggested by some authors, the LPBe may be part of a downstream circuit in which information on energy balance may interact with ascending visceral signals, promoting a positive affective status in calorie-depleted animals (Garfield et al., 2015).

Opiates have been found to play an important role in intake through their differential action on mu/kappa receptors in the LPBe (Carr et al., 1991; Moufid-Bellancourt et al., 1996) which undergo neuroadaptation under special conditions of chronic food deprivation (Carr & Papadouka, 1994; Wolinsky et al., 1996). These data may be compatible with our aforementioned findings of preferences for taste stimuli associated with electrical stimulation (Simon et al., 2007; 2008), an effect that was completely blocked by naloxone administration (Simón et al., 2007; 2011).

Intake-modulating effects have also been reported for the intra-parabrachial administration of benzodiazepines (midazolam) (Söderpalm and Berridge, 2000), cannabinoids (DiPatricio and Simansky, 2008), and fenfluramine (Simansky and Nicklous, 2002). Among other effects, these drugs may modify the assessment of certain 'innately preferred' substances, acting on palatability (Soderpalm and Berridge, 2000; Wilson et al., 2003; DiPatricio and Simansky 2008). They may also increase the

hedonic properties of nutrients or diminish the state of 'discomfort' generated by homeostatic imbalance (Carr et., 1991, Carr, 2002).

The rewarding effect of LPBe electrical stimulation is observed not only in taste tasks but also in a **concurrent conditioned place preference (**cCPP), a variant of CPP in which the animal can move freely throughout the maze but only receives stimulation when it enters a previously selected compartment containing environmental (visual) cues (Simon et al., 2007; 2009; 2011; García et al., 2014; Agüera et al., 2016). These results suggest that preferences might not be specific to a single sensory modality (Simon et al., 2007; 2009; 2011; 2013; García et al., 2014; Agüera et al., 2016). In fact, some of these experiments showed that animals manifesting preferences for a taste stimulus associated with electrical LPBe stimulation after two CTP association trials consistently maintained this preference in a second phase in which they underwent a cCPP task (Simon et al., 2007) or a second CTP procedure with different taste stimuli (Simon et al., 2008).

In the case of the experiments involving CTP procedures, there was no change in the left/right positioning of burettes with/without the stimulus associated with stimulation (Simon et al., 2007; 2008). We therefore explored whether the preferences established were related to taste stimuli or proprioceptive stimuli (right or left position of burettes). For this purpose, a new group of animals were trained in a similar CTP procedure and then underwent a second test in which the left/right position of the burettes was inverted. According to the results obtained, the learning of animals was related to the place and not the taste stimuli. (García et al. 2014). On the other hand, another experiment in which the position of each burette varied among trials found that the animals acquired the learning but needed a larger number of trials (Simon et al., 2013). Overall, these findings suggest that animals are capable of developing a

preference for either type of stimulus when associated with electrical stimulation of the LPBe but appear to have a biological predilection towards spatial cues (Simon et al., 2013; García et al., 2014).

These data are compatible with other studies in which animals showed preference for the place associated with the intragastric infusion of liquids or foods or even with the presence of a sexual partner when the stimuli were administered immediately before confining the animals within a specific T-maze compartment (Arnold and Agmo, 1999; Spiteri et al., 2000; Garcia-Horsman et al., 2008). They are also in agreement with experiments that used this place procedure to explore the rewarding effects of substances of abuse (McBride et al., 1999; Tzschentke, 2007).

Although LPBe electrical stimulation appears to generate preferences for associated taste or place stimuli in most animals (Simon et al., 2007; 2008; 2009; 2011; 2013; García et al., 2014), we observed a small number of animals that consistently preferred the taste or place that was not associated with stimulation (Simon et al., 2007; 2008; 2009). In other words, the electrical stimulation may have had an **aversive effect** in some animals. In this regard, the LPBe contains relay fibers of the spino-(trigemino) ponto-amygdaloid bundle, known to be specifically involved in processing the affective-emotional, autonomic, and visceral components of pain (Bernard et al., 1991; 1996; Gauriau and Bernard, 2002; Li et al., 2006). It is therefore possible that a negative affective status was generated in some animals through the activation of nociceptive neurons in this system, explaining their avoidance behavior (Simon et al., 2007; 2008; 2009; 2011).

The LPBe has been described as playing a key role in concurrent taste aversion learning induced by aversive visceral stimuli administration (Mediavilla et al., 2000) and as participating in a descending visceral system involved in appetite suppression

and the processing of 'unpleasant feelings' in unfavorable conditions for eating (Carter et al., 2013). Moreover, the activation of kappa opiate receptors, also present in this LPBe region, appears to have aversive effects, contrasting with the effects of mu receptor activation (Moufid-Bellancourt et al., 1996; Darcq and Kieffer, 2018).

It is possible that small changes in the placement of electrodes or in the current used for electrical stimulation may differentially activate positive or negative cells that react distinctly to the affective/hedonic properties of taste stimuli (Yamamoto et al., 1994). It also feasible that the stimulation affects rewarding and/or aversive motivational systems that are anatomically very close (Moufid-Bellancourt et al., 1996; Wolinsky et al., 1996), as may be the case for the aforementioned visceral pathways generating signals of positive satiety (Garfield et al., 2015) and negative discomfort (Carter et al., 2013).

Different experiments involving the LPBe have also been used in operant behavior learning aimed at the self-administration of current pulses to this region while avoiding the activation of an aversive system. Although the majority of animals did not display aversive behavior, it was not possible to induce ICSS behavior, contrasting with the ready induction of this behavior using the LH (Simon et al., 2011). This result suggests that electrical stimulation of the LPBe may be related to the activation of affective mechanisms rather than goal-directed behaviors (as observed with the LH).

Similar dissociation has also been found at other sites such as the thalamus, where the anterior region of the medial parafascicular subnucleus was positive for ICSS behavior, while stimulation of its posterior part improved learning by facilitating the acquisition and retention of two-way active avoidance conditioning (Vale-Martinez et al, 1999). Likewise, it has been reported that some drugs (e.g., lysergic acid diethylamide [LSD], buspirone, and pentylenetetrazole) can induce place preferences

but not self-administration behaviors, whereas others (e.g., pentobarbital or phencyclidine) cannot induce conditioned place preference but can sustain self-administration behaviors (Bardo and Bevins, 2000).

Preferences for tastes and places associated with electrical stimulation have also been observed in other brain areas anatomically connected to the LPBe, such as the insular cortex (Cubero and Puerto, 2000; García et al., 2013). At the same time, findings in the VTA have revealed neuronal populations with different electrophysiological properties responsible for either reward or aversion, pain or analgesia, escape or self-stimulation according to the precise localization of the electrode (Prado and Roberts, 1985; Salamone, 1994; Hikida et al., 2016; Morales and Margolis, 2017).

Stimuli associated with LPBe electrical stimulation were presented in a **concurrent** or contiguous manner in all studies by our group on preference/aversion (Simon et al., 2007, 2008, 2009, 2011, 2013), and these effects disappeared when a time delay was introduced (García et al., 2014). This finding suggests that the acquisition and retention processes might involve a rigid (implicit) learning procedure (García et al., 2014), explaining why animals benefited from an increase in the number of trials (Simon et al., 2013) and why the learning was not acquired when there was a time delay (García et al., 2014).

The effects of LPBe electrical stimulation are consistent with results obtained using natural stimuli (Mediavilla et al., 2000; 2005-for a review-; Yamamoto and Sawa 2000a; 2000b; Zafra et al., 2002; 2016). These effects may activate the same circuits as those observed with acute or chronic stress, exposure to emotionally arousing material, or even drug addiction (Schwabe et al., 2010; Darcq and Kiefer, 2018). These have been proposed to involve activation of a visceral pathway, promoting the generation of stereotyped behaviors and inducing implicit learning (Schwabe et al., 2010).

Conversely, lesions of the LPBe, one of the first central relays in this viscero-vagal-LPBe pathway, appear to selectively impair implicit learning (Mediavilla et al., 2005).

The well-documented presence of opiate receptors in the LPBe (Mansour et al., 1995; Chamberlin et al., 1999; Wolinsky et al., 1996) suggests that it not only generates nutritional preferences/aversions in taste and place conditioning procedures but might also participate in the processing of substances of abuse (Bechara et al., 1993). Indeed, some authors have attributed the LPBe with a key role in processing the discriminative properties of morphine (Jaeger and Van der Kooy, 1993) and probably the aversive properties derived from peripheral visceral effects (Bechara et al., 1993; Nader et al., 1996). However, the LPBe may also be essential for the rewarding properties of drugs that act on the opiate system (Simon et al., 2007; 2011; Hurtado and Puerto, 2018).

As already noted, our experimental groups learned to associate both places and flavors with electrical stimulation of the LPBe, but they showed a greater propensity for spatial cues. This result may be related to the important role for addicted individuals of the places in which the drugs are taken (Koob & Le Moal, 2000; Koob et al., 2014) and to the development of dependency and/or tolerance with repeated administrations (See, 2002). In this regard, a tolerance effect has been observed after repeated stimulation of the LPBe, especially when administered passively (not contingently) by the experimenter (Hurtado and Puerto, 2016; 2018) These findings are in agreement with the report by other authors that withdrawal reactions were precipitated by a peripherally acting opioid antagonist that generated activation throughout the visceral pathway, specifically in the PBle (Hamlin et al., 2001).

Finally, other studies in our laboratory showed that naloxone blocked the rewarding effects of stimulation in a cCPP procedure when the task was conducted **in a new maze** but not when conducted in the same setting as that of the initial learning

acquisition (Simon et al., 2007; 2011; García et al. 2014). These findings suggest that opiates present in this parabrachial area may act *via* a circuit that is independent of dopamine (Simon et al., 2007; 2011), which was not the case with LH stimulation (Simon et al., 2011). Furthermore, administration of this opiate antagonist eliminated the hedonic component without affecting the motivation that keeps animals in an expectant state when placed in the same experimental setting (Simon et al., 2007; 2011; García et al. 2014). In a similar way, other studies have shown that naloxone injection eliminated the rewarding reactions but not the motivation of animals that had previously received heroin or cocaine (McFarland and Ettenberg, 1998).

However, the complexity of the processing of drugs of abuse suggests that these sistems may induce long-term neuroadaptations and may recruit new systems (Koob and Le Moal, 2000; Hamlin, 2001; Koob et al., 2014). For example, these stimuli may also activate circuits involved in incentive attribution processes, by which animals progressively acquire an improved estimation of the circumstances and actions from retrospective experience and make a motivational/affective reevaluation of these circumstances and/or actions based on prevailing states of the body and brain, as proposed by some authors (Dayan and Berridge, 2014).

8- Interpretation and future guidelines

Globally, our experiments have shown that electrical stimulation of the LPBe in combination with CTP and cCPP behavioral procedures generates preferences (and aversions) toward stimuli with which it is associated in a contiguous manner. This effect is totally blocked by naloxone when animals are placed in a new maze (Simon et al. 2007; 2008; 2009; 2011; 2013; 2016; Garcia et al., 2014).

These data may be compatible with some current theories on reward mechanisms and addiction such as the Opponent Process theory (Solomon and Corbitt,

1974 -cited by Koob et al., 2014-), initially referred to classical substances of abuse but later to binge eating and other behavioral disorders. According to this theory, a continuum from occasional and limited use of substances to a chronically relapsing disorder may be explained by interactions of mechanisms responsible for developing habits/incentive salience with those involved in executive control and affective regulation. Mechanisms that support addictive behavior, characterized by compulsive seeking behavior, loss of control, and a negative emotional state, would be temporarily connected in an opponent loop containing: an *A-process*, giving rise to unconditional affective reactions (euphoria) that quickly decay, producing 'tolerance'; and a *B-process,* emerging immediately after the first process but dependent on a different neurobiological mechanism, generating an aversive craving state that is amplified with repeated exposure (Koob and Le Moal, 2000; Moore et al., 2017). This conceptual framework, subsequently developed by Koob and Le Moal, focuses on motivational-affective circuits/systems and hypothesizes that transition towards compulsive use and loss of control is accompanied by chronic perturbations of homeostatic systems (allostasis) and by neuroadaptations, leading to behavioral sensitization (Koob and Le Moal, 2000; Koob, et al.,, 2014). Accordingly, the presence in the brainstem of regions such as the LPBe, which process opiate-mediated positive (and/or aversive) affective information (Simon et al., 2007; 2008; 2009; 2011; 2013; García et al., 2014) and where a tolerance effect has been observed (Hurtado and Puerto, 2016; Hurtado et al., 2016), suggests that this region may possibly form part of wider hedonic-affective circuits that may be hierarchically controlled by anterior prosencephalic regions (Roitman et al., 2004; Berridge and Kringelbach, 2015) and whose components require further elucidation.

According to other researchers, desire, pleasure, and the learning of associations are elements that simultaneously intervene in any gratifying experience (due to natural stimuli, stimulation, or drugs) and may be dissociated (Waraczynski, 2006; Berridge and Robinson, 1998; Berridge and Kringelbach, 2015). According to this hypothesis, the neurobiological mechanisms that sustain addiction evolved to support homeostatic behaviors (e.g., food, water intake or sexual behavior) important for individual or species survival (Kelley and Berridge, 2002). Rewards also act as incentives, generating neural representations that not only allow the learning of crucial associations for survival but also govern the search for these rewards (Kelley and Berridge, 2002; Hyman et al., 2006; Darcq and Kieffer, 2018). In this theoretical framework, dopamine-independent opiate-mediated transmission would be part of the circuit involved in the subjective experience of pleasure ('*liking*'), while the mesolimbic dopaminergic pathway would be part of the seeking circuits ('*wanting*'), integrating attention and sensorimotor mechanisms and promoting the formation of 'habits' and the generation of 'compulsive' seeking behaviors (Kelley and Berridge, 2002; Berridge and Kringelbach, 2015). From this perspective, the mesolimbic system and its connections, widely distributed throughout the brain, would sustain motivation, while another smaller circuit would encode the hedonic component, possibly overlapping with pathways involved in processing aversive effects (Kelley and Berridge, 2002; Berridge and Kringelbach, 2015). LPBe may be part of this second system that encodes the hedonic/aversive aspects of stimulation.

Publications by Salamone support the idea that dopamine regulates components other than pleasurable feelings (Salamone and Correa, 2012; Salamone et al., 2016; 2018): They noted that its release not only in positive but also in aversive/stressful situations suggests that it may be more related to ‘motivation’, including activational,

attentional, and motor aspects (Salamone and Correa, 2012; Salamone et al., 2016). They observed that dopaminergic antagonists affect behavioral activation and produce changes in response allocation, with the selection of lower-cost behavior in both discriminative learning tasks (T-maze) and operant procedures (lever-pressing or effort-based selection), (Salamone et al., 1991; 2016). Conversely, drugs that increase dopaminergic transmission tend to enhance behaviors requiring a high effort (Salamone et al., 2016). Recording of the dopaminergic signal of the mesolimbic system at different time scales indicated that this system may integrate information from different motivational microcircuits, serving as a sensory-motor interface (Salamone & Correa, 2012; Salamone et al., 2016). Our experimental findings for the LH are consistent with these data, given that ICSS induced plastic changes in dopaminergic receptors and naloxone, unlike in the case of the LPBe, was not able to block this effect (Simon et al., 2011; 2016). Further research is warranted on the differential characteristics of the two systems.

Shizgal et al developed 'neuroeconomical models' of decisionmaking, based on objective variables that indicate the extent to which an individual, after learning the association of a behavior with its consequences, participates in seeking behaviors and reward pursuit rather than alternative behaviors such as resting or grooming, which could compete with reward acquisition (Solomon et al., 2017). In this regard, a tonic increase in dopamine release was found to potentiate the pursuit of brain stimulation reward (Hernandez et al., 2012), although these changes in dopamine tone did not correlate well with variables related to affective aspects (Scardochio et al., 2015).

Our behavioral and neurochemical results obtained in the LPBe and LH support these dissociations: As has already been mentioned, taste/place preferences induced by LPBe electrical stimulation were completely blocked by naloxone (Simon et al., 2007;

2011), whereas preferences for cues associated with LH electrical stimulation were not (Simon et al., 2011). In addition, ICSS operant behavior was readily obtained with LH stimulation but notwith PBLe stimulation (Simon et al., 2011).

The above data on behavioral and neurochemical dissociations support the idea that heterogeneous substrates encoding activational and affective (and perhaps other) aspects of reward may overlap in some brain areas (Roitman et al., 2004; Berridge and Kringelbach, 2015).

In conclusion, the brainstem LPBe receives peripheral gustatory and visceral information (Fulwiler and Saper, 1984; De Lacalle and Saper, 2000) that is connected to anterior brain areas such as the so-called "extended amygdala" (Li et al., 2006; Gauriau and Bernard, 2002), and it may play an important role in processing affective reward components other than those that involve the mesolimbic system (Kelley and Berridge, 2002). Given that basic hedonic feelings can be generated in decerebrated animals at brainstem level (Grill and Norgren, 1978; Castro et al., 2015) and opiates are present in the LPBe (Wolinsky et al., 1996; Chamberlin et al., 1999), the effects of natural rewards, electrical stimulation, and even drugs of abuse may be neurobiologically related to an affective reward mechanism in this area. The combination of behavioral and pharmacological procedures with novel techniques such as optogenetics or other genetic manipulations (e.g., gene activation/silencing, transgenics) can improve our global understanding of this system and advance our knowledge of possible long-term neuroadaptations within and between systems.

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Figure 1: Sagittal section of rat brain depicting some areas known to support Intracraneal Self-Stimulation behavior [Adapted from Phillips and Fibiger, 1989].

Figure 2: Quantitative Autoradiography of D1 receptors.

Left: Coronal sections showing significant changes in D1 receptor expression in an animal (13E) from the LH-ICSS group. Right: Schematic representation of areas with significant labeling, from the corresponding section of the atlas of Paxinos and Watson.

Figure 2B (OPTIONAL, EDITORIAL TEAM DECISSION): Quantitative Autoradiography of D1 receptors showing D1 receptor expression in an animal (2C) from the Control Group.

Figure 3: Specific ^{3}H-DAMGO mu-receptor binding in nine coronal rat brain sections in self-stimulated (n=9) and control (n=8) animals. Data were analyzed with a 2-tailed Student's t-test for unrelated samples and expressed as means ± SEM. LH-ICSS animals showed significantly higher Mu receptor binding in the IP nucleus alone (t=2.485 14 *df*, p<0.026*) [Reprinted from: Neurobiology of Learning and Memory, 127. Simon et al. Changes in D1 but not D2 dopamine or mu-opioid receptor expression in limbic and motor structures after lateral hypothalamus electrical stimulation: A quantitative autoradiographic study, page 20 (©2016), with permission from Elsevier].

Abbreviations:

Sections: PFC: prefrontal cortex, NAC: nucleus accumbens, BNST: bed nucleus of the stria terminalis, HC: hippocampus, VTA: ventral tegmental area, CG: central grey area, DR: dorsal rafe, NPB: parabrachial area, LC: locus coeruleus.

Specific nuclei and subnuclei: AI: agranular insular cortex; O: orbital cortex; Cg: cingulate cortex; L: limbic cortex; M1A-M1B: primary motor cortex; AcbSh: nucleus accumbens, shell; AcbC: nucleus accumbens, core; CPu1: caudate putamen, matrix; CPu2: caudate putamen, striosomas; BNSTm: bed nucleus of the stria terminalis, medial part; BNSTl: bed nucleus of the stria terminalis, lateral part; LSI: lateral septal nucleus, intermediate part; CA1-3: fields of hippocampus; LHb: lateral habenular nucleus; MDM-T: mediodorsal thalamic nucleus, medial part; MDC-T: mediodorsal thalamic nucleus, central part; MDL-T: mediodorsal thalamic nucleus, lateral part; IML-T: intermediolateral cell column; CM-T: central medial thalamic nucleus; VPL-T: ventral posterolateral thalamic nucleus; VPM-T: ventral posteromedial thalamic nucleus; STh: subthalamic nucleus; ZI: zona incerta; BLA: basolateral amygdaloid nucleus, anterior part; Ce: central amygdaloid nucleus; ACo: anterior cortical amygdaloid nucleus; DM: dorsomedial hypothalamic nucleus; LH: lateral hypothalamic area; VMH: ventromedial hypothalamic nucleus; PVP-T: paraventricular thalamic nucleus, posterior part; SN: substantia nigra; VTA: ventral tegmental area; LPAG: lateral periaqueductal grey; SuG: superficial gray layer of the superior colliculus; InG: intermediate gray layer of the superior colliculus; IP: interpeduncular nucleus; MG: medial geniculate nucleus; DR: dorsal raphe nucleus; MnR: median raphe nucleus; LC: locus coeruleus.

Figure 4: Electrical stimulation of the lateral hypothalamus in a concurrent CPP task and effect of the administration de 4.0 and 10.0 mg/Kg of the opiate antagonist naloxone. LH-ES: stimulated group; LH-I: implanted non-stimulated group; LH-C: intact control group [Reprinted from Behavioral Brain Research, 225. Simon et al., Concurrent stimulation-induced place preference in lateral hypothalamus and parabrachial complex: differential effects of naloxone, page 313 (© 2011), with permission from Elsevier].

Figure 5: Schematic representation of gastrointestinal input to the brainstem *via* the vagal-parabrachial pathway.

Figure 6: Histological localization of the electrode in LPBe-stimulated animals.

Table 1

Comparison of brain ^{3}H-SCH-23390 (D1R antagonist) and ^{3}H-YM-09151-2 (D2R agonist) between ICSS experimental and control groups, using the Student's t-test for unrelated samples [t=value of t in the Student's test; df=degree of freedom; p=probability of t in a 2-way Student's t-test. Results are expressed as nCi].

Examined sections (abbreviations):

1) Level of the prefrontal cortex (PFC, +3.20 mm. from bregma): PrL-IL: prelimbic-infralimbic cortex; Cg: cingulate cortex; M2: secondary motor cortex; M1: primary motor cortex; AI: agranular insular cortex; LO: lateral orbital cortex; VO: ventral orbital cortex; DEn: dorsal endopiriform nucleus; AOP: anterior olfactory nucleus, posterior part; AcbSh: accumbens nucleus, shell.

2) Level of the nucleus accumbens (NAC, +1.70 mm. from bregma): CPu1: caudate putamen, matrix; CPu2: striosomas of the caudate putamen; Cg: cingulate cortex; Motor Cx: motor cortex; AcbSh: accumbens nucleus, shell; AcbC: accumbens nucleus, core; LS: lateral septal nucleus; VP: ventral pallidum; CI: claustrum; DEn: dorsal endopiriform nucleus.

3) Level of the bed nucleus of the stria terminalis (BNST, -0,30 mm. from bregma): CPu1: caudate putamen, matrix; CPu2: striosomas of the caudate putamen; VP: ventral pallidum, LS: lateral septal núcleus; Tu: olfactory tubercle.

4) Level of the hippocampus (HC, -2.80 mm. from bregma): CA1-3: fields of hippocampus; Hb: habenular nucleus; CPu: caudate putamen; BLA: basolateral amygdaloid nucleus, anterior part; PRh: perirhinal cortex; DEn: dorsal endopiriform nucleus MGP: medial globus pallidus.

5) Level of the ventral tegmental area (VTA, -4.80 mm. from bregma): PiRe: pineal recess; Hbc: habenular commissure; CA1 field of the hippocampus; DG: dentate gyrus; PRh: perirhinal cortex; DEn: dorsal endopiriform nucleus; SNR: substantia nigra, reticular part; SNC: substantia nigra, compact part; VTA: ventral tegmental area; V2MM: secondary visual mediomedial cortex.

6) Level of the central gray (CG, -5.80 mm. from bregma): SNR: substantia nigra, reticular part; SuG: superficial gray layer of the superior colliculus PRh: perirhinal cortex; DEn: dorsal endopiriform nucleus.

Figure 3B (OPTIONAL, EDITORIAL TEAM DECISSION):

Brain differences in ^{3}H-SCH-23390 binding (D1R antagonist) between ICSS experimental and control groups, using the Student's t-test for unrelated samples [t=value of t in the Student's test; df=degree of freedom; p=probability of t in a 2-way Student's t-test. Results are expressed as nCi].

Examined sections (abbreviations):

1) Level of the prefrontal cortex (PFC, +3.20 mm. from bregma): PrL-IL: prelimbic-infralimbic cortex; Cg: cingulate cortex; M2: secondary motor cortex; M1: primary motor cortex; AI: agranular insular cortex; LO: lateral orbital cortex; VO: ventral orbital cortex; DEn: dorsal endopiriform nucleus; AOP: anterior olfactory nucleus, posterior part; AcbSh: accumbens nucleus, shell.

2) Level of the nucleus accumbens (NAC, +1.70 mm. from bregma): CPu1: caudate putamen, matrix; CPu2: striosomas of the caudate putamen; Cg: cingulate cortex; Motor Cx: motor cortex; AcbSh: accumbens nucleus, shell; AcbC: accumbens nucleus, core; LS: lateral septal nucleus; VP: ventral pallidum; CI: claustrum; DEn: dorsal endopiriform nucleus.

3) Level of the bed nucleus of the stria terminalis (BNST, -0,30 mm. from bregma): CPu1: caudate putamen, matrix; CPu2: striosomas of the caudate putamen; VP: ventral pallidum, LS: lateral septal núcleus; Tu: olfactory tubercle.

4) Level of the hippocampus (HC, -2.80 mm. from bregma): CA1-3: fields of hippocampus; Hb: habenular nucleus; CPu: caudate putamen; BLA: basolateral amygdaloid nucleus, anterior part; PRh: perirhinal cortex; DEn: dorsal endopiriform nucleus MGP: medial globus pallidus.

5) Level of the ventral tegmental area (VTA, -4.80 mm. from bregma): PiRe: pineal recess; Hbc: habenular commissure; CA1 field of the hippocampus; DG: dentate gyrus; PRh: perirhinal cortex; DEn: dorsal endopiriform nucleus; SNR: substantia nigra, reticular part; SNC: substantia nigra, compact part; VTA: ventral tegmental area; V2MM: secondary visual mediomedial cortex.

6) Level of the central gray (CG, -5.80 mm. from bregma): SNR: substantia nigra, reticular part; SuG: superficial gray layer of the superior colliculus PRh: perirhinal cortex; DEn: dorsal endopiriform nucleus.

RESPONSE TO REVIEWERS

We are very grateful to our reviewers for their comments and insights, which have helped us to strengthen and clarify our paper. We include our point-by point responses below.

In addition, we have modified the **Abstract** to incorporate **headings,** as requested by the Editor:

ABSTRACT

Background: *Since the discovery of rewarding intracranial self-stimulation by Olds and Milner, extensive data have been published on the biological basis of reward. Although participation of the mesolimbic dopaminergic system is well documented, its precise role has not been fully elucidated, and some authors have proposed the involvement of other neural systems in processing specific aspects of reinforced behavior.*

Aims and methods: *We reviewed published data, including our own findings, on the rewarding effects induced by electrical stimulation of the lateral hypothalamus (LH) and of the external lateral parabrachial area (LPBe) -a brainstem region involved in processing the rewarding properties of natural and artificial substances-, and compared its functional characteristics as observed in operant and non-operant behavioral procedures.*

Results: *Brain circuits involved in the induction of preferences for stimuli associated with electrical stimulation of the LBPe appear to functionally and neurochemically differ from those activated by electrical stimulation of the LH.*

Interpretation: *We discuss the possible involvement of the LPBe in processing emotional-affective aspects of the brain reward system.*

Conflict of interest*: none*

REVIEWER 1

As requested, we have reduced our discussion on the dopaminergic system of reward in section 2, which is followed by a section on the utilization of non-operant procedures to study the brain reward system and then by sections on the role of opiates in ICSS behaviors and in homeostatic systems. This is followed by discussion on the involvement of the Vagal-Parabrachial system in rewarding processes (section 6).

1. Abstract, L24: "...processing natural and artificial substances..."

We have adopted the more correct wording suggested by the reviewer:

", a brainstem region involved in processing the rewarding properties of natural and artificial substances, "

2. P3L18: References of Berthoud & Münzberg, 2011; Stauffer et al., 2016.

This paragraph was designed to refer in general to brain mechanisms that have evolved to identify stimuli that favor maintenance of homeostasis and enable the generation of affective reactions and the acquisition of learning, in line with psychological theories on incentive motivation. We have now REPLACED these with the following references, which are more closely related to this point:

Bindra, 1974; Dayan & Balleine, 2002; and Berridge, 2018 (page 2).

3. P5L16: "The specific role of dopamine in this context":

We have try to clarify this affirmation, which now READS as follows (page 4)*:*

"The specific role of dopamine in relation to this process remains a controversial issue and warrants further research (Waraczynski, 2006; Hernandez et al., 2007; Ikemoto, 2010; Smith et al., 2011; Salamone and Correa, 2012; Berridge and Kringelbach, 2015; Morales and Margolis, 2017)

4. P5L53: "Conversely"; P6L7 "In contrast".

We agree with the reviewer and have REPLACED "*Conversely*" with *"In addition*", and "*In contrast*" with "*It has also been reported that":(Page 5)*

In addition, mice with a genetic disruption of dopamine transporter [DAT] and a consequent increase in synaptic DA, not only required fewer trials to learn an incentive runway task, but also ran faster to the goal and were better at avoiding distractions (Peciña et al., 2003).

It has also been reported that pharmacological dopamine blockade or even complete destruction of the DA mesolimbic system did not diminish facial expressions of hedonic impact (positive affective reactions), measured in a "taste reactivity test", a procedure that allows the recording of orofacial reactions to innately and learned gustative stimuli in human infants and animals (Grill and Norgren, 1978; Peciña et al., 1997; Berridge and Robinson, 1998).

5. P7L18: References of "Carlezon & Chartoff, 2007", "Vlachou & Markou, 2011".

These citations relate to the second part of the paragraph, which addresses ICSS) modulation. According to these studies, chemical substances and electrical self-stimulation might act on the same mechanism/circuit/neurobiological substrate to change its motivational and/or affective consequences in ways that have not yet been elucidated.

We have resolved this issue by placing of all these references at the end of the sentence, which now reads as follows: (page 8, paragraph 2):

Drugs of abuse that cause addiction in humans (e.g., cocaine, amphetamine, heroin, nicotine, etc. can be self-administered by laboratory animals in operant procedures and modulate ICSS behavior by changing rate/frequency curves and brain stimulation thresholds (Carlezon and Chartoff, 2007; Vlachou and Markou, 2011; Negus and Miller, 2014).

6. P7L24: "the most representative addictive substances".

We have MODIFIED this phrase in the revised text. It now reads (section 4, paragraph 3, page 8):

Opiates are among these highly addictive substances, which have potentially serious health consequences (Bodnar, 2017 -for a review).

7. P7L29: "positive effects"

We have changed this term to "reinforcing effects", as suggested (section 4, paragraph 3, page 8).

8. P8L9. "titration point".

We now define the "titration point" as the lowest stimulation frequency needed to maintain ICSS behavior. We apologize for our error in describing the experiment of Easterling & Holzman. We have rewritten this description, which now READS as follows (page 9, second paragraph):

In another study, Easterling and Holtzman demonstrated that acute morphine administration produced a small decrease in the titration point for ICSS behavior (the lowest stimulation frequency needed to maintain this operant behavior), reporting that this effect progressively diminished over time (Easterling and Holzman, 1997). In addition, they found that cumulative doses of naltrexone (opioid antagonist) during the course of ICSS only generate minimal dose-independent increases in the titration point, observing that this effect also decreased with longer time (Easterling and Holtzman, 1997; 2004). These results suggest a weak and non-determinant role of opiates in ICSS of the LH, that disappear over time and that opiate antagonists do not completely block this-behavior, even at high doses (Schaefer, 1988; Cazala & Davis, 1991; Easterling and Holzman, 1997; 2004; Bielajew et al., 2003; Wiebelhaus et al., 2016).

9.P8L24-37: "effect of opiates on ICSS"

This paragraph has been deleted.

10. P8L40: Changes in the expression of opioid receptors in LHSS.

The quantitative autoradiography study by Simon et al., (2016) shows that plastic changes in D1 (and D2, to a lesser degree) but not in mu receptors occur after

repeated self-stimulation of the LH, but we cannot know whether these receptors are presynaptic and/or postsynaptic; therefore, as suggested by the reviewer, we cannot rule out the presence of other pre-synaptic adaptations in other systems.

11. P8L46-48: "relevant plastic changes".

We have MODIFIED the text to improve our expression of this idea, as follows (page 10, second paragraph):

*[...]After ICSS of the LH, administration of the opiate agonist ^{3}H-DAMGO showed no significant differences in the concentration of mu receptors between self-stimulated and control animals across a wide range of brain sections from the whole rostrocaudal axis; however, significant differences were observed after administration of the specific D1-receptor antagonist ^{3}H-SCH-23390 in the NAC shell, caudate-putamen, ventral pallidum, and medial globus pallidus (Simon et al., 2016) (See **Figure 3**).*

12. P9L11-L24. Conclusive paragraph of the section 3.

This paragraph has been MODIFIED and MOVED to section 4 (page 10 last paragraph). It now reads as follows:

Taken together, these data on the involvement of opioids in self-stimulation of the LH might be compatible with a dual action on dopamine-dependent and dopamine-independent mechanisms of reward (Wassum et al., 2009; Fields and Margolis, 2015; Ide et al., 2017) that cannot be completely blocked by the effect of antagonists in this region.

13. P10.L48. Relevance of the Robinson et al., 2014 study to this section.

We have now MOVED this study to section 3 under the heading 'reward induced by non-operant procedures' (page 7, paragraph 2), because its aim was to dissociate hedonic effects (choice test) from motivational effects (operant behavior).

In this experiment, the animals had to choose between two operant behaviors to obtain optostimulation+sucrose or sucrose. Given the failure to develop the operant behavior if not associated with a source of external reinforcement, it can be concluded that the effect of optostimulation of the CeA is a motivational facilitator of the behavior. According to the choice test, the hedonic value is greater for the first option than the second, which is attributable to the sucrose and not to the instrumental behavior, which is present in both cases.

14. P12-L13-25: Incomplete paragraph. Adaptations in DA and glutamate receptors in NAC in rats after food restriction, found by Carr et al.

We have now COMPLETED this paragraph as requested, with the following addition (page 11 and 12):

In this line, the manipulation of motivational mechanisms, such as chronic food restriction, has been found to activate opioid receptors in an opioid dopaminergic-dependent system, which in turn produces changes in dopaminergic D1 and glutamergic receptors of the NAC (Haberny & Carr, 2005; Ouyang et al., 2017). In fact, this effect can be blocked by the administration of both general (naltrexone) and selective agonists (for mu and kappa receptors) (Berman et al., 1994; Carr and Papadouka, 1994; Carr, 2002) and may correspond to the generation of adverse neuroadaptations and locomotor-activating effects in striatal dopaminergic neurons (Carr, 2011).

15. P12. L35: "plastic changes" related to the motivational component of reward.

Different types of stimulus (electric stimulation, natural reinforcers, drugs of abuse) appear to have the capacity to activate the dopaminergic mesolimbic system, but they may differ in the generation of more or less transient release patterns due to the activation of specific microcircuits of dopaminergic neurons related to motivational behavior, consistent with observations by authors such as Salamone or Carelli (Salamone & Correa, 2012; Cameron et al., 2014). This sentence HAS BEEN MODIFIED to improve our expression of this idea, as follows (page 12 first paragraph):

[*In summary, various studies have demonstrated that not only the electrical stimulation of certain brain regions such as the LH but also natural reinforcers (food) and drugs of abuse can share the capacity to induce increases in DA release in the NAC (Salamone, 1994; Sokolowski et al., 1998; Cameron et al., 2014). Their differential release pattern may be more or less transient according to the activation of microcircuits of dopaminergic neurons that appear to be related to the motivational or seeking component of reward (Spanagel et al., 1992; Olson et al., 2007; Cameron et al., 2014; Fields and Margolis, 2015).*

Hence, Carelli....]

16. P13L13-31. "different behaviours associated with natural and drug reward ar not surprising considering the very different sensory-motor properties involved in the intake of these rewards".

We agree with our reviewer about the highly different sensory-motor properties involved in the intake of natural stimuli and drugs.

In the experiments cited on the association of behaviors with morphine (Spiteri et al., 2000) or amphetamine (Vezina & Steward, 1987) and in our studies on the association of behaviors with electrical stimulation, behavioral analysis has shown an increase in self-centered behaviors typical of affective reactions (sniffing the floor or walls), whereas when associated with the availability of food, an increase is produced in the number of visits and therefore in approaching/searching behaviors (less related to affective reactions).

When drugs are used in CPP procedures, animals associate one localization with a physiological reaction to the drug injection and another with its absence. The learning is more complex when natural stimuli are used, because the animal associates one localization with the availability of the food and another with its absence, but the animal must consume the food to experience the physiological reaction; therefore an operant behavior is required, although it acts after the trials as an incentive through anticipation of the hedonic reaction.

17. P13. L34-46: Conclusion of the section 5 "involvement of opioids in rewarding homeostatic mechanisms".

In this section, which gathers evidence on the role of opioids in the LH, we have improved the expression of our conclusions, which now READ as follows (page 13):

In conclusion, studies on the role of opioids in homeostatic LH-related mechanisms indicate their possible relationship with activation of a dopamine-related system, possibly connected to goal-directed behaviors. However, as already noted, some authors have also observed the presence of opiate hedonic hotspots (NAC shell, ventral pallidum) embedded in this mesolimbic system, which may generate and/or increase affective reactions to rewarding taste or smell stimuli from food (Wassum et al., 2009; Peciña and Smith, 2010; Smith et al., 2011).

18. Section 6: "Involvement of the Vagal-Parabrachial system in rewarding processes".

Since the first report by our group on the rapid analysis of nutritional products that arrive in the gastrointestinal system (Puerto et al., 1976) based on information sent to the brain *via* the vagal pathway (Zafra et al., 2007), there has been considerable research into the functional characteristics of this pathway and the first relays at central level, leading us to investigate the lateral parabrachial nucleus (Mediavilla et al., 2005- for a review-).

These initial paragraphs are devoted to an explanation of these procedures (concurrent vs. sequential) that have been developed to study the functional properties of this system (also relevant to temporal aspects of the effect addressed on page 22) and the presence of different neurotransmitters that may support its participation in reward.

19. P21L28-44: "involvement of the LPBe in "implicit learning".

In flavor or spatial learning procedures, the flexibility of learning and the possibility of allowing delay are among the factors that have allowed us to differentiate between the involvement of explicit or implicit memory mechanisms (Mediavilla et al., 2005). Explicit memory is flexible, enabling animals to respond adequately in

situations that have been modified and guiding them to execute tasks in a different context from that in which the learning took place.

In the work of Garcia et al., 2014, animals were trained to associate a burette containing flavored water in a fixed position (left or right) with subsequent electrical stimulation of the LPBe and a different flavor in the opposite position with no stimulation. A choice test was conducted after two association trials, simultaneously presenting the two burettes with the flavor stimuli in the same position but with no application of stimulation. In a second choice test after a delay of 6 hours, the positions of the burettes were inverted. Given that the animals then preferred the stimulus associated with the initial position and not that associated with the flavor, it can be concluded that: 1) Proprioceptive cues were more important than flavor cues, in agreement with other studies (e.g., Simon et al., 2013); and 2) the animals based their choice on the proprioceptive cue (turn to the left or right), which was identical to that presented during the acquisition phase, indicating a rigid learning that does not admit delay.

20. P22.L31-48

We have rewritten this paragraph (page 23, paragraph 1) to clarify our description of the importance of spatial cues in the generation of tolerance and dependency and the possible participation of the LPBe in this process. It now reads as follows:

As already noted, our experimental groups learned to associate both places and flavors with electrical stimulation of the LPBe, but they showed a greater propensity for spatial cues. This result may be related to the important role for addicted individuals of the places in which the drugs are taken (Koob & Le Moal, 2000; Koob et al., 2014) and to the development of dependency and/or tolerance with repeated administrations (See, 2002). In this regard, a tolerance effect has been observed after repeated stimulation of the LPBe, especially when administered passively (not contingently) by the experimenter (Hurtado and Puerto, 2016; 2018) These findings are in agreement with the report by other authors that withdrawal reactions were precipitated by a peripherally acting opioid antagonist that generated activation throughout the visceral pathway, specifically in the PBle (Hamlin et al., 2001).

21. P27L5-9: absence of self-stimulation behavior in the LPBe

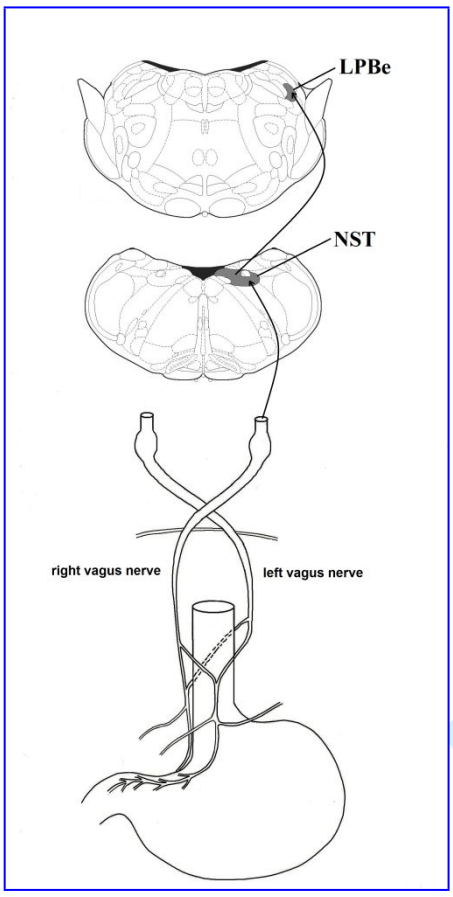
This issue is NOW ADDRESSED in the revised Discussion (page 21, paragraphs 1 and 2).

22. Figure 5: Erratum

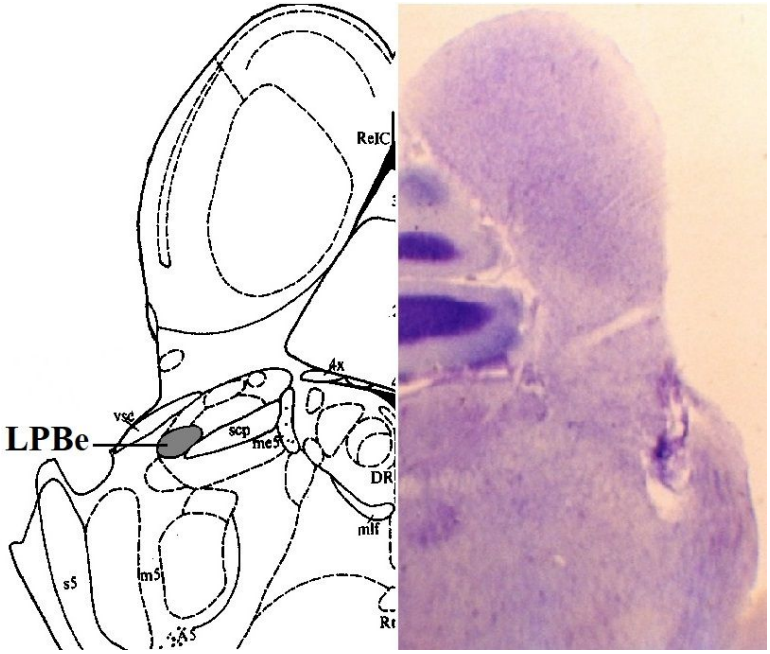
WE HAVE CORRECTED the error in figure 5

23. P16L5. Figure 6

Figure 6, which depicts the localization of the LPBe, was unfortunately missing from the original submission and has now been INCLUDED.



◀Figure 5



▲Figure 6

REVIEWER 2

We are very grateful to this reviewer for the comments and corrections, which have helped us to improve the quality of our article. Figure 6, which depicts the localization of the LPBe, was unfortunately missing from the original submission and has now been included. We have corrected typographic and other errors detected by the reviewer.

Page 4, L3-9: We have DELETED this sentence.

Page 4, L14-17: We have CLARIFIED this sentence (page 3 last paragraph):

Although ICSS can stimulate neurons containing different neurotransmitters (Stein & Wise, 1969; Yeomans et al., 1993; Ikemoto, 2010;Vlachou and Markou, 2011).

Page 4, L16: The study by Stein is now correctly cited (INTRODUCED IN THE TEXT, page 3, last paragraph.

Page 5: Table 1 and Figure 2:

We have modified Table 1 and footnotes as recommended by this reviewer:

TABLE 1:
D1 and D2 changes in dopamine receptors after self-stimulation of the Lateral Hypothalamus

Region		***D1 (t)***	***df***	***Sig. (bilat)***	***D2 (t)***	***Df***	***Sig. (bilat)***
Prefrontal Cortex (PFC)	**DEn**	0.698	14	0.497	**-2.101↓**	**14**	**0.05***
	AcbSh	**-2.409 ↓**	**6**	**0.05***	--	--	--
N. Accumbens (NAC)	**CPu 2**	**2.429 ↑**	**15**	**0.028***	1.649	14	0.121
	AcbSh	**2.047**	**13**	**0.061**	0.967	14	0.350
	VP	**4.309 ↑**	**11**	**0.001***	1.249	13	0.234
Bed Nu of the S.T.(BNST)	**CPu 2**	**3.622 ↑**	**5**	**0.015***	--	--	--
Hippocampus (HC)	**CPu**	**2.264 ↑**	**15**	**0.039***	1.520	15	0.149
	MGP	**2.403 ↑**	**11**	**0.035***	0.189	14	0.853

Table 1

C*omparison of brain ^{3}H-SCH-23390 (D1R antagonist) and ^{3}H-YM-09151-2 (D2R agonist) between ICSS experimental and control groups, using the Student's t-test for unrelated samples [t=value of t in the Student's test; df=degree of freedom; p=probability of t in a 2-way Student's t-test. Results are expressed as nCi].*

Abbreviations by region.

Prefrontal cortex (PFC, +3.20 mm. from bregma): PrL-IL: prelimbic-infralimbic cortex; Cg: cingulate cortex; M2: secondary motor cortex; M1: primary motor cortex;

AI: agranular insular cortex; LO: lateral orbital cortex; VO: ventral orbital cortex; DEn: dorsal endopiriform nucleus; AOP: anterior olfactory nucleus, posterior part; AcbSh: accumbens nucleus, shell.

Nucleus accumbens (NAC, +1.70 mm. from bregma): CPu1: caudate putamen, matrix; CPu2: striosomas of the caudate putamen; Cg: cingulate cortex; Motor Cx: motor cortex; AcbSh: accumbens nucleus, shell; AcbC: accumbens nucleus, core; LS: lateral septal nucleus; VP: ventral pallidum; CI: claustrum; DEn: dorsal endopiriform nucleus.

Bed nucleus of the stria terminalis (BNST, -0,30 mm. from bregma): CPu1: caudate putamen, matrix; CPu2: striosomas of the caudate putamen; VP: ventral pallidum, LS: lateral septal núcleus; Tu: olfactory tubercle.

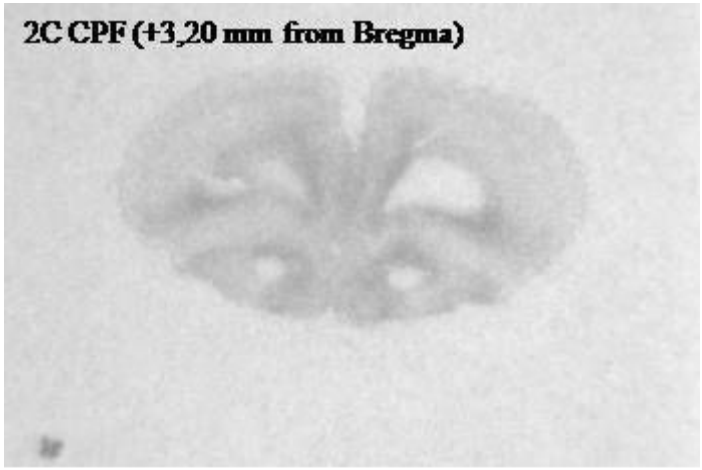
Hippocampus (HC, -2.80 mm. from bregma): CA1-3: fields of hippocampus; Hb: habenular nucleus; CPu: caudate putamen; BLA: basolateral amygdaloid nucleus, anterior part; PRh: perirhinal cortex; DEn: dorsal endopiriform nucleus MGP: medial globus pallidus.

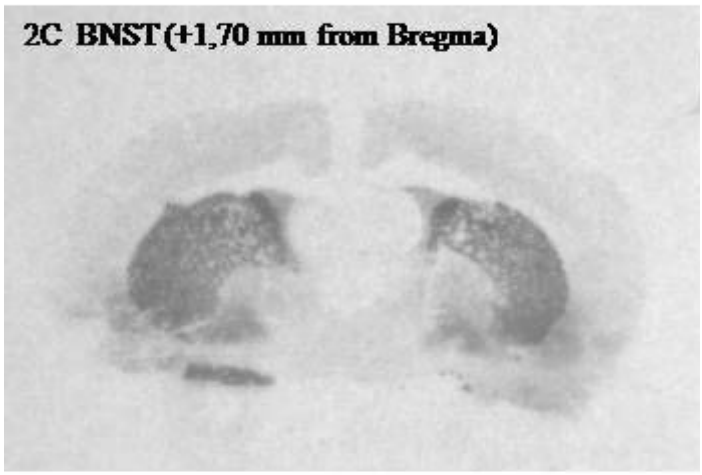
Ventral tegmental area (VTA, -4.80 mm. from bregma): PiRe: pineal recess; Hbc: habenular commissure; CA1 field of the hippocampus; DG: dentate gyrus; PRh: perirhinal cortex; DEn: Dorsal endopiriform nucleus; SNR: substantia nigra, reticular part; SNC: substantia nigra, compact part; VTA: ventral tegmental area; V2MM: secondary visual mediomedial cortex.

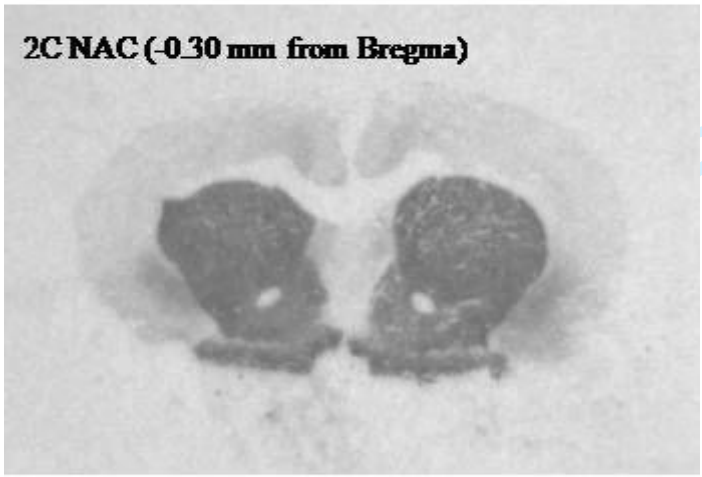
Central gray (CG, -5.80 mm. from bregma): SNR: substantia nigra, reticular part; SuG: superficial gray layer of the superior colliculus PRh: perirhinal cortex; DEn: Dorsal endopiriform nucleus.

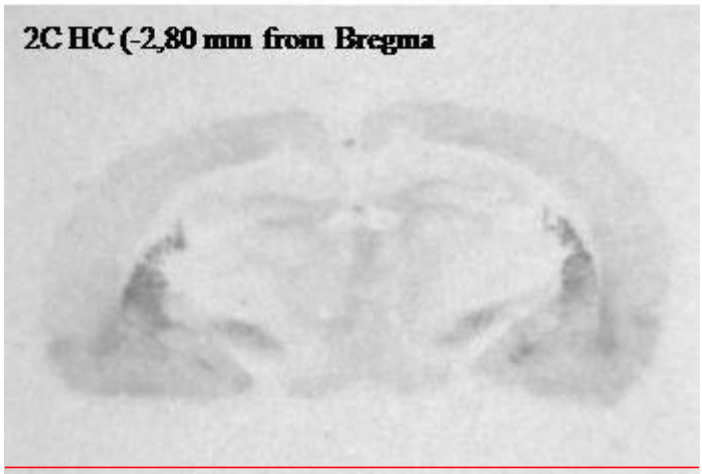
Figure 2:

It is difficult to detect differences among autoradiographs of different groups because the human eye has limited sensitivity to subtle differences in shades of gray, which is why we use automated quantification methods. However, we have included with this manuscript a set of 4 images from equivalent sections of an animal in the control group. We will accept the decision of the editorial team on the addition or not of this figure (as Figure 2B).


2C CPF (+3,20 mm from Bregma)


2C BNST (+1,70 mm from Bregma)


2C NAC (-0.30 mm from Bregma)

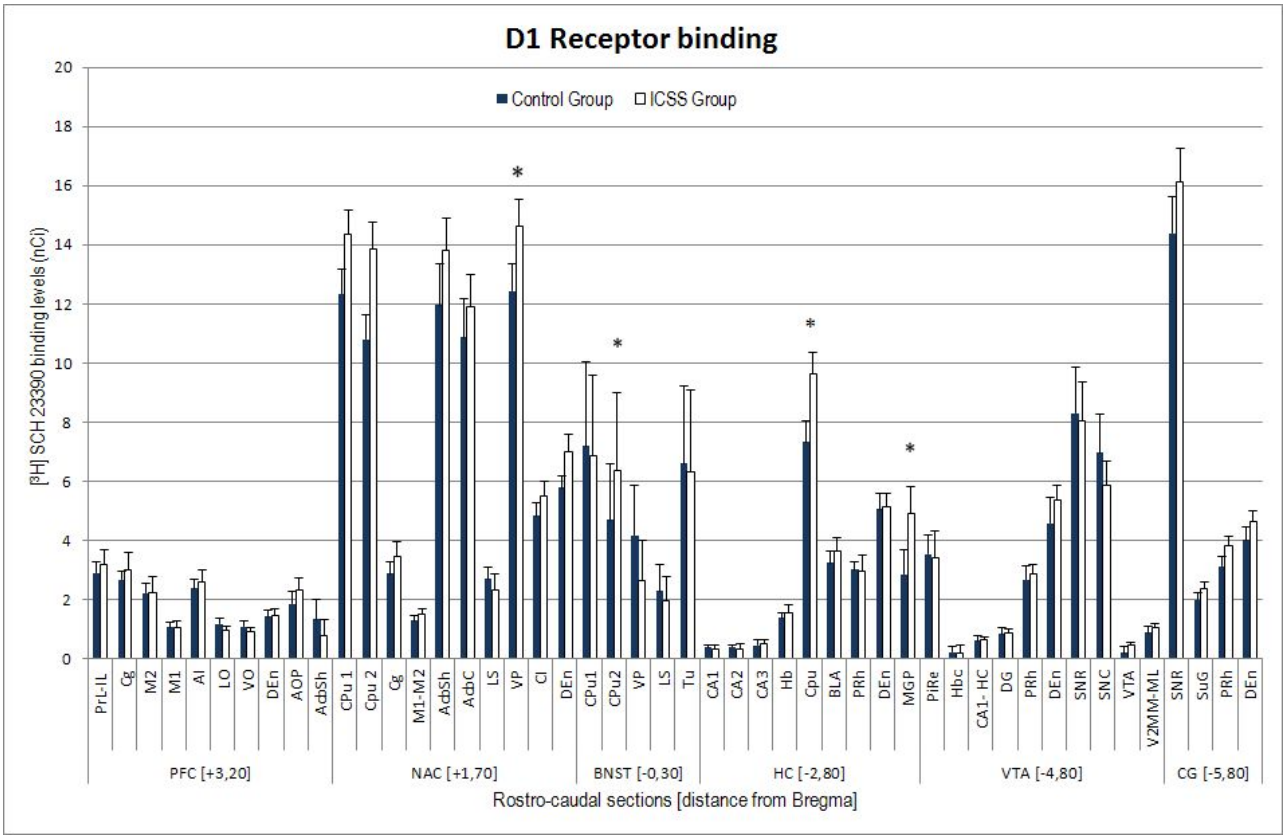

2C HC (-2,80 mm from Bregma

Page 6, L18-22: **Reference to D2 receptors**

This sentence HAS BEEN DELETED from the revised version, as recommended.

Page 8, L55

A figure on the effect of [^{3}H]SCH-23390 was not included because these data are already reported in Table 1. In the light of this comment, we have now added this figure (Figure 3B) in this revised version. We are content to leave the DECISION to include or exclude this figure to the EDITORIAL TEAM.



Page 9, L9: Reference.

WE HAVE AMENDED this error: the correct reference is *"Gross-Isserof et al., 1992" (page 10, second paragraph).*

Page 10, L48:

This change has been made (page 7 paragraph 2):

In a study involving CPP in combination with optogenetic stimulation of the CeA....

Page 12, L20 and L24:

WE HAVE CORRECTED this reference (*Berman et al., 1995 (page 12, first paragraph) and ADDED the study by* Carr (2011) in the list of references.

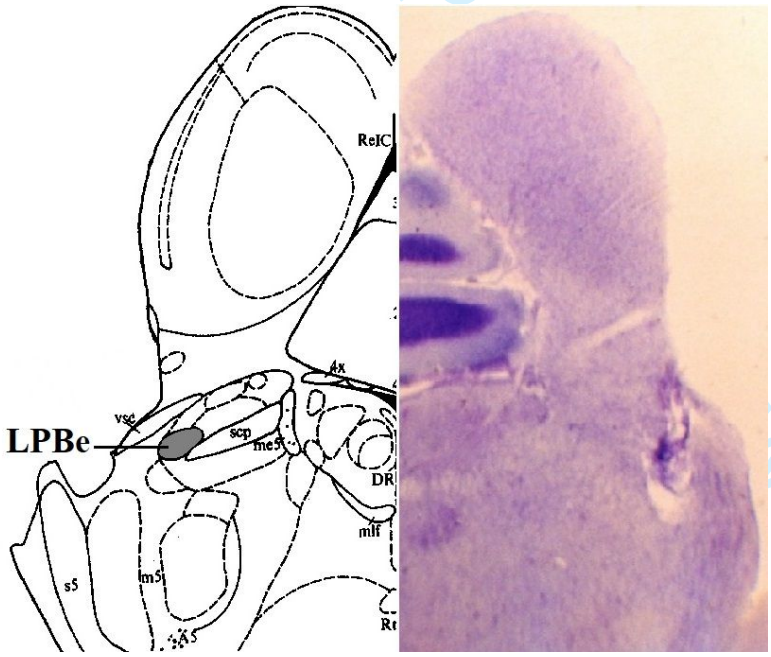
Carr KD (2011) Food scarcity, neuroadaptations, and the pathogenic potential of dieting in an unnatural ecology: Binge eating and drug abuse. Physiol Behav 104, 162-167.

Page 13, L46:

WE HAVE CORRECTED this citation: "*Peciña and Smith, 2010*" (Page 13, paragraph 2)

Page 16, L4:

Figure 6 was unfortunately missing from the original submission and has now been included.



Page 18, L16:

WE HAVE CORRECTED the year in this reference *(Carr et al., 1991) (Now on page 18, first paragraph).*

Page 22, L48:

WE HAVE CORRECTED THE citation of *Koob et al., 2014 (page 24, first paragraph)*.

Page 23, L55: Reference of Solomon and Corbitt.

As suggested, WE HAVE INCLUDED THE ORIGINAL REFERENCE for Solomon & Corbitt (Page 41).

Solomon RL, Corbitt JD (1974) An opponent-process theory of motivation. I. Temporal dynamics of affect. Psych Rev 81(2), 119-145.

Page 25, L9, L52.

WE HAVE CORRECTED this typographic error and added the reference to Salamone et al., 2018 (pag 38).

Salamone JD, Correa M, Yang JH, Rotolo R, Presby R (2018) Dopamine, Effort-Based Choice, and Behavioral Economics: Basic and Translational Research. Front Behav Neurosci 12, 52.

Page 26, L20:

The reference of Salamone et al. 2012 HAS BEEN CORRECTED (page 27, on the top). It now reads: *Salamone & Correa, 2012*.

Reference Jaeger and Van der Kooy:

This reference has been deleted.

REVIEWER 3:

We have restructured the first part of our review in accordance with the recommendations of this reviewer. The section on the dopaminergic mesolimbic system has been summarized and is followed by a section on the use of non-operant procedures to study reward components. This is then followed by sections on the role of opioids in ICSS and in homeostatic behaviors. The sections on the vagal-parabrachial axis are largely unchanged.

References:

Berman Y, Devi L, Carr KD (1995). Effects of chronic food restriction on prodynorphin-derived peptides in rat brain regions. *Brain Res* 664(1-2): 49-53.

Berridge KC (2018). Evolving concepts of emotion and motivation. *Front Psychol* 9, 1647.

Bindra, D. (1974). A motivational view of learning, performance and behavior modification. *Psychol Rev* 81(3): 199-213.

Cameron et al. 2014

Carlezon WA, Chartoff EH (2007). Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation. *Nat Protoc* 2: 2987-2995.

Carr KD (2002). Augmentation of drug reward by chronic food restriction: behavioral evidence and underlying mechanisms. *Physiol Behav* 76(3): 353-364.

Carr KD (2011). Food scarcity, neuroadaptations, and the pathogenic potential of dieting in an unnatural ecology: Binge eating and drug abuse. *Physiol Behav* 104, 162-167.

Carr KD, Papadouka V (1994). The role of multiple opioid receptors in the potentiation of reward by food restriction. *Brain Res* 639(2): 253-60.

Carr et al., 2001

Dayan P, Balleine BW (2002). Reward, motivation, and reinforcement learning. *Neuron* 36(2): 285-298.

Easterling KW, Holtzman SG (1997). Intracranial self-stimulation in rats: sensitization to an opioid antagonist following acute or chronic treatment with mu opioid agonists. *J Pharmacol Exp Ther* 281(1): 188-99.

Fields HL, Margolis EB (2015). Understanding opioid reward. *Trends Neurosci* 38(4): 217-225.

Garcia R, Simon MJ, Puerto A (2014). Rewarding effects of the electrical stimulation of the parabrachial complex: taste or place preference?. *Neurobiol Learn Mem* 107: 101-107.

Gross-Isseroff R, Cohen E, Shavit Y (1992),Comparison of mu opioid receptors in brains of rats bred for high or low rate of self-stimulation. *Physiol Behav* 51(5): 1093-1096.

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accumbens of ad libitum fed and food-restricted rats. *Mol Brain Res* 141: 121-127.

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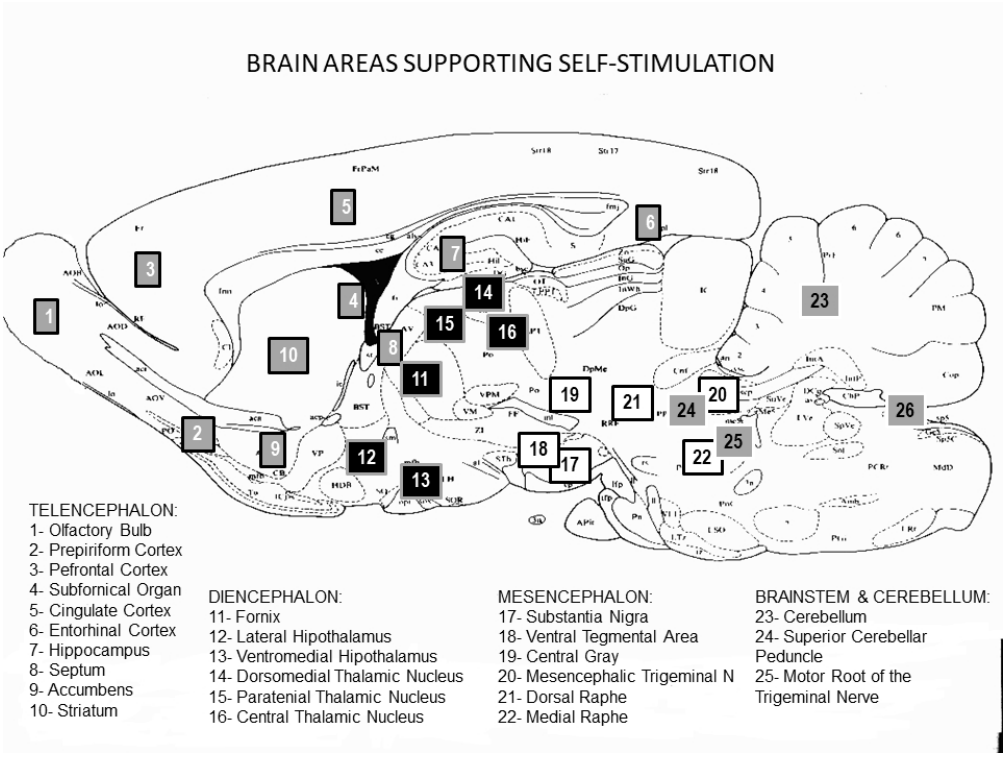

Figure 1:Sagittal section of rat brain depicting some areas known to support Intracraneal Self-Stimulation behavior [Adapted from Phillips and Fibiger, 1989].

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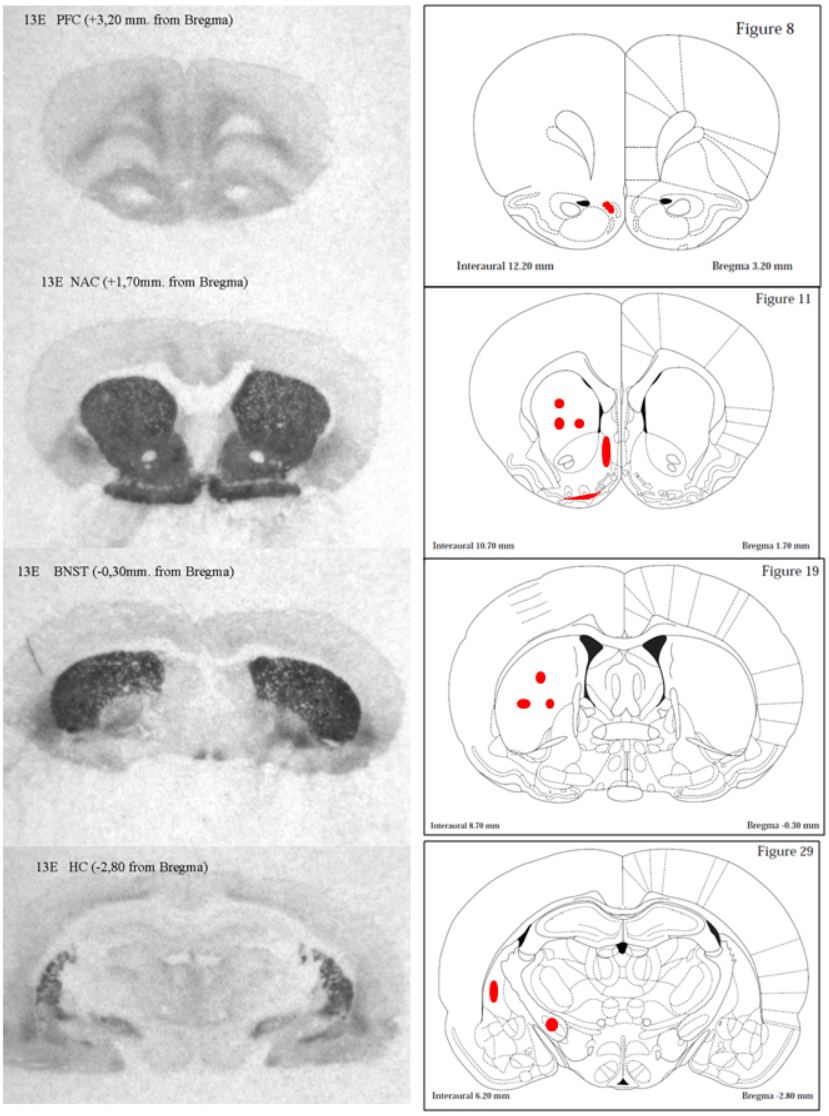


Figure 2:Quantitative Autoradiography of D1 receptors.
Left: Coronal sections showing significant changes in D1 receptor expression in an animal (13E) from the LH-ICSS group. Right: Schematic representation of areas with significant labeling, from the corresponding section of the atlas of Paxinos and Watson.

190x254mm (96 x 96 DPI)

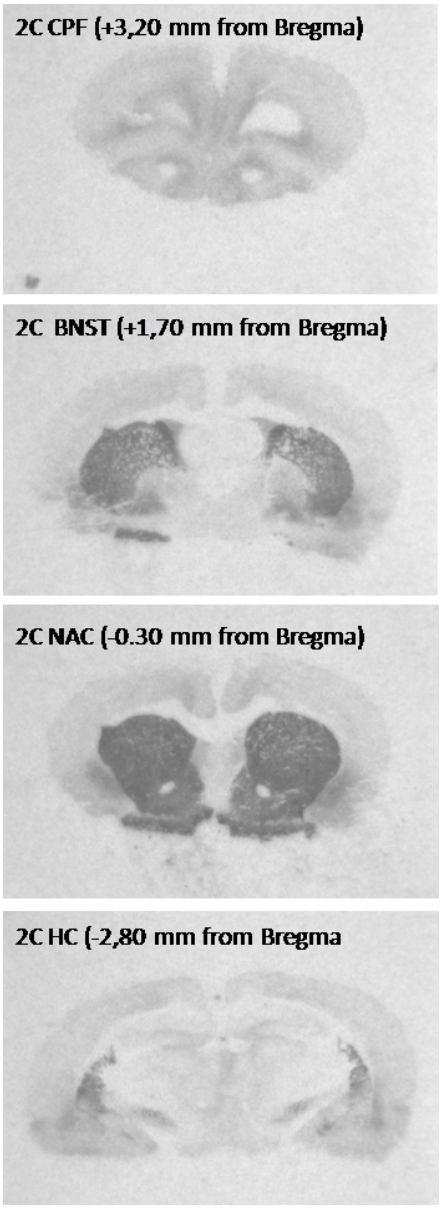


Figure 2B: Quantitative Autoradiography of D1 receptors showing D1 receptor expression in an animal (2C) from the Control Group.

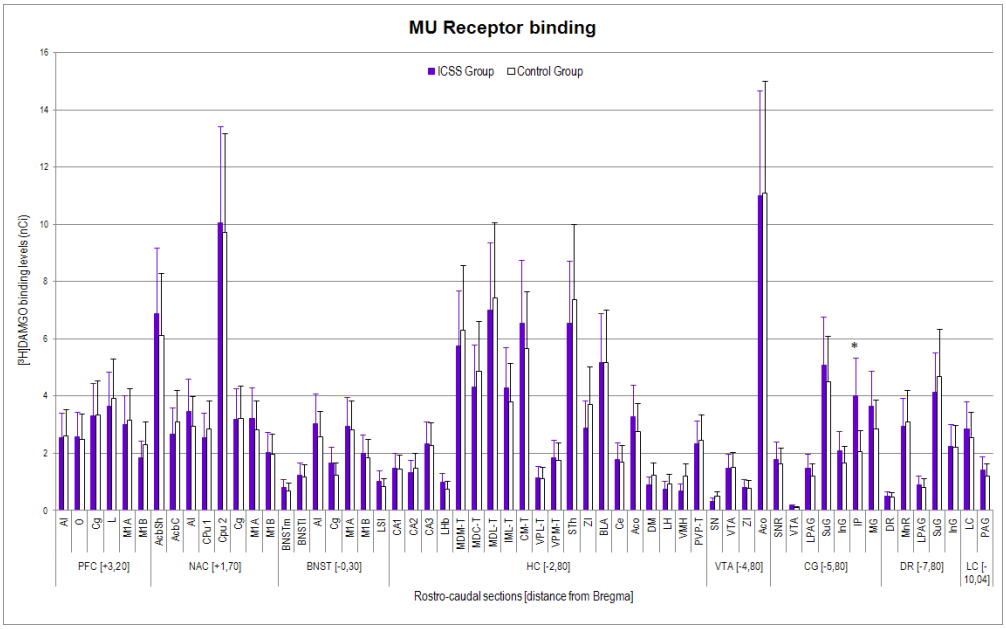
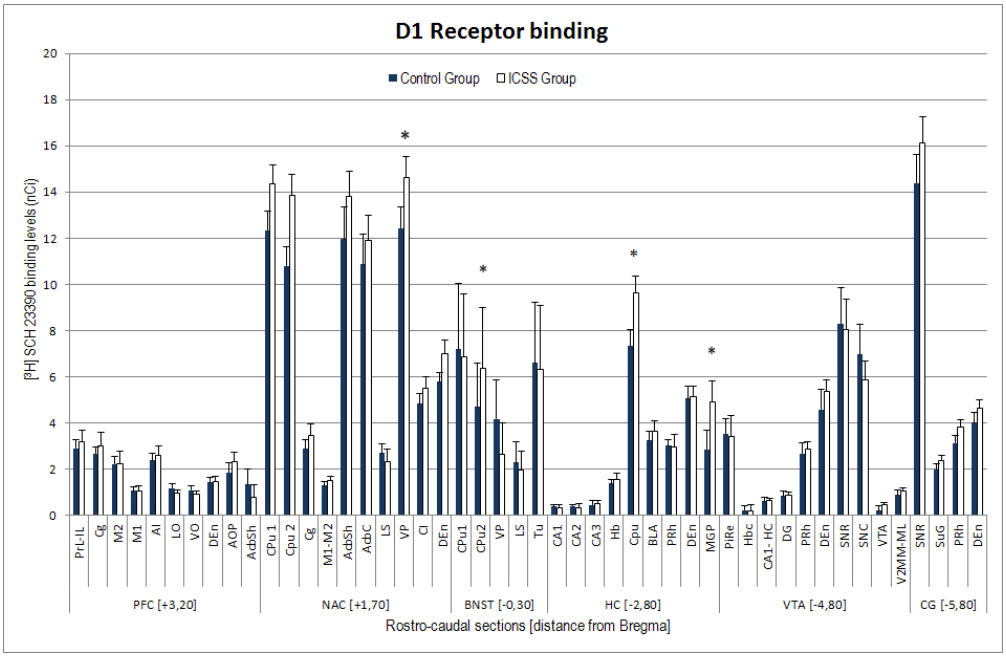


Figure 3: Specific3H-DAMGO mu-receptor binding in nine coronal rat brain sections in self-stimulated (n=9) and control (n=8) animals. Data were analyzed with a 2-tailed Student's t-test for unrelated samples and expressed as means ± SEM. LH-ICSS animals showed significantly higher Mu receptor binding in the IP nucleus alone (t=2.485 14df, p<0.026*).[Reprinted from: Neurobiology of Learning and Memory, 127. Simon et al. Changes in D1 but not D2 dopamine or mu-opioid receptor expression in limbic and motor structures after lateral hypothalamus electrical stimulation: A quantitative autoradiographic study, page 20 (©2016), with permission from Elsevier].

303x188mm (96 x 96 DPI)



Brain differences in 3H-SCH-23390 binding (D1R antagonist) between ICSS experimental and control groups, using the Student's t-test for unrelated samples [t=value of t in the Student's test; df=degree of freedom; p=probability of t in a 2-way Student's t-test. Results are expressed as nCi].

Examined sections (abbreviations):

1) Level of the prefrontal cortex (PFC, +3.20 mm. from bregma): PrL-IL: prelimbic-infralimbic cortex; Cg: cingulate cortex; M2: secondary motor cortex; M1: primary motor cortex; AI: agranular insular cortex; LO: lateral orbital cortex; VO: ventral orbital cortex; DEn: dorsal endopiriform nucleus; AOP: anterior olfactory nucleus, posterior part; AcbSh: accumbens nucleus, shell.

2) Level of the nucleus accumbens (NAC, +1.70 mm. from bregma): CPu1: caudate putamen, matrix; CPu2: striosomas of the caudate putamen; Cg: cingulate cortex; Motor Cx: motor cortex; AcbSh: accumbens nucleus, shell; AcbC: accumbens nucleus, core; LS: lateral septal nucleus; VP: ventral pallidum; CI: claustrum; DEn: dorsal endopiriform nucleus.

3) Level of the bed nucleus of the stria terminalis (BNST, -0,30 mm. from bregma): CPu1: caudate putamen, matrix; CPu2: striosomas of the caudate putamen; VP: ventral pallidum, LS: lateral septal núcleus; Tu: olfactory tubercle.

4) Level of the hippocampus (HC, -2.80 mm. from bregma): CA1-3: fields of hippocampus; Hb: habenular nucleus; CPu: caudate putamen; BLA: basolateral amygdaloid nucleus, anterior part; PRh: perirhinal cortex; DEn: dorsal endopiriform nucleus MGP: medial globus pallidus.

5) Level of the ventral tegmental area (VTA, -4.80 mm. from bregma): PiRe: pineal recess; Hbc: habenular commissure; CA1 field of the hippocampus; DG: dentate gyrus; PRh: perirhinal cortex; DEn: dorsal endopiriform nucleus; SNR: substantia nigra, reticular part; SNC: substantia nigra, compact part; VTA: ventral tegmental area; V2MM: secondary visual mediomedial cortex.

6) Level of the central gray (CG, -5.80 mm. from bregma): SNR: substantia nigra, reticular part; SuG: superficial gray layer of the superior colliculus PRh: perirhinal cortex; DEn: dorsal endopiriform nucleus.

252x163mm (96 x 96 DPI)

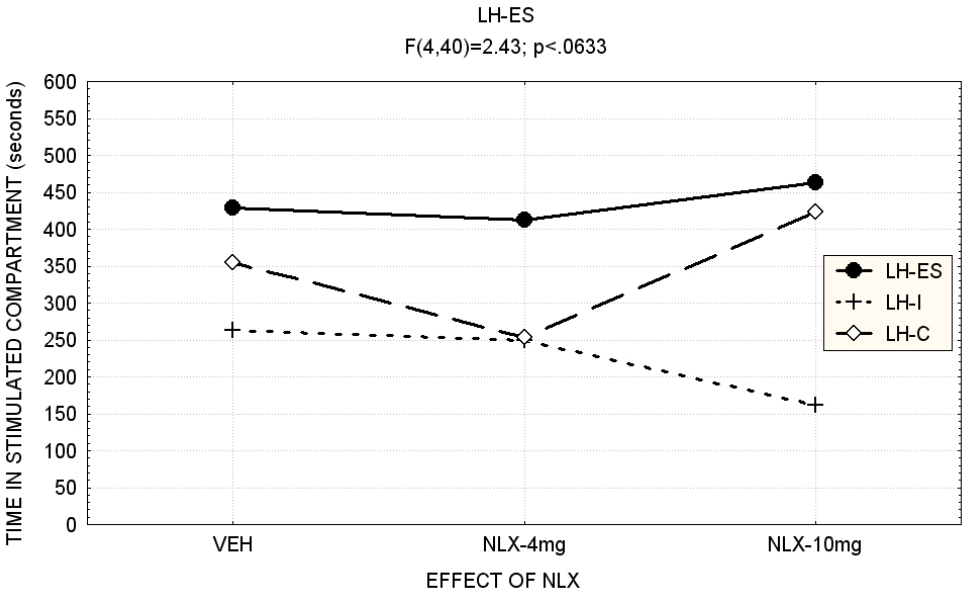


Figure 4: Electrical stimulation of the lateral hypothalamus in a concurrent CPP task and effect of the administration de 4.0 and 10.0 mg/Kg of the opiate antagonist naloxone. LH-ES: stimulated group; LH-I: implanted non-stimulated group; LH-C: intact control group[Reprinted from Behavioral Brain Research, 225. Simon et al., Concurrent stimulation-induced place preference in lateral hypothalamus and parabrachial complex: differential effects of naloxone, page 313 (© 2011), with permission from Elsevier].

167x102mm (150 x 150 DPI)

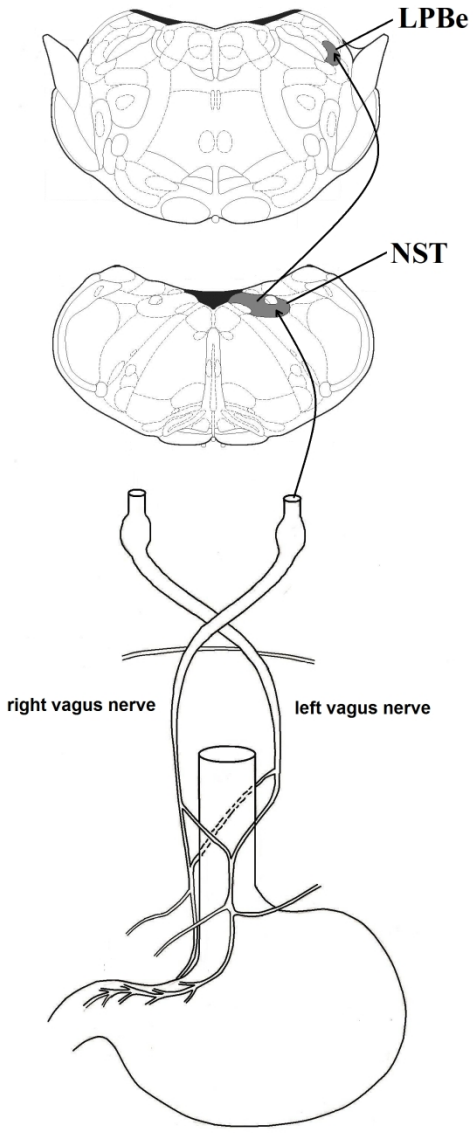


Figure 5: Schematic representation of gastrointestinal input to the brainstem via the vagal-parabrachial pathway.

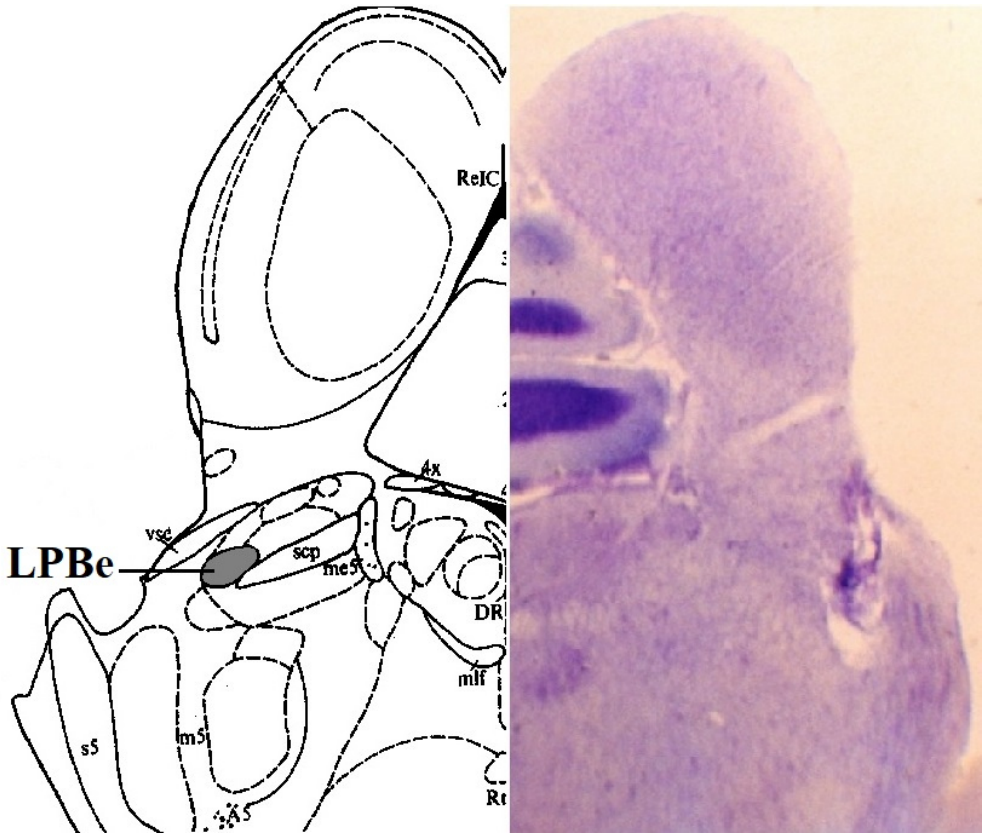


Figure 6: Histological localization of the electrode in LPBe-stimulated animals.

TABLE 1:
D1 and D2 changes in dopamine receptors after self-stimulation of the Lateral Hypothalamus

Region		*D1 (t)*	*df*	*Sig. (bilat)*	*D2 (t)*	*df*	*Sig. (bilat)*
Prefrontal Cortex (PFC)	**DEn**	0.698	14	0.497	**-2.101↓**	**14**	**0.05***
	AcbSh	**-2.409 ↓**	**6**	**0.05***	--	--	--
N. Accumbens (NAC)	**CPu 2**	**2.429 ↑**	**15**	**0.028***	1.649	14	0.121
	AcbSh	**2.047**	**13**	**0.061**	0.967	14	0.350
	VP	**4.309 ↑**	**11**	**0.001***	1.249	13	0.234
Bed Nu of the S.T.(BNST)	**CPu 2**	**3.622 ↑**	**5**	**0.015***	--	--	--
Hippocampus (HC)	**CPu**	**2.264 ↑**	**15**	**0.039***	1.520	15	0.149
	MGP	**2.403 ↑**	**11**	**0.035***	0.189	14	0.853