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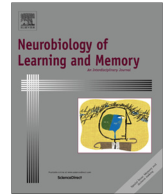

Changes in D1 but not D2 dopamine or mu-opioid receptor expression in limbic and motor structures after lateral hypothalamus electrical self-stimulation: A quantitative autoradiographic study

Maria J. Simon [a,*], A. Higuera-Matas [b], D. Roura-Martinez [b], M. Ucha-Tortuero [b], R. Santos-Toscano [b], C. Garcia-Lecumberri [b], E. Ambrosio [b], A. Puerto [a]

[a] Department of Psychobiology, University of Granada, Campus Cartuja s/n, 18071 Granada, Spain
[b] Department of Psychobiology, National Distance Education University (UNED), C/ Juan del Rosal 10, 28040 Madrid, Spain

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ABSTRACT

Intracranial self-stimulation (ICSS) of the lateral hypothalamus (LH) is involved in the activation of neuroanatomical systems that are also associated with the processing of natural and other artificial rewarding stimuli. Specific components of this behavior (hedonic impact, learning, and motor behavior) may involve changes in different neurotransmitters, such as dopamine and opioids. In this study, quantitative autoradiography was used to examine changes in mu-opioid and D1/D2-dopamine receptor expression in various anatomical regions related to the motor and mesolimbic reward systems after intracranial self-stimulation of the LH. Results of the behavioral procedure and subsequent radiochemical assays show selective changes in D1 but not D2 or mu receptors in Accumbens-Shell, Ventral Pallidum, Caudate–Putamen, and Medial Globus Pallidus. These findings are discussed in relation to the different psychobiological components of the appetitive motivational system, identifying some dissociation among them, particularly with respect to the involvement of the D1-dopamine subsystem (but not D2 or mu receptors) in goal-directed behaviors.

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1. Introduction

Electrical brain self-stimulation has been used as a powerful model for understanding appetitive motivated behaviors (De Haan, 2010; Olds & Milner, 1954; White & Milner, 1992). Some of its main anatomical reward systems pass through the medial forebrain bundle (MFB) and the lateral hypothalamus (LH), a site known to support robust self-stimulation behavior (Gallistel, Leon, Lim, Sim, & Waraczynski, 1996; Marchant, Millan, & McNally, 2012; Shizgal, 1989; Waraczynski, 2006; Wise, 1996; Wise & Rompré, 1989; Yeomans, 1990).

Studies of electrical self-stimulation in the LH have commonly focused on the mesolimbic system (Gallistel et al., 1996; Shizgal, 1989; Yeomans, Mathur, & Tampakeras, 1993) and the neurotransmitters related to this pathway, including dopamine and opioids (Sagara, Sendo, & Gomita, 2010; Salamone & Correa, 2012; Schaefer, 1988; Smith, Berridge, & Aldridge, 2011; Vlachou & Markou, 2011). The functions of these neural systems are likely related to the processing of natural stimuli essential for survival (e.g., food, drink, sex), allowing individuals to learn to detect and evaluate them and to generate responses aimed at achieving these goals (Berthoud & Münzberg, 2011; Mogenson, Jones, & Yim, 1980; Sagara et al., 2010; Waraczynski, 2006). However, these anatomical systems can also be artificially activated by intracranial electrical stimulation and probably by the action of various drugs of abuse (Berridge, 2012; Berthoud & Münzberg, 2011; Hyman, Malenka, & Nestler, 2006; Kelley & Berridge, 2002; Marchant et al., 2012; Sagara et al., 2010; Waraczynski, 2006).

It has been considered that the different components of appetitive motivation, i.e., hedonic impact, learning, and goal-directed behavior, may be processed by distinct anatomical systems with likely interactions and common elements. These systems may therefore involve different neurotransmitters, including dopamine and opioids, among others (Ikemoto & Panksepp, 1999; Salamone & Correa, 2012; Smith et al., 2011; Waraczynski, 2006).

Although the specific functions of dopamine remains controversial, it has been specifically associated with: reward prediction, a concept related to the codification of unexpectedly outcomes of a positive event (Garris et al., 1999; Minerowicz & Schultz, 1996; Schultz, 2010); reinforcing processes, understood as the attribution of incentive salience to a previously neutral

* Corresponding author. Fax: +34 958246186.
E-mail address: mjsimon@ugr.es (M.J. Simon).

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stimuli (Berridge & Robinson, 1998; Hyman et al., 2006); and/or goal-directed behaviors, an interface between motivation and action including a purposive behavior aimed at achieving a particular goal (Hernandez, Breton, Conover, & Shizgal, 2010; Horvitz, 2000; Ikemoto & Panksepp, 1999; Koch, Schmid, & Schnitzler, 2000; Mogenson et al., 1980; Phillips, Stuber, Heien, Wightman, & Carelli, 2003; Roitman, Stuber, Phillips, Wightman, & Carelli, 2004; Salamone, Cousins, & Snyder, 1997; Sokolowski, Conlan, & Salamone, 1998; Stuber, Roitman, Phillips, Carelli, & Wightman, 2005).

Hence, this neurotransmitter is considered to be involved in learning processes and sensorimotor integrations that facilitate flexible approach responses (Berridge, 2012; Hernandez et al., 2010; Salamone & Correa, 2012). Taken together, these results support the hypotheses of authors who have attributed to the dopaminergic transmission in the mesoaccumbens system a key role in the regulation or motivational modulation of seeking behaviors (Brown, McCutcheon, Cone, Ragozzino, & Roitman, 2011; Roitman et al., 2004; Stuber et al., 2005) or who have related it to downstream actions that enable these behaviors and, ultimately, make intracranial self-stimulation (ICSS) possible (Hernandez, Trujillo-Pisantry, Cossette, Conover, & Shizgal, 2012; Hernandez et al., 2006).

In general, it has been reported that opioids are involved in hedonic reactions of "pleasure" (Kelley & Berridge, 2002; Smith & Berridge, 2007; Smith et al., 2011; Wassum, Ostlund, Maidment, & Balleine, 2009). Authors using opioid antagonists (e.g., naloxone or naltrexone) suggested that these opioid systems may modulate ICSS behavior (Cazala, 1991; Easterling & Holtzman, 1997a; Easterling & Holtzman, 1997b; Easterling & Holtzman, 2004). However, in our laboratory, administration of the opioid antagonist naloxone blocked the rewarding effects induced by electrical stimulation of regions such as the nucleus parabrachial lateral external (LPBe) or insular cortex in a concurrent conditioned place preference task (Garcia, Simon, & Puerto, 2013; Simon, Garcia, Zafra, Molina, & Puerto, 2007) but not those induced by LH stimulation, even at high naloxone doses (Simon, Garcia, & Puerto, 2011). These later findings suggest that the rewarding effects observed after activation of this hypothalamic region involve neurochemical systems that may in some way differ from those identified in previous studies (Simon et al., 2011).

Some authors have examined the expression of D1 and D2 dopaminergic receptors in operant behaviors for feeding (Haberny & Carr, 2005; Narayanan, Land, Solder, Deisseroth, & DiLeone, 2012) and in the selection of instrumental responses related to reward (Koch et al., 2000); the expression of both DA and opioid receptors have also been examined in the self-administration of morphine and other drugs (Biscaia et al., 2008; Higuera-Matas et al., 2010; Le Marec, Marie-Claire, Noble, & Marie, 2011; Sanchez-Cardoso et al., 2007, 2009). There have also been mapping studies of brain areas in which metabolic activity was modified in response to unilateral rewarding self-stimulation of the MFB and dopaminergic antagonist administration (Gallistel, Gomita, Yadin, & Campbell, 1985), and which also showed the involvement of endogenous opioid activity in different rat strains (Gross-Isseroff, Cohen, & Shavit, 1992).

With this background, the objective of the present study was to use quantitative autoradiography to examine the importance of the opioid (centering on mu receptors) and dopaminergic (investigating D1 and D2 receptor expression) systems in LH-induced self-stimulation behavior, in which both are reportedly involved. The high spatial resolution offered by the quantitative autoradiography method allowed us to analyze the brain regions that could potentially participate in this appetitive motivated behavior, determining and comparing the relevance of opioid and dopaminergic neurotransmission systems.

2. Materials and methods

2.1. Subjects and surgery

The study used 31 male Wistar rats weighing 330–415 g from Harlan Interfauna Ibérica S.A. (Barcelona, Spain). Upon arrival at the laboratory, they were individually housed and maintained at a constant temperature (22 °C) under a 12-h/12-h light/dark cycle (lights on at 8:00 h), with free access to water and food (commercial rodent chow A04/A03; Panlab, Barcelona, Spain). Every attempt was made to minimize animal suffering. All experimental procedures complied with guidelines established by the European Union (86/609/EEC) and Spanish Law (1201/2005) and were approved by the Ethics Board of the National Distance Education University (UNED).

Surgery was performed under ketamine/diazepam anesthesia (ketamine: 40 mg/kg, 1 mL/kg, Pfizer; diazepam: 10 mg/kg, 1 mL/kg, Roche, intraperitoneally -i.p.- administrated) using a stereotactic instrument (Narisighe, SR5R, Japan). Twenty-three animals were chronically implanted in the LH with a 00 stainless steel monopolar electrode insulated except at the tip (coordinates: AP = +5.8; V = +2.8 and L = ±2.8 in the atlas of De Groot (1959)). Eight additional rats were implanted only with the reference electrode and served as a neurologically intact control group. As a prophylactic measure, oxytetracycline powder was added to the water (16 mg/mL; Pfizer) during the first post-surgery week.

2.2. Apparatus

Electrical stimulation was delivered by a CS220 two-channel stimulator connected to two ISU-165 isolation units (both from Cibertec, Madrid, Spain), monitoring the current on a BOAR oscilloscope (model 3502, Korea).

The self-stimulation procedure to test the rewarding effect of electrical LH stimulation was conducted in six operant chambers (Model E10-10RF, Coulbourn Instruments, Allentown, PA) with two levers mounted on the front wall and a green stimulus light located above the active lever. The active lever was connected to the stimulator and oscilloscope, while the inactive lever presses were recorded but had no programmed consequences. Metallic grids on the floor and lateral walls of the operant chamber boxes were covered with a thin wood panel to avoid short-circuits.

2.3. Behavioral procedure

After recovery, LH-implanted and neurologically intact animals were subjected to a 4-session autoshaping procedure to accelerate their learning of the operant lever-pressing behavior. Rats were deprived of food for 22 h and submitted to a fixed ratio 1 (FR1) daily schedule of food reinforcement in which a single press of the lever turned on a stimulus light above the lever that signaled pellet delivery (45 mg; Noyes Pellets, NH, USA).

After two days with free access to water and food, the animals underwent exploratory current intensity tests and standard shaping procedures to establish the optimal current parameters for LH self-stimulation behavior. Low currents (<150 μA) were initially applied and then raised, when necessary, until signs of interest (sniffing and approach) or aversion were observed. Rats showing signs of aversion were excluded from the experiment, while those showing approach behavior were trained to press the lever in a continuous schedule to elicit strong instrumental responses. The fixed stimulation parameters were 0.25-s trains of rectangular cathodic pulses at 0.1 ms and 66.6 Hz. The current ranged from 500 to 900 μA (Means = 755 μA). After seven days of shaping, animals showing a self-stimulation rate <2 presses/min were elimi-

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nated, and the remainder underwent a behavioral procedure over 14 consecutive days. Each animal was placed for 1 h in the operant chamber and was connected to the stimulator for self-stimulation behavior in a 1FR schedule. Control animals were put in the same operant chamber for a similar period of time, but lever presses were not followed by electrical stimulation. The total number of lever presses was recorded and stored in an IBM computer (Fig. 1).

2.4. Histochemical analysis

After sacrifice of the animals, their brains were removed, frozen in isopentane, cooled in dry ice, and stored until neurochemical assays were performed. Coronal brain sections (20 μm thickness) were obtained according to the Paxinos and Watson (2005) atlas and were then mounted on gelatin-coated slides and stored at −80 °C until they were assayed. Nine levels with 63 regions of interest were chosen for opioid receptor expression: prefrontal cortex (PFC, +3.20 mm from bregma), nucleus accumbens (NAC, +1.70 mm from bregma), bed nucleus of the stria terminalis (BNST, −0.30 mm from bregma), hippocampus (HC, −2.80 mm from bregma), ventral tegmental area (VTA, −4.80 mm from bregma), central grey area (CG, −5.80 mm from bregma), dorsal raphe (DR, −7.80 mm from bregma), parabrachial area (NPB, −8.80 mm from bregma), and locus coeruleus (LC, −10.04 mm from bregma). Six levels and a total of 31 regions were analyzed for D1 and D2 dopamine receptor expression: PFC (+3.20 mm from bregma), NAC (+1.70 mm from bregma), BNST (−0.30 mm from bregma), HC (−2.80 mm from bregma), VTA (−4.80 mm from bregma), and CG (−5.80 mm from bregma). The choice of these regions was based on the expression levels of D1 and D2 receptors reported in the literature.

2.5. Quantitative mu-opioid receptor autoradiography

The method described by Mansour, Khachaturian, Lewis, Akil, and Watson (1987) was followed. Briefly, mounted brain sections were pre-incubated in 100 mM Tris–HCl buffer (pH = 7.4) for 6 min, and then incubated at 25 °C with 3 nM ^{3}H-DAMGO (Amersham, Madrid, Spain) in 50 mM Tris–HCL buffer (pH 7.4) for 1 h. Incubation was either in the presence or absence of 10 nM unlabeled DAMGO (Sigma, Madrid, Spain) in order to determine the non-specific and total binding, respectively. Subsequently, slides were washed twice (2 × 6 min) in cold Tris–HCl buffer (50 mM, pH = 7.4), briefly rinsed in the same buffer, washed twice in distilled water, and dried under a stream of cool air.

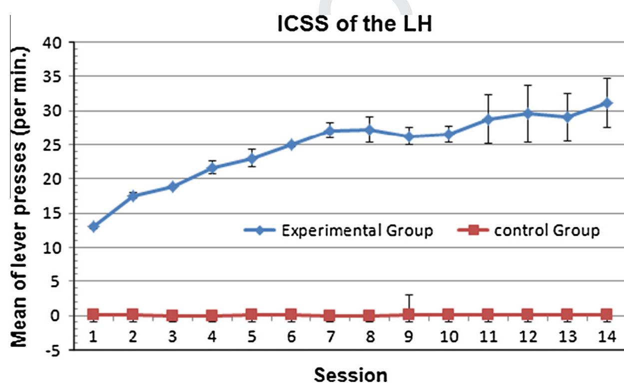

Fig. 1. Curve for ICSS rate in LH stimulated and control animals. The *X* axis shows daily progression and the *Y* axis shows the mean of lever presses per minute.

2.6. Quantitative D1-like and D2-like receptor autoradiography

Coronal brain sections adjacent to those used for mu-opioid receptor autoradiography were obtained at different levels. Duplicate tissue sections were incubated at room temperature with 1nM ^{3}H-SCH-23390 (D1R antagonist; 85 Ci/mmol) or 1 nM ^{3}H-YM-09151-2 (D2R agonist; 71.4 Ci/mmol: Perkin Elmer, Madrid, Spain) in 50 mM Tris–HCl buffer [pH 7.4] containing 120 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, and 4 mM $MgCl_2$ for 60 min. Non-specific binding was determined in the presence of 10 μM SCH-23390 (D1 antagonist) or 10 μM (+)Butaclamol (D2 antagonist: Sigma, Spain). Following these incubations, sections were quickly dipped into 50 mM Tris–HCl incubation buffer (0–4 °C) and then into distilled water. Finally, the sections were blown dry under cold dried air.

2.7. Exposure, development, and quantification of autoradiographs

Slices were apposed to tritium-sensitive film ([^{3}H]-Hyperfilm, Amersham Biosciences/GE Healthcare, Spain) in standard X-ray cassettes for 8–10 weeks at 4 °C. Films were developed for 5 min at 20 °C in Kodak D-19 developer, fixed for 10 min with Agfa fixer, and finally rinsed in water and air-dried. Autoradiographs were digitized and analyzed using the public domain NIH Image program (http://rsb.info.-nih.gov/nih-image), and tritium-labeled standards were used to calibrate the non-linear response of the film. Density measurements (calculated for each animal from 4 to 8 measurements in consecutive brain sections) were pooled and the values averaged. Specific binding was determined by subtracting non-specific binding (2–4 measurements per animal) from the total binding.

2.8. Statistical analysis

Autoradiographic mu, D1, and D2 data were analyzed with a two-tailed *t*-test. Square root transformations were applied when appropriate to correct skewness in data distribution and lack of homogeneity of variances. Statistical significance was set at $\alpha = 0.05$, and SPSS 15.0 (IBM plc, Chicago, IL) was used for the statistical analysis.

3. Results

Three animals implanted with intracerebral monopolar electrodes died after surgery and eight did not meet the behavioral criterion for self-stimulation after shaping and were therefore excluded from the experimental procedure. During the experimental phase, the electrode became detached in two animals, and one animal died after the 8th experimental session. Finally, the experimental procedure was completed by nine self-stimulated animals and eight neurologically intact rats. Fig. 1 shows the mean pressings/min by the animals during the behavioral procedure. It can be seen that the rate of pressings/min increased over the 14 days of the procedure, suggesting a gradual improvement in instrumental learning by the animals.

3.1. Mu-opioid receptors

Fig. 2 depicts the autoradiographic results for mu receptors. The highest mu-receptor binding levels were observed in the nucleus accumbens shell (AcbSh), striosomas of the caudate putamen (CPu-s), central medial thalamic nucleus (CM-T), and basolateral amygdaloid nucleus (BL); however, results *versus* controls only reached significance (2-tailed test) for the interpeduncular nucleus (IP) ($t = 2.485$ 14*df*, $p < 0.026^{*}$). The number of mu opioid receptors from the mediodorsal thalamic nucleus, medial part (MDM-T),

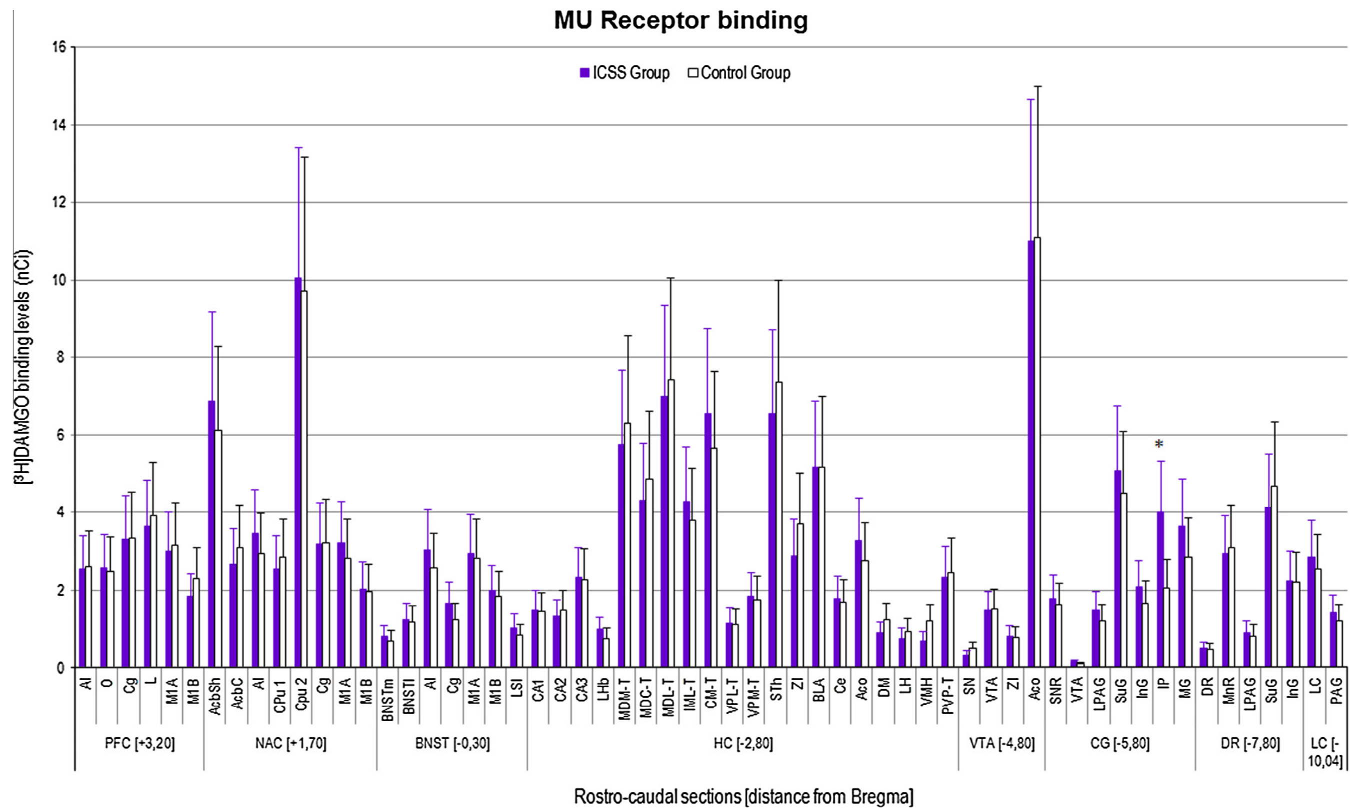

Fig. 2. Specific ^{3}H-DAMGO mu-receptor binding in nine coronal sections of the rat brain in self-stimulated ($n = 9$) and control ($n = 8$) animals. Data were analyzed by means of a two-tailed Student's t-test for unrelated samples and expressed as means ± SEM. LH-ICSS animals showed significantly higher Mu receptor binding in the IP nucleus alone ($t = 2.485$ 14df, $p < 0.026$*). 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 periacueductal 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; LPBE: lateral parabrachial nucleus, external part; DCIC: dorsal cortex of the inferior colliculus; DTgN: Dorsal Tegmental Nucleus; LC: locus coeruleus.

mediodorsal thalamic nucleus, central part (MDC-T), ventral posteromedial thalamic nucleus (VPM-T), zona incerta (ZI), and caudal part of the anterior cortical amygdaloid nucleus (ACo) was lower in the self-stimulated animals than in the control group, although the differences were not significant.

3.2. D1R and D2R dopamine levels

In the self-stimulated animals, a significant decrease in the number of D1 receptors (*vs.* controls) was observed in the most rostral part of the AcbSh ($t = -2.409$, 6df, $p < 0.05$), whereas there was a non-significant trend toward an increase in the most caudal part ($t = 2.047$, 13df, $p < 0.06$). Significant differences were also observed in rostral [+1.70 mm from bregma] ($t = 2.429$ 15df, $p < 0.028$*), intermediate [−0.30 mm from bregma] ($t = 3.622$, 5GL, $p > 0.015$*), and caudal [−2.80 mm. from bregma] ($t = 2.264$, 15df, $p < 0.039$*) sections of the caudate–putamen complex. We highlight the change observed in the ventral pallidum (VP) region ($t = 4.309$ 11df, $p < 0.001$*) and, most notably, in the medial globus pallidus (MGP) nucleus in caudal sections ($t = 2.403$, 11df, $p < 0.035$*) (see Tables 1 and 2).

Fig. 3 depicts microphotographs of rostrocaudal sections from an animal in the LH-ICSS group, corresponding to the regions showing the greatest differences in labeling with respect to the equivalent sections from the control group animals.

Finally, no differences in D2 receptors were found in any studied region except for the dorsal endopiriform nucleus ($t = -2.101$ 14df, $p < 0.05$*). Tables 1 and 2 summarize the main changes observed in D1 and D2 dopamine receptors, respectively, after 15 days of LH-ICSS (see Fig. 4).

4. Discussion

The results of this experiment support the involvement of the dopaminergic system, specifically the D1 receptor subtype, in rewarding self-stimulation of the LH. In contrast, despite the highly heterogeneous distribution of mu-opioid binding in the rat brain, no significant differences in mu opioid receptors were found between self-stimulated and control animals.

This study also demonstrates that ICSS of the LH selectively activates certain dopaminergic brain regions, including the nucleus accumbens shell, ventral pallidum, caudate–putamen, and medial globus pallidus. These results are in line with previous reports on the participation of these regions in eliciting dopamine-related goal-directed behaviors (Berridge, 2012; Kelley & Berridge, 2002;

Table 1
Distribution of D1 dopamine receptors in LH self-stimulated and control rats.

Region	LH-SS Mean ±	SEM	Control Mean ±	SEM	*t*	*df*	*p* (bilat)
Prefrontal Cortex (PFC)							
PrL-IL	3.5648	0.3442[8]	2.9068	0.3687[8]	1.305	14	0.213
Cg	3.3559	0.5249[8]	2.6680	0.2923[8]	1.145	14	0.271
M2	2.5225	0.5203[8]	2.2080	0.3233[8]	0.513	14	0.616
M1	1.1703	0.2031[8]	1.0529	0.1639[8]	0.450	14	0.660
AI	2.8939	0.3543[8]	2.3759	0.2996[8]	1.116	14	0.283
LO	1.0546	0.1370[8]	1.0316	0.1310[7]	0.120	13	0.906
VO	1.0128	0.1282[8]	1.0743	0.1947[8]	−0.264	14	0.796
DEn	1.6170	0.1867[8]	**1.4329**	0.1865[8]	0.698	14	0.497
AOP	2.6169	0.3428[8]	2.1846	0.2605[7]	0.981	13	0.345
AcbSh	**1.6330**	**0.5501[4]**	3.0500	**0.2079[4]**	−**2.409**↓	**6**	**0.05***
N. Accumbens (NAC)							
CPu 1	14.3401	0.8263[9] 12,3381	12.3381	0.8554[8]	1.681	15	0.114
CPu 2	**13.8446**	**0.9076[9]**	10.8055	**0.8462[8]**	**2.429**↑	**15**	**0.028***
Cg	3.4542	0.5078[9]	2.8744	0.3848[8]	0.892	15	0.387
Motor Cx	1.6453	0.1518[8]	1.2816	0.1908[8]	1.491	14	0.158
AcbSh	**15.3044**	**0.6619[7]**	11.9556	**1.4108[8]**	**2.047**	**13**	**0.061**
AcbC	11.8858	1.1195[9]	10.8705	1.2931[8]	0.597	15	0.560
LS	2.0509	0.4051[8]	2.3250	0.2488[7]	−0.566	13	0.588
VP	**15.4002**	**0.5334[6]**	11.7014	**0.6482[7]**	**4.309**↑	**11**	**0.001***
CI	5.5199	0.4770[9]	4.8214	0.4572[8]	1.050	15	0.310
DEn	6.9818	0.5884[9]	5.7784	0.3935[8]	1.655	15	0.119
Bed Nucleus of the S.T.							
CPu 1	15.4548	0.6920[4]	14.3648	1.9308[4]	0.531	6	0.614
CPu 2	**15.7757**	**0.3927[3]**	9.3680	**1.4693[4]**	**3.622**↑	**5**	**0.015***
VP	6.0032	2.0809[4]	8.3490	1.1718[4]	−0.982	6	0.364
LS	4.4262	0.5125[4]	4.5817	0.5412[4]	−0.209	6	0.842
Tu	14.1955	2.8757[4]	13.1795	1.9859[4]	0.291	6	0.781
Hippocampus (HC)							
CA1	0.3157	0.0741[7]	0.3850	0.0804[8]	−0.627	13	0.542
CA2	0.4489	0.1307[8]	0.4065	0.0639[8]	0.291	14	0.775
CA3	0.5061	0.1021[7]	0.5860	0.1091[7]	−0.534	12	0.603
Hb	1.3512	0.2263[8]	1.3721	0.1908[8]	−0.071	14	0.945
CPu	**9.6223**	**0.7178[9]**	7.3274	**0.7094[8]**	**2.264**↑	**15**	**0.039***
BLA	3.6578	0.4419[9]	3.2495	0.3734[8]	0.696	15	0.497
PRh	3.3864	0.4064[9]	3.2199	0.2125[7]	0.347	13	0.734
DEn	5.1263	0.4804[9]	5.0561	0.5386[8]	0.098	15	0.924
MGP	**5.6228**	**0.6344[8]**	3.5752	**0.3194[5]**	**2.403**↑	**11**	**0.035***
Ventral Tegmental Area (VTA)							
PiRe	5.0768	0.5367[6]	4.0284	0.5251[7]	1.390	11	0.192
Hbc	0.8012	0.2337[5]	0.5158	0.1768[5]	0.974	8	0.359
CA1	0.6170	0.1024[9]	0.7003	0.1351[7]	−0.501	14	0.624
DG	0.8784	0.1306[9]	0.9379	0.1993[7]	−0.259	14	0.799
PRh	2.8596	0.3096[9]	3.0530	0.3411[7]	−0.418	14	0.682
DEn	5.3478	0.5073[9]	5.2283	0.6411[7]	0.148	14	0.884
SNR	8.0253	1.3169[9]	9.4864	1.1697[7]	−0.803	14	0.435
SNC	5.8686	0.8237[9]	7.9521	0.9926[7]	−1.629	14	0.126
VTA	0.4750	0.0855[9]	0.5170	0.1080[5]	−0.299	12	0.770
V2MM	1.0343	0.1694[9]	1.0137	0.1657[7]	0.085	14	0.933
Central Grey (CG)							
SNR	16.1446	1.1206[9]	14.3919	1.2454[8]	1.049	15	0.311
SuG	2.3823	0.2030[9]	1.9960	0.2427[8]	1.230	15	0.238
PRh	3.8061	0.3127[9]	3.1169	0.3300[8]	1.515	15	0.150
DEn	4.6502	0.3611[9]	4.0002	0.4431[8]	1.147	15	0.269

This table shows means ± SEM of specific ^{3}H-SCH-23390 (D1R antagonist) binding in brains (number in brackets) in LH-SS experimental and control groups, as determined by quantitative autoradiography. [*t* – value of *t* in a Student's *t*-test for unrelated samples, *df* – degree of freedom; *p* – probability of *t* in a two-way Student's *t*-test. Results are expressed as nCi].
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 raphe, NPB: Parabrachial area, LC: locus coeruleus.
Specific nuclei and subnuclei: 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; CPu1: caudate putamen, matrix; CPu2: striosomas of the caudate putamen; AcbSh: accumbens nucleus, shell; AcbC: accumbens nucleus, core; LS: lateral septal nucleus; VP: ventral pallidum; CI: claustrum; Tu: olfactory tubercle; CA1-3: fields of hippocampus; Hb: habenular nucleus; BLA: basolateral amygdaloid nucleus, anterior part; PRh: perirhinal cortex; MGP: medial globus pallidus; PiRe: pineal recess; hbc: habenular commissure; DG: dentate gyrus; SNR: substantia nigra, reticular part; SNC: substantia nigra, compact part; VTA: ventral tegmental area; V2MM: secondary visual mediomedial cortex; SuG: superficial gray layer of the superior colliculus.

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Sagara et al., 2010; Smith et al., 2011; Stuber, Britt, & Bonci, 2012; Waraczynski, 2006).

Indeed, some studies on the functional organization of the limbic reward circuit suggest that distinct neural circuits could be activated during goal-directed behaviors (Carelli & Wightman, 2004), as in the case of the ventral striatopallidal circuit, which carries information from the nucleus accumbens to the ventral pallidum (Kalivas & Nakamura, 1999; Mingote et al., 2008; Panagis

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Table 2
Distribution of D2 dopamine receptors in LH self-stimulated rats.

Region	LH-SS Mean ±	SEM	Control Mean ±	SEM	*t*	*df*	*p* (bilat)
Prefrontal Cx. (PFC)							
PrL-IL	2.2737	0.1694[9]	2.1499	0.1944[8]	0.482	15	0.637
Cg	2.2096	0.1255[9]	2.4725	0.2675[8]	−0.924	15	0.370
M2	2.0999	0.1272[9]	2.0575	0.1143[8]	0.245	15	0.810
M1	1.4280	0.0642[8]	1.4000	0.1843[8]	0.143	14	0.888
AI	1.8893	0.1361[9]	1.9106	0.1930[8]	−0.092	15	0.928
LO	1.7918	0.2172[9]	1.7786	0.1567[8]	0.048	15	0.962
VO	1.6366	0.1080[8]	1.5637	0.1172[7]	0.458	13	0.655
DEn	**0.8527**	**0.0623[8]**	**1.0946**	**0.0965[8]**	**-2.10-2.10002.1012.101↓**	**14**	**0.05***
AOP	1.9750	0.2133[9]	1.8214	0.1874[8]	0.535	15	0.601
AcbSh	–	–	–	–	–	–	–
N. Accumbens (NAC)							
CPu 1	9.9370	0.7519[9]	8.7708	0.7647[8]	1.085	15	0.295
CPu 2	8.9455	0.4534[8]	7.8201	0.5099[8]	1.649	14	0.121
Cg	2.5077	0.5974[9]	2.1204	0.5163[8]	0.484	15	0.635
Motor Cx	–	–	–	–	–	–	–
AcbSh	6.5078	0.8528[9]	5.4521	0.5640[7]	0.967	14	0.350
AcbC	6.6466	0.7747[9]	6.4134	0.8207[8]	0.207	15	0.839
LS	–	–	–	–	–	–	–
VP	7.1226	0.4353[7]	5.9148	0.0.8173[8]]	1.249	13	0.234
CI	–	–	–	–	–	–	–
DEn	–	–	–	–	–	–	–
Bed N. of the S.T.							
CPu 1	–	–	–	–	–	–	–
CPu 2	–	–	–	–	–	–	–
VP	–	–	–	–	–	–	–
LS	–	–	–	–	–	–	–
Tu					–	–	–
Hippocampus (HC)							
CA1	2.6104	0.4745[9]	1.8608	0.4681[8]	1.120	15	0.280
CA2	2.7966	0.4283[9]	1.8479	0.4448[8]	1.534	15	0.146
CA3	2.1642	0.2895[9]	1.5065	0.3763[8]	1.403	15	0.181
Hb	–	–	–	–	–	–	–
CPu	9.9950	0.7566[9]	8.2873	0.8338[8]	1.520	15	0.149
BLA	–	–	–	–	–	–	–
PRh	–	–	–	–	–	–	–
DEn	–	–	–	–	–	–	–
MGP	0.4371	0.0754[9]	0.4094	0.1362[7]	0.189	14	0.853
V. Tegm. Area (VTA)							
PiRe	–	–	–	–	–	–	–
Hbc	–	–	–	–	–	–	–
CA1	2.6740	0.3197[9]	1.9309	0.5285[8]	1.235	15	0.236
DG	3.3972	0.4927[9]	2.8520	0.9450[8]	0.529	15	0.605
PRh	–	–	–	–	–	–	–
DEn	–	–	–	–	–	–	–
SNR	0.7257	0.1503[9]	1.9309	0.5285[8]	1.692	15	0.111
SNC	–	–	–	–	–	–	–
VTA	1.4832	0.2387[6]	2.8043	1.0219[4]	−1.534	8	0.164
V2MM	–	–	–	–	–	–	–
Central Grey (CG)							
SNR	2.2396	0.1495[9]	1.9005	0.3154[8]	1.009	15	0.329
SuG	4.0121	0.3735[9]	3.0521	0.5695[8]	1.441	15	0.170
PRh	–	–	–	–	–	–	–
DEn	2.9711	0.3098[7]	2.2840	0.5745[6]	1.097	11	0.296

This table shows means ± SEM of specific ^{3}H-YM-09151-2 (D2R agonist) binding in brains (number in brackets) in LH-SS experimental and control groups, as determined by quantitative autoradiography. [*t* – value of *t* in a Student's *t*-test for unrelated samples, *df* – degree of freedom; *p* – probability of *t* in a two-way Student's *t*-test. Results are expressed as nCi].

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: 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; CPu1: caudate putamen, matrix; CPu2: striosomas of the caudate putamen; AcbSh: accumbens nucleus, shell; AcbC: accumbens nucleus, core; LS: lateral septal nucleus; VP: ventral pallidum; CI: claustrum; Tu: olfactory tubercle; CA1-3: fields of hippocampus; Hb: habenular nucleus; BLA: basolateral amygdaloid nucleus, anterior part; PRh: perirhinal cortex; MGP: medial globus pallidus; PiRe: pineal recess; hbc: habenular commissure; DG: dentate gyrus; SNR: substantia nigra, reticular part; SNC: substantia nigra, compact part; VTA: ventral tegmental area; V2MM: secondary visual mediomedial cortex; SuG: superficial gray layer of the superior colliculus.

357 et al., 1997; Smith et al., 2011; Stuber et al., 2012; Waraczynski & 358 Demco, 2006). Recent investigations in the nucleus accumbens 359 suggest that neuroadaptations of medium spiny interneurons in 360 the ventral pallidum, which express D1/D2 receptors, do not influence basal locomotor activity but may have activating/inhibiting 361 effects on locomotion in animals receiving repeated cocaine injec- 362 tions (Stuber et al., 2012; Unterwald, Rubenfeld, & Kreek, 1994). 363 These results suggest that NAC-VP connections may be involved 364

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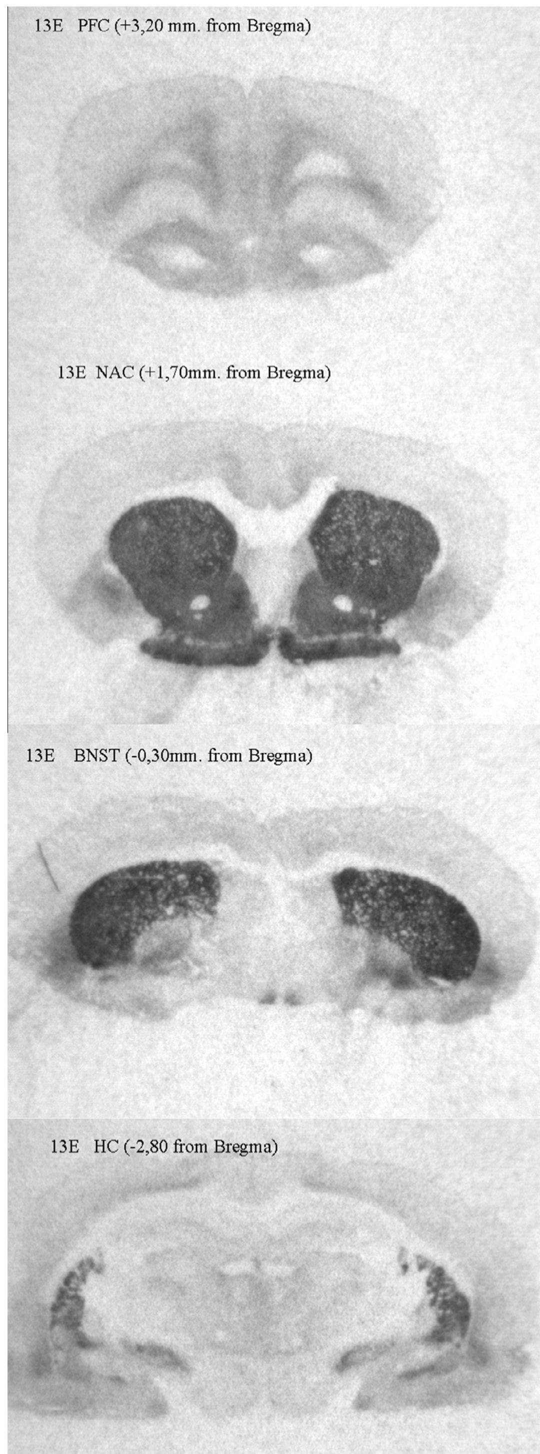

Fig. 3. Coronal sections showing significant changes in D1 receptor expression in an animal (13E) from the LH-ICSS group. Abbreviations: *Sections:* PFC: prefrontal cortex, NAC: nucleus accumbens, BNST: bed nucleus of the stria terminalis, HC: hippocampus, VTA: ventral tegmental area, CG: central grey area, DR: dorsal rafe, NPB: Parabrachial area, LC: locus coeruleus. *Specific nuclei and subnuclei:* 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; CPu1: caudate putamen, matrix; CPu2: striosomas of the caudate putamen; AcbSh: accumbens nucleus, shell; AcbC: accumbens nucleus, core; LS: lateral septal nucleus; VP: ventral pallidum; CI: claustrum; Tu: olfactory tubercle; CA1-3: fields of hippocampus; Hb: habenular nucleus; BLA: basolateral amygdaloid nucleus, anterior part; PRh: perirhinal cortex; MGP: medial globus pallidus; PiRe: pineal recess; hbc: habenular commissure; DG: dentate gyrus; SNR: substantia nigra, reticular part; SNC: substantia nigra, compact part; VTA: ventral tegmental area; V2MM: secondary visual medio-medial cortex; SuG: superficial gray layer of the superior colliculus.

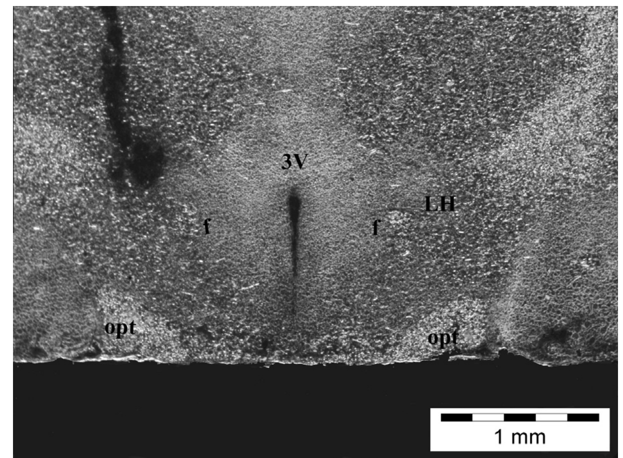

Fig. 4. Representative image of the localization of the electrode tip in ICSS-LH implanted animals (3V: third ventricle; f: fornix; opt: optic tract; LH: lateral hipothalamus).

in linking reward to responding rather than to detecting or computing reward value (Leung & Balleine, 2013; Waraczynski & Demco, 2006).

Mingote et al. reported changes in adenosine A_{2A} receptors of neurons that connect the Nucleus Accumbens with the Ventral Pallidum in a high effort-related choice task (Mingote et al., 2008). However, the retrograde labeling of fibers from the VP terminated in the "core" region of the NAC in their study, whereas it was produced in the shell region of the NAC in the present study of ICSS of the LH, a behavior also characterized by its vigor and persistence.

Finally, The role of the ventral pallidum as a link between expected reward and the regulation of subsequent motor activity has also been suggested by results obtained in other species, e.g., primates (Tachibana & Hikosaka, 2012) and even in humans (Fitzgerald, Schwartenbeck, & Dolan, 2014).

Various authors have shown long-lasting increases in NAC dopamine that remain stable during the self-stimulation period (Hernandez et al., 2006, 2010, 2012), suggesting a stimulation-induced neural signal responsible for maintaining performance. They conducted a three-dimensional analysis of intracranial electric self-stimulation behavioral parameters and the effect on these of drugs of abuse such as cocaine, and they suggested that changes observed in the dopaminergic system may be attributable to a trans-synaptic activation of dopaminergic neurons in the NAC (Hernandez et al., 2010; Yeomans et al., 1993). Thus, taking into account the experimental procedures employed in the present study, our results may possibly be explained by a change in tonic dopamine that might regulate goal selection over long time periods, e.g., by energizing or depressing decision-making under appetitive or aversive/stressing conditions (Hernandez et al., 2010, 2012; Leung & Balleine, 2013; Mannella, Gurney, & Baldassarre, 2013; Tachibana & Hikosaka, 2012).

Several studies have also described dopaminergic receptors of the NAC as being involved in the selection of neuronal ensembles that encode specific goal-directed behaviors (Carr, Cabeza de Vaca, Sun, & Chau, 2009; Nicola, Surmeier, & Malenka, 2000; Ranaldi & Beninger, 1994; Shen, Frajolet, Greengard, & Surmeier, 2008). Thus, recent studies using fast scan cyclic voltammetry demonstrated that, in the case of ICSS, dynamic changes in dopamine release in the NAC shell can take place over multiple time scales, e.g., a transient release that rapidly declines and, at the same time, a transient increase prior to operant behavior (during learning acquisition by animals), which persists during the maintenance-delay phase (Owesson-White, Cheer, Beyene,

Carelli, & Wightman, 2008). Thus, Carelli's group observed a transient but significant increase in NAC dopamine levels before operant behavior that resulted in the receipt of food or the self-administration of cocaine (Brown et al., 2011; Carelli & Wightman, 2004; Roitman et al., 2004; Stuber et al., 2005).

The present findings on D1 dopaminergic involvement in various anatomical structures related to the mesocorticolimbic pathway are consistent with previous reports that self-stimulation of the VTA is facilitated by administering D1-receptor agonists (but not D2 agonists) into the NAC (Ranaldi & Beninger, 1994). Likewise, the direct injection of SKF-82958 (D1 agonist) into the medial AcbSh reduced the threshold for ICSS of the LH, more markedly in food-restricted animals, and increased the locomotor activity of animals (Carr et al., 2009). These results suggest that the action of dopamine on medium spiny cell D1-receptors may enhance the activity of cells that receive highly convergent excitatory inputs while decreasing both the background activity and the activity of cells receiving less temporally coherent inputs (Nicola et al., 2000).

We found changes in D1 dopaminergic receptors in the caudate–putamen, and some authors have proposed that this system might facilitate the response selection process (Balleine, Delgado, & Hikosaka, 2007) and that its injury impairs habit formation (Yin, Knowlton, & Balleine, 2004). Thus, both unpredicted reward and rewarding predictive cues evoked phasic dopamine in the dorsal striatum in rats trained in a discriminative stimulus paradigm that predicted the appearance of a lever-press to obtain food, possibly due to the recruitment of additional dopaminergic neurons during the acquisition of greater experience of the task (Brown et al., 2011).

Some studies have identified fibers from the nucleus accumbens that connect with the internal segment of the MGP (Mogenson et al., 1980), the main skeletomotor output region of the basal ganglia, which has shown autoradiographic changes in our study and may modulate the vigor of performance according to motivational factors or contribute to motor learning (Da Cunha et al., 2009; Turner & Desmurget, 2010; Wickens, 2008).

With regard to D2 receptors, different authors have reported changes in their density after intermittent morphine administration and have described tolerance and sensitization after a chronic administration regime that induced changes in D2, D1, and Mu receptors (Le Marec et al., 2011). Animals can self-administer a mixture of D1–D2 agonists but not D1 or D2 agonists separately (Ikemoto, Glazier, Murphy, & McBride, 1997).

The present results, obtained by electrical stimulation of the LH, appear to differ in part from the rewarding effects reported by some fellow researchers using self-administration techniques with drugs of abuse such as cocaine or cannabinoids (Higuera-Matas et al., 2010; Sanchez-Cardoso et al., 2007, 2009). This discrepancy may be attributable to subtle differences between reward-seeking behaviors generated by electrical self-stimulation (the present case) and those produced by the self-administration of drugs, as described by Cameron, Wightman, and Carelli (2014), Carelli (2002), Carelli, Ijames, and Crumling (2000). Electrophysiology and voltammetry studies by Carelli's group have repeatedly shown small differences in the pattern and dynamics of rapid dopamine release between goal-directed behaviors for cocaine *versus* food (natural reward) (Cameron et al., 2014; Carelli, 2002; Carelli et al., 2000), which may involve particular interactions with distinct types of post-synaptic receptor and the induction of specific patterns of synaptic plasticity (Jung & Shim, 2011; Kravitz, Tye, & Kreitzer, 2012; Surmeier, Ding, Day, Wang, & Shen, 2007). Further research is required to explore these issues.

In addition, various studies have behaviorally, anatomically, and neurochemically dissociated between pleasant effects and the activation of dopamine-related or goal-directed behaviors (Berridge, 2012; Kelley & Berridge, 2002; Sagara et al., 2010; Smith et al., 2011; Stuber et al., 2012; Waraczynski, 2006). Thus, the activation of dopaminergic receptors in our study is compatible with reports of a selective behavioral activation by dopamine (Sagara et al., 2010; Salamone, Correa, Farrar, & Mingote, 2007). In fact, Cannon & Palmiter found a deficit in goal-directed behaviors in genetically manipulated animals unable to synthesize dopamine, but they still demonstrated preferences for rewarding stimuli such as sucrose or saccharin (Cannon & Palmiter, 2003). Likewise, studies from our laboratory evidenced that administration of the opiate antagonist naloxone blocks reward but does not prevent subjects from selecting the compartment in which they had previously learned to receive rewarding electrical stimulation of the parabrachial-insular axis (Garcia et al., 2013; Simon et al., 2007, 2011).

We found a weak labeling of mu opioid receptors, which was strongest in the NAC and caudate–putamen, in line with findings by Gross-Isseroff et al. (1992), although the comparison with controls was significant in their study but not in ours. Other authors observed no significant changes in [^{3}H]-DAMGO binding of the ventral tegmental area after intermittent morphine administration (Le Marec et al., 2011), whereas it significantly decreased after chronic administration, a reinforcing effect that may theoretically be compatible with LH stimulation.

In contrast, other studies found that the administration of mu and delta opioid agonists lowered the threshold for brain stimulation reward of the ventral tegmental area, which was explained as an increase in the sensitivity of animals to rewarding brain stimulation (Duvauchelle, Fleming, & Kornetsky, 1996). Bielajew et al. also reported that naloxone specifically blocked the rewarding electrical stimulation of the VTA, which is considered an essential part of the mesoaccumbens system (Bielajew, Diotte, & Miliaressis, 2003).

However, in agreement with our results for mu receptors, previous experiments in our laboratory showed that naloxone, even at high doses, did not block the CPP induced by electrical stimulation of the LH (Simon et al., 2011). Likewise, other authors observed that, although activation of mu opioid receptors in the nucleus accumbens or ventral pallidum generated "wanting" for food reward and hedonic pleasure or their "liking" (Smith & Berridge, 2007; Smith et al., 2011), food intake was not inhibited by the administration of naloxone in the VP (Smith & Berridge, 2007; Smith et al., 2011).

In summary, the results of this study on the biological bases of ICSS of the LH evidence a significant involvement of the dopaminergic activity of D1 receptor but not of D2 or mu opioid receptors. These findings suggest that the neural activation induced by rewarding electrical self-stimulation of the LH is selectively reflected in certain anatomical structures related to the mesolimbic dopaminergic system. These include the CPU and MGP and especially the AcbSh and VP, which are involved in the selection of neuronal ensembles that encode specific goal-directed behaviors (Surmeier et al., 2007). Finally, the present finding of a lack of significant changes in mu receptors may explain why naloxone is unable to block the rewarding effect of LH stimulation (Simon et al., 2011) and contributes neurochemical evidence of dissociations among different components or subsystems of the brain reward system.

5. Uncited reference

Wise (2004).

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818

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