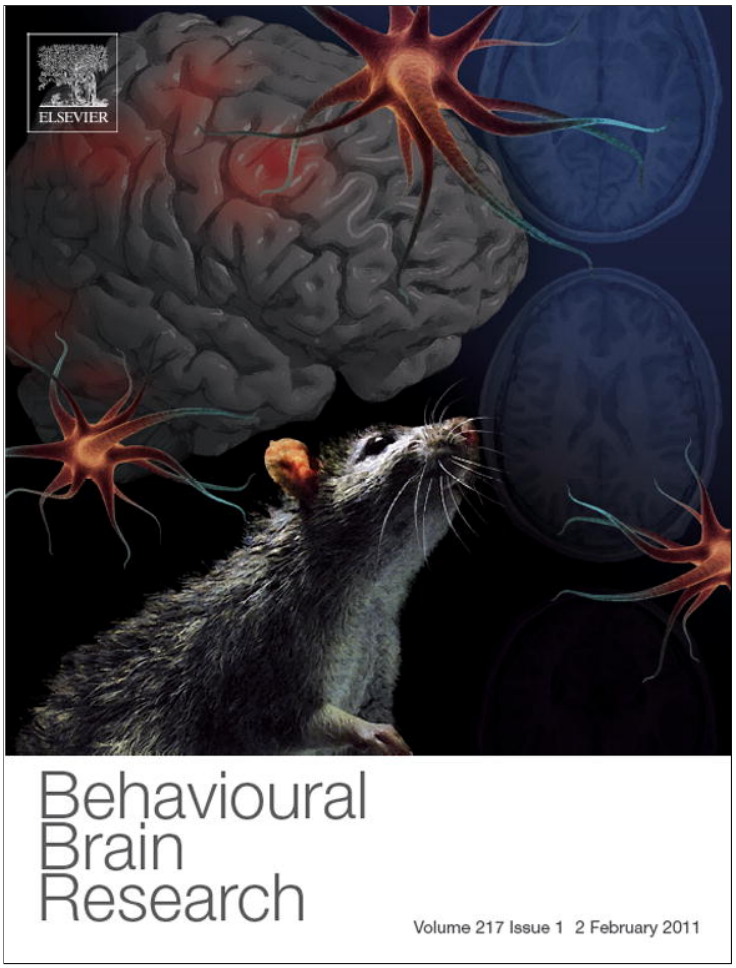

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Research report

Individual differences in schedule-induced polydipsia: Neuroanatomical dopamine divergences

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ABSTRACT

Autoradiography analysis of D1 and D2 dopamine receptors and c-Fos activity were performed in brain of rats classified as low drinkers (LD) and high drinkers (HD) according to schedule-induced polydipsia (SIP) performance. Previous studies have shown that groups selected according to their rate of drinking in SIP differ in behavioral response to dopaminergic drugs. This study reports differences between LD and HD rats in dopamine D1 and D2 receptor binding through different mesocorticolimbic brain areas. LD and HD rats showed opposite patterns of binding in dopamine D1 and D2 receptors in the nucleus accumbens, medial prefrontal cortex, amygdala, ventral tegmental area and substantia nigra. Whereas LD rats showed higher binding than HD rats for D1 receptors, HD rats showed higher binding than LD rats for D2 receptors (except in substantia nigra that were roughly similar). These neuroanatomical differences in dopamine receptor binding were also associated with an elevated c-Fos count in the medial prefrontal cortex of HD rats. In tandem with previous evidence, our results suggest a different dopaminergic function between LD and HD, and points to SIP as a behavioral model for distinguishing populations possibly vulnerable to dopaminergic function disorders.

1. Introduction

Adjunctive behavior occurs as complementary behavior in situations where strongly motivated appetitive behavior is involved [9]. The best studied example of adjunctive behavior is schedule-induced polydipsia (SIP) in laboratory rats, whereby hungry, though not thirsty, animals exposed to intermittent presentations of food consume large amounts of water during inter-food interval periods [8].

The 'excessiveness' and 'persistence' of drinking behavior evinced in the SIP task has led some authors to suggest that SIP might be a suitable behavioral model to study the development of alcohol consumption and vulnerability to other drugs of abuse [13,32,38,51]. SIP has also been proposed as a model of anxiety-related disorders, such as obsessive–compulsive behavior, in order to study the potential therapeutic role of pharmacological treatments [15,36,40,42,53,54].

Previous studies have pointed to the implication of dopaminergic pathways as the neurochemical basis of SIP development [18,29,37]. Lesion studies using 6-OHDA on main dopaminergic brain structures, such as substantia nigra, significantly reduced SIP, whereas electrical stimulation of lateral hypothalamus increased SIP acquisition [30]. Stimulants such as amphetamines generally produce, at low to moderate doses, either no effect or decreases in established adjunctive drinking [10,41,52]. Furthermore, doses of D-amphetamine which failed to alter the overall response rate nevertheless increased the proportion of licks that occurred at the beginning of inter-food intervals [11,34,41].

Selective dopaminergic compounds have also been tested on adjunctive drinking. In general, both dopamine agonists and antagonists reduce schedule-induced polydipsia in a dose-dependent manner [6,7,19,29,44,48]. For example, both SKF38393 (a selective agonist of dopamine D1-like receptors) and quinpirole (a selective agonist of dopamine D2-like receptors) produced reductions in adjunctive drinking [29]. Similarly, SCH23390 (a selective antagonist of dopamine D1-like receptors) and haloperidol (an antagonist of dopamine D2-like receptors) reduced the volume of water consumed [48]. Since dopamine agonists and antagonists both decrease schedule-induced polydipsia, one possibility is that changes in adjunctive drinking caused by administration of dopaminergic compounds might be the result of changes in the balance of activation of dopamine D1-like and D2-like receptors away from some optimum level, rather than just changes in overall levels of dopaminergic neurotransmission [29].

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SIP acquisition displays important individual differences, with a considerable variability in the amount of water consumed among animals [4,5,16,24,25,27,28,31,35,47]. These individual differences in SIP development have been related to behavioral and neurochemical differences linked to the dopaminergic system. SIP acquisition is associated with faster learning of an active avoidance task and less freezing in a defeat test compared to rats that do not develop SIP [5]. SIP acquisition also correlates with higher locomotor responses to novelty and development of amphetamine self-administration in rats [35]. Animals with a high locomotor response to novelty also show faster acquisition of SIP versus low-response rats [16]. Moreover, rats classified according to SIP acquisition in high drinkers (HD) and low drinkers (LD), show differences to pharmacological challenge with dopaminergic drugs. Whereas SIP in HD rats was reduced with 0.5 mg/kg D-amphetamine and 10 mg/kg cocaine, SIP in LD rats was only reduced with the much higher dose of 2 mg/kg D-amphetamine [24]. These data furnish additional evidence of the individual differences in behavior that occur in the SIP paradigm, and suggest that the dopaminergic system may underlie such individual differences in drinking.

Taken together, all these findings are consistent with the suggestion that the dopaminergic system is involved in individual differences or behavioral predisposition to exhibit SIP. Accordingly, the main aim of the studies reported here was to explore and characterize the possible neuroanatomical differences between LD and HD polydipsic rats in brain areas of dopamine reward circuitry [17]. To this end, we used autoradiography to measure the density and tissue distribution of the main dopamine receptor subtypes (D1 and D2) in different mesocorticolimbic brain structures of LD and HD rats. Using immunochemistry, we also evaluated possible changes in c-Fos protein in three dopamine brain reward areas, namely, the medial prefrontal cortex, ventral tegmental area and nucleus accumbens. This study could help characterize the neuroanatomical basis of individual differences in SIP and contribute to a better understanding of SIP as a potential model of the above-mentioned psychopathologies, which could discriminate between populations with different risks of vulnerability.

2. Materials and methods

2.1. Animals

Thirty-two experimentally naïve male Wistar rats were used. They were obtained from Charles River (Lyon, France), and were approximately 70 days old and had a mean free-feeding weight of 485 g (range: 475–505 g). One of the rats was withdrawn from the experiment due to health problems. Rats were individually housed in a room with controlled environmental conditions (21 °C ambient temperature, 60% relative humidity, and 08.00 h/20.00 h light/dark cycle). All animals were monitored and fed daily until 90 days of age, when their weights were gradually reduced to and maintained at 85% of their free-feeding weights through food restriction. Water was freely available in the home cages. All animal-use procedures were in accordance with the European Communities Council Directive 86/609/EEC and Spanish Royal Decree 223/1998.

2.2. Schedule-induced polydipsia (SIP)

2.2.1. Apparatus

The experiment was conducted in eight identical 29 cm × 24.5 cm × 35.5 cm, Letica Instruments LI-36 rodent-conditioning chambers (Barcelona, Spain). Each chamber was enclosed in sound-proofed housing, which was equipped with a ventilation system and a small observation window in the left panel. The front panel of each chamber was made of aluminum, the left-hand wall and roof of transparent Plexiglas, and the remaining sides of black Plexiglas. A water bottle was attached to the external side of the right wall, with its spout accessible to the animal through a 3.2 cm × 3.9 cm aperture, located 20 cm from the front wall and 7 cm above the floor. The spout was positioned 2 cm from the wall aperture in such a way that the rat could lick it but not maintain permanent contact with it. Licks at the spout were detected when the electric circuit between the 16 parallel metal bars encircling the grid floor and the drinking bottle spout was completed via contact with the animal's tongue. The chambers were illuminated by two internal 3-W bulbs placed on the upper part of the front panel to either side of the food hopper and a 25-W ambient light fitted to the external housing. The ambient noise produced by the ventilation was 60 dB, which served to mask any other possible external sounds. With the aid of a Letica Instruments dispenser fitted to the outside of the front panel, 45-mg food pellets (Bio-Serv, Frenchtown, NJ, USA) could be dropped into a small internal receptacle situated on the front wall at a height of 3.7 cm above the floor, which served as a food tray. Events were scheduled and recorded using an Archimedes microcomputer (Paul Fray Ltd.) installed with the Arachnid software package.

2.2.2. Procedure

On being stabilized at 80–85% of their free-feeding weights, all rats were subjected to a water-intake test in their own home cages. For two consecutive days, 60 food pellets of 45 mg each were simultaneously deposited in a tray, and the amount of water consumed over 30 min was measured. The rats were then exposed to an adaptation session in the conditioning chambers for 30 min, with 20 food pellets being previously deposited in the food tray, and with ventilation and illumination being provided, but with no experimental contingency in operation. During this session, the water bottles were not installed. Thereafter, the rats were exposed to 30-min sessions with a fixed time (FT) 30-s schedule of food-pellet presentation, for a total of 80 sessions. One food pellet was regularly delivered into the food tray at 30-s intervals regardless of the animal's behavior. Before each session, bottles containing 100 ml of fresh tap water were installed in each chamber as described above. Lights were switched on at the beginning and switched off at the end of each session. In each session, the following was recorded for each rat: total number of licks to the bottle spout, transformed into licks per min; and total amount of water consumed (ml). These measures were used to separate rats into high (HD) and low (LD) drinkers according to whether licks/min were above or below the group median, respectively, resulting in 15 HD and 16 LD. A measure of lick efficiency, as number of licks per ml consumed, was also calculated for these two groups of rats.

2.3. Receptor autoradiography

Following the SIP procedure, 23 rats were randomly selected and sacrificed by rapid decapitation for receptor autoradiography. Brains were swiftly dissected, frozen for 1 min in isopentane (approximately −40 °C), and stored at −80 °C until processed. Using Paxinos and Watson's Atlas as a guide [33], equivalent coronal sections (20 μm-thick) for all brains were collected in a cryostat Reichert Jung, Model 2800 Frigocut N (Letica Instruments, Barcelona, Spain) from different levels, to map different brain areas of mesocorticolimbic circuitry (see Fig. 1), namely: medial prefrontal cortex and olfactory tubercle (from 2.70 mm to bregma); nucleus accumbens and dorsal striatum (from 1.60 to bregma); hippocampus and amygdala (from −2.12 to bregma); and, ventral tegmental area and substantia nigra (from −4.8 to bregma). Sections were immediately mounted on gelatin-coated slides and stored at −35 °C until used.

2.3.1. D1 receptor autoradiography

Slides were incubated for 60 min at room temperature with 1 nM [^{3}H] SCH23390 (Perkin Elmer, Madrid, Spain) in a buffer containing 50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$ and 4 mM $MgCl_2$ (pH 7.4).

2.3.2. D2 receptor autoradiography

Slides were incubated for 60 min at room temperature with 1 nM [^{3}H] Spiperone (Perkin Elmer, Madrid, Spain). Specific binding for D2 receptors was obtained by incubation with 1 nM [^{3}H] YM09151 (Perkin Elmer, Madrid, Spain), and adding 1 nM 7-OH-DPA and 500 nM L745870 to the buffer in order to block binding to D3 and D4 receptor subtypes. Non-specific binding was defined by adding 10 nM Butaclamol (Sigma Química, Madrid, Spain).

After incubation, slides were washed twice in ice-cold buffer (4 °C), and rinsed twice in ice-cold distilled water before drying at 4 °C. The slides were then desiccated with drierite desiccants (Sigma Química, Madrid, Spain). The next day, slides were apposed to Hyperfilm-^{3}H (Amersham, Madrid, Spain) for 6–8 weeks. Thereafter, the autoradiography films were developed with Kodak-D19 liquid. Autoradiograms were analysed with a Hewlett-Packard computer using the NIH Image program (US, National Institutes of Health, USA). For receptor autoradiography, optical densities were converted to bound ligand densities (fmol/mg gray matter) using plastic standards and the specific activity of the radioligands. Unspecific binding from adjacent sections was subtracted.

2.4. c-Fos protein immunochemistry

After the SIP procedure, the remaining 8 rats were assigned to c-Fos protein immunochemistry. Animals anesthetized with tribon 0.01 ml/g, were transcardially perfused with 0.9% saline for 5 min, followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) pH 7.4, for 20 min. Brains were then extracted and stored in PBS with paraformaldehyde, for 18 h at 4 °C. Thereafter, brains were washed three times in PBS every 10 min, and maintained for 2 days in sucrose at 4 °C. Using Paxinos and Watson's Atlas [33], equivalent coronal sections (40 μm-thick) for all brains were collected in the cryostat, at: the medial prefrontal cortex (from 2.70 mm to bregma); ventral tegmental area (from −.80 to bregma); and nucleus

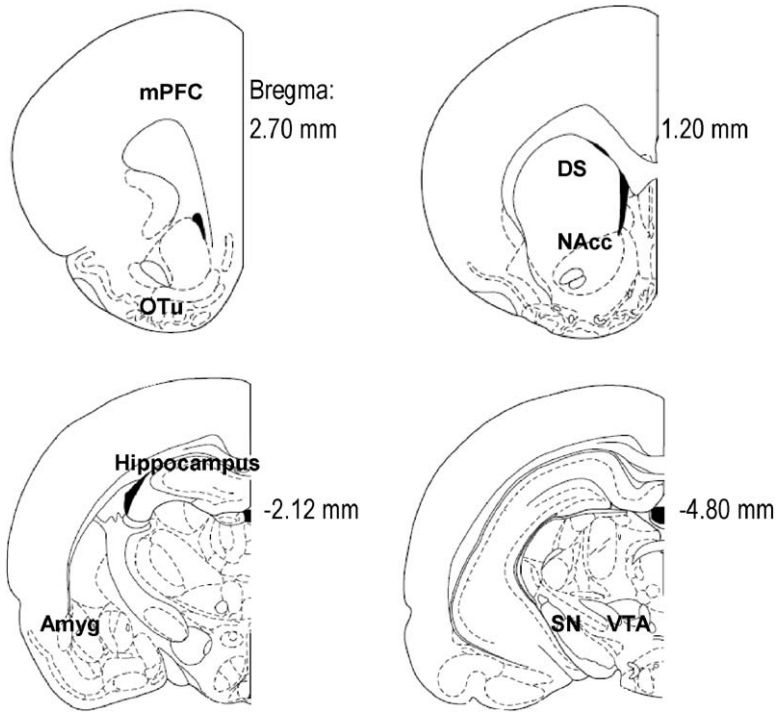

Fig. 1. Representation of various brain areas from which D1, D2 autoradiograms and c-Fos counts were taken. Numbers represent millimeters anterior or posterior to bregma. mPFC: medial prefrontal cortex; OTu: olfactory tubercle; DS: dorsal striatum; NAcc: nucleus accumbens; Amyg: amygdala; SN: substantia nigra; VTA: ventral tegmental area. The figures were adapted from Paxinos and Watson's Atlas [32].

accumbens (from 1.20 mm to bregma) (see Fig. 1). Sections mounted on slides were washed three times in PBS. Brain sections were incubated for 20 min in 0.3% H_2O_2 to block endogenous peroxidase activity, followed by three washes in PBS. The sections were then incubated for 45 min in 10% goat serum + 3% Triton-X in PBS, 20 min in 2% goat serum, and 48 h in 0.1 mg/ml c-Fos antibody, at 4 °C, 1:15,000 (polyclonal rabbit anti-cFos: Santa Cruz Biotechnology, CA, USA) + 2% normal goat serum + 0.1% bovine serum albumin in PBS. After two washes in PBS, sections were incubated for 90 min in the secondary antibody, Anti-Rabbit IgG (1:250) (Vector Laboratories, Burlingame, CA USA). Fos and anti-Fos complex were visualized by Vector's Vectastain Elite protocol, ABC system (Vector Laboratories, Burlingame, CA USA), by incubation in 3,3′-diaminobenzidine (DAB). DAB procedure was used, following the Vector DAB Substrate Kit protocol for peroxidase horseradish–peroxidase complex. Sections were rinsed with PBS and mounted onto gelatin-coated slides, dehydrated and cover slipped with DPX glue, and finally dried for 3 days. To control for variation in the immunohistochemical reaction for c-Fos, tissue from different groups was allowed to react together. c-Fos-positive cell counts were recorded by visual inspection under 40× magnification of the histological glass slides, using a Leitz Dialux 20 microscope. Cells were counted from two adjacent sections for each brain area for each rat and restricted to twice an area defined by a 1 cm × 1 cm grid located in the binoculars. Counts were made by trained raters, blind to experimental groups.

2.5. Statistical analyses

In order to explore the general pattern of relationships between SIP behavioral measures and neurochemical data, Pearson correlation coefficients and significations were calculated. Although SIP measures were not strict independent variables in this study, multivariate repeated measures ANOVAs (MANOVAs) were performed in order to contrast the general pattern of relationships between data suggested by the analysis of correlations. The MANOVA with binding DA receptors data included two repetition factors: "receptor" with two levels (D1 and D2) and "brain structure" with eight levels (dorsal striatum, olfactory tubercle, nucleus accumbens, medial prefrontal cortex, hippocampus, amygdala, ventral tegmental area, and substantia nigra), and a between-subjects factor "drinking group" with two levels (low and high). In the second order post hoc analysis, MANOVAs for each brain structure were performed with "receptor" as repetition factor and "drinking group" as between-subjects factor, further followed by post hoc *t*-tests. The MANOVA with c-Fos count cells data included the repetition factor "brain structure" with three levels (medial prefrontal cortex, ventral tegmental area, and nucleus accumbens) and the between-subjects factor "drinking group" with two levels (low and high), and *t*-tests as post hoc. All analyses were performed using the SPSS v.15 software package.

3. Results

3.1. Schedule-induced polydipsia

The FT 30-s food delivery schedule resulted in individual differences in the acquisition level of adjunctive licking and drinking behaviors. After 80 sessions, the animals were divided into HD and LD, according to whether licking rates were above or below the median split of 17.23 licks per minute, respectively. HD rats registered a statistically significant higher rate of licking [$F(1,21) = 18.11$, $p < 0.001$], a statistically significant higher consumption of water [$F(1,21) = 10.88$, $p < 0.01$] and a statistically lower lick efficiency – significantly more licks per ml of water consumed [$F(1,21) = 11.05$, $p < 0.01$] than did their LD counterparts (see Table 1).

Table 1
Mean (± S.E.M.) licks per minute, water intake (ml) and lick efficiency (licks/ml) in the first and last sessions of exposure to the FT 30-s food delivery schedule.

	First session		Last (80th) session	
	LD	HD	LD	HD
Licks/min	0.21 ± 0.11	0.25 ± 0.11	7.67 ± 1.25	46.30 ± 8.99**
Water intake (ml)	4.91 ± 0.63	4.54 ± 0.75	5.97 ± 0.89	15.70 ± 2.88*
Lick efficiency (licks/ml)	1.29 ± 0.31	1.65 ± 0.54	38.54 ± 5.27	88.47 ± 12.64**

* $p < 0.05$, statistically significant difference between LD and HD groups.
** $p < 0.01$, statistically significant difference between LD and HD groups.

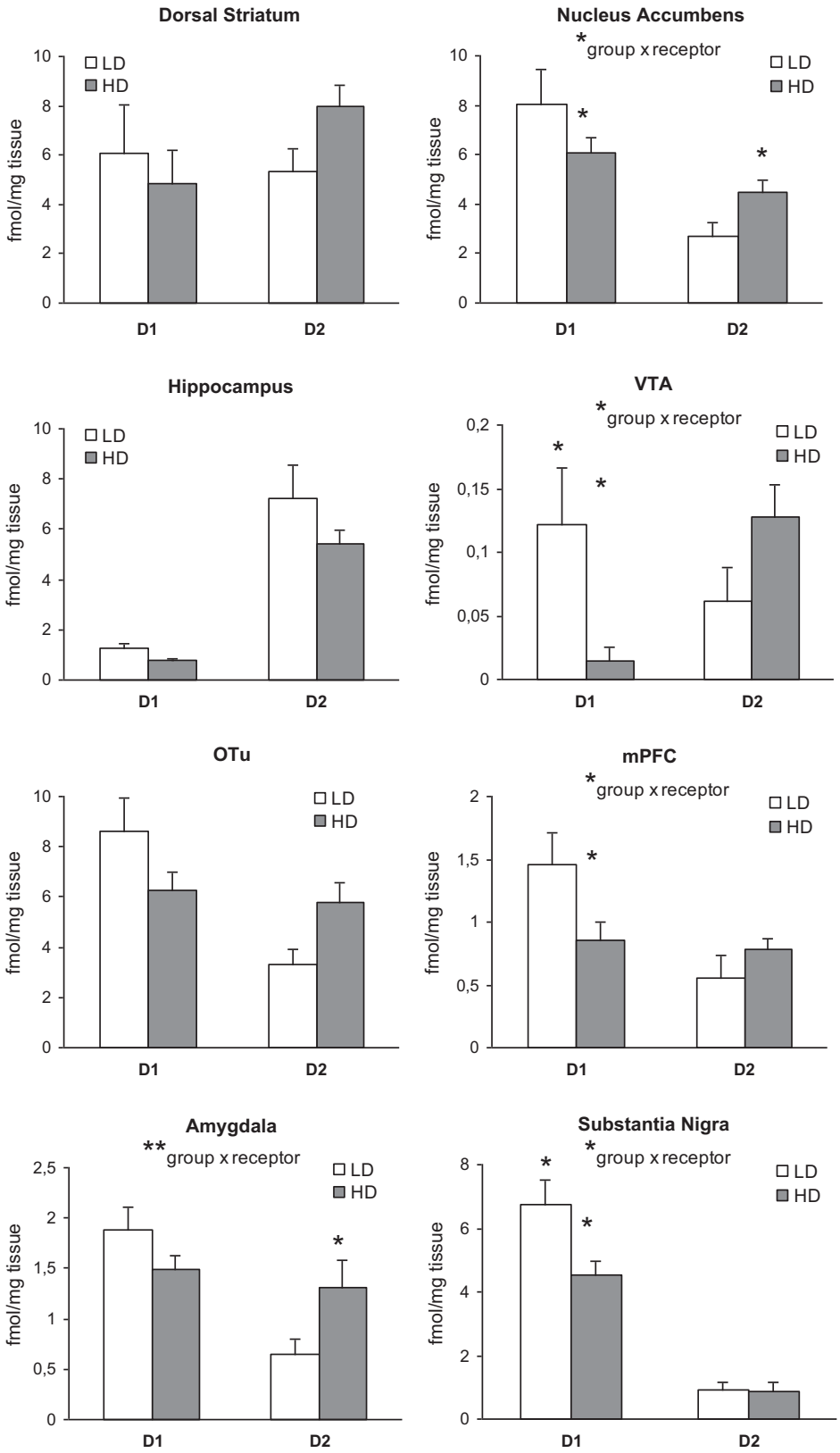

Fig. 2. Dopamine D1 and D2 receptor binding (mean ± S.E.M.) of low-drinker (LD) and high-drinker (HD) rats in: dorsal striatum, nucleus accumbens, hippocampus, ventral tegmental area (VTA), olfactory tubercle (OTu), medial prefrontal cortex (mPFC), amygdala, and substantia nigra. Statistically significant "drinking group" × "type of DA receptor" interaction: $^{*}p < 0.05$, $^{**}p < 0.01$. *Statistically significant difference between the HD and LD groups ($p < 0.05$).

Table 2
Pearson correlations between SIP measures and DA receptors binding (D1: upper table, D2: lower table) in dorsal striatum (DS), olfactory tubercle (OTu), nucleus accumbens (NAcc), medial prefrontal cortex (mPFC), hippocampus (Hip), amygdala (Amyg), ventral tegmental area (VTA), and substantia nigra (SN). *r* is in the first line, and bilateral signification of *r* is in the second line of each cell.

	DS D1	OTu D1	NAcc D1	mPFC D1	Hip D1	Amyg D1	VTA D1	SN D1
Licks/min	−.122	−.198	−.375	−.332	−.354	−.295	−.351	−.415*
	.580	.366	.078	.121	.098	.172	.100	.049
Ml consumed	−.232	−.339	−.377	−.137	−.116	−.137	−.259	−.292
	.288	.113	.076	.532	.599	.534	.233	.177
Licks/ml	−.423	−.222	−.369	−.438	.488*	−.377	−.462*	−.470
	.063	.347	.110	.053	.029	.102	.040	.036
	DS D2	OTu D2	NAcc D2	mPFC D2	Hip D2	Amyg D2	VTA D2	SN D2
Licks/min	.363	.249	.338	.071	−.116	.255	.522*	.217
	.097	.251	.114	.746	.598	.241	.011	.321
Ml consumed	.293	.242	.333	.034	.033	.107	.269	.106
	.186	.266	.121	.878	.880	.627	.214	.629
Licks/ml	.514*	.455*	.499*	.120	−.282	.258	.640**	.048
	.024	.044	.025	.616	.284	.273	.002	.841

* $p < 0.05$.
** $p < 0.01$.

3.2. D1 and D2 receptor autoradiography in HD and LD rats

The analysis of correlations between SIP measures and DA receptor autoradiography showed a general pattern of negative correlations between SIP measures and D1 binding receptors in all areas studied (except licks/ml in the hippocampus), and positive correlations between SIP measures and D2 binding receptors in all brain areas (except the hippocampus in licks/min and licks/ml). Although licks/min and lick efficiency (licks/ml) were in general better correlated than water intake with neurochemical measures, all *r* coefficients showed mostly similar trends for a given dopamine-type receptor (see Table 2 for coefficients and significations). Linear and quadratic fits to the data (not shown) were analysed, and in all cases the linear adjustment showed a better fit.

Taking together the eight brain structures studied, the MANOVA showed a statistical significant interaction between "type of receptor" (D1 or D2) and "drinking group" (LD or HD) [$F(1,21) = 6.03$, $p = 0.02$]. Post hoc analysis showed that the general pattern of negative relationships between SIP and D1 receptors and/or positive relationships between SIP and D2 receptors were significant (by MANOVA interactions "type of receptor" × "drinking group") in the nucleus accumbens ($F = 4.84$, $p = 0.03$), medial prefrontal cortex ($F = 5.01$, $p = 0.04$), amygdala ($F = 11.76$, $p < 0.01$), ventral tegmental area ($F = 7.28$, $p = 0.01$), and substantia nigra ($F = 7.09$, $p = 0.02$). Dorsal striatum and olfactory tubercle showed similar statistical trends than the other structures, but analyses did not reach significance ($p > 0.05$). The only area that therefore did not show this kind of interaction between drinking level and dopamine binding was the hippocampus, where LD showed higher binding than did HD for both D1 and D2 receptors. Post hoc *t*-tests showed significant higher D1 binding for low drinkers in ventral tegmental area [$t(21) = 2.57$, $p = 0.02$] and substantia nigra [$t(21) = 2.75$, $p = 0.01$], and significant higher D2 binding for high drinkers in nucleus accumbens [$t(21) = -2.52$, $p = 0.02$] and amygdala [$t(21) = -2.18$, $p = 0.04$]. Fig. 2 depicts histograms with the aforementioned results.

3.3. c-Fos activity counts in HD and LD

Correlations between some SIP measures (licks/min and water intake) and c-Fos count cells showed a high, positive and significant *r* in the medial prefrontal cortex and ventral tegmental area, and almost no relationship in the case of the nucleus accumbens. No statistical significant correlations were obtained for lick efficiency (licks/ml) (see Table 3 for coefficients and significations).

Table 3
Pearson correlations between SIP measures and c-Fos count cells in nucleus accumbens (NAcc), medial prefrontal cortex (mPFC), and ventral tegmental area (VTA). *r* is in the first line of each cell, bilateral signification of *r* is in the second line.

	mPFC	VTA	NAcc
Licks/min	.747*	.736*	.018
	.033	.037	.966
Ml consumed	.822*	.743*	−.159
	.012	.035	.707
Licks/ml	.248	−.241	.175
	.553	.556	.679

* $p < 0.05$.

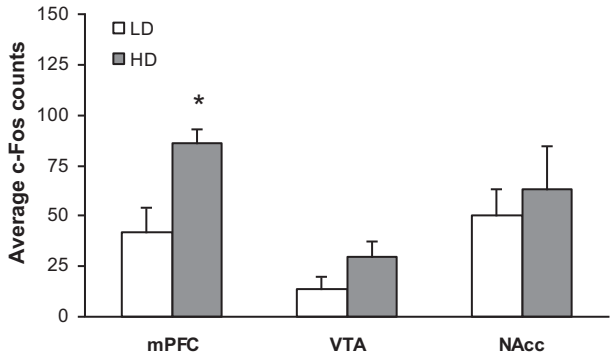

Fig. 3. Mean (±S.E.M.) c-Fos counts in medial prefrontal cortex (mPFC), ventral tegmental area (VTA) and nucleus accumbens (NAcc), in LD and HD groups of rats. *Statistically significant difference between the HD and LD groups ($p < 0.05$).

Taken together the three brain structures studied for c-Fos, the MANOVA analyses with the between subjects factor "drinking group" showed differences between LD and HD groups [$F(1,6) = 13.76$, $p = 0.01$]. Post hoc *t*-test revealed that, as compared to LD rats, HD rats showed a statistically significant increase in c-Fos activity in the medial prefrontal cortex [$t(6) = -2.91$, $p = 0.03$] (see Fig. 3). Although no statistical differences were found in c-Fos counts in ventral tegmental area and nucleus accumbens, in both cases HD rats registered a higher activity than did LD rats as can be also seen in Fig. 3.

4. Discussion

This study shows that SIP acquisition correlates positively with dopamine D2 receptor binding and negatively with dopamine D1

receptor binding through different mesocorticolimbic brain areas. LD and HD rats classified by level of SIP acquisition differed in D1 and D2 patterns of receptor binding in the nucleus accumbens, medial prefrontal cortex, amygdala, ventral tegmental area and substantia nigra. While LD rats showed higher D1 receptor binding in these areas, HD rats showed higher D2 receptor binding (except in substantia nigra), and *vice versa*. These neuroanatomical differences in dopamine receptor binding were also associated with a positive correlation between SIP acquisition and c-Fos count cells in the medial prefrontal cortex and ventral tegmental area, and an elevated c-Fos count in the medial prefrontal cortex in HD rats.

Our findings confirm those of a large body of the literature, showing the involvement of dopamine neuronal systems in established SIP performance [18,29,37]. Previous pharmacological studies have reported the implication of dopamine D1 and D2 receptor subtypes in SIP, where administration of dopaminergic agonists and antagonists changes the balance of activation of dopamine D1 and D2 receptors, resulting in SIP disruption [6,7,10,19,29,44,49,52]. However, the different quantification patterns of dopamine D1 and D2 receptors for HD and LD rats described here suggest a different implication of dopamine receptor subtypes in SIP. In this same vein, some studies have observed different effects resulting from dopamine D1 and D2 receptor manipulation. For example, chronic administration of the dopamine D1 receptor agonist, SKF38393, did not affect water intake, while chronic treatment with the dopamine D2 agonist, quinpirole, increased water intake, an effect reinstated by amphetamine and partially prevented by the D2 antagonist, domperidone [12]. Other studies have also suggested different roles for dopamine D1 and D2 receptors in SIP. The dopamine agonist, apomorphine, produced motor deficits that interfered with consummatory behavior implicated in the initiation of drinking, while the dopamine D2 antagonist, haloperidol, interfered with the sensory feedback necessary to sustain consummatory behavior, thus accelerating the cessation of drinking [44]. The pattern of results obtained here seems to suggest that D2 receptors might be related to the motor aspects of drinking, as has been suggested for the increases in feeding produced by D2 agonists [26], while D1 receptors are involved in motivational aspects more strongly than motoric [26]. In line with this, the higher lick efficiency of LD rats might mean that the excess of licking of HD rats could be stereotyped, an idea that will be in keeping with the stereotypes shown after high or repetitive doses of D2 agonists [12,45].

The implication of central catecholaminergic neurons in SIP has also been shown by the increase in dopamine synthesis and utilization in the limbic area during SIP performance [50]. Dopamine efflux has been observed to increase in specific brain areas, such as the nucleus accumbens, throughout SIP sessions, though such activity does not appear to be necessary for SIP development [51]. Behavioral studies have shown, however, that once SIP is established, animals display lower central dopaminergic system activity, for example, by reduced locomotor response to amphetamine [46]. Hence, the increased binding of dopamine D2 receptors of HD rats in brain areas such as nucleus accumbens and medial prefrontal cortex could point to dopamine D2-receptor upregulation, and might explain the desensitization response to locomotor effects of amphetamine linked to SIP acquisition. The prefrontal cortex seems to be strongly implicated in the development of SIP, shown by dose-dependent selective effects in SIP reduction by cocaine infusion, without having substantial effects on either locomotor activity or panel pressing, compared to those had by the nucleus accumbens [18]. In reporting an increased c-Fos count in HD versus LD rats, our study also confirms the special implication of prefrontal cortex in SIP. c-Fos is a group of intermediate-early genes transcribed in response to both inhibitory and excitatory synaptic transmission [43]. Despite the fact that the prefrontal cortex is innervated by dopamine neurons originating in the ventral tegmental area [20,22] and that lesion studies have previously shown the implication of ventral tegmental area in SIP [49], no differences were found in c-Fos expression between HD and LD rats in this structure. Nonetheless, increased c-Fos activity in the prefrontal cortex in HD versus LD rats suggests an elevated signal transmission in HD rats that might be caused by differences in dopamine D1 receptor binding between LD and HD rats. These differences in D1 receptor activity could thus act in the facilitation of c-Fos induction [21,43]. In line with this, the implication in the response-selection process of the dopamine D1 receptor in the prefrontal cortex [14,39] could have a decisive role in the differences between LD and HD rats observed in SIP.

Previous behavioral studies have shown the implication of the dopaminergic system in individual differences or behavioral predisposition to exhibit SIP, for example, via the different effects of dopaminergic drugs on HD and LD rats [24]. The present study adds a different quantification pattern of dopamine D1 and D2 receptors in different brain areas of HD versus LD rats, thereby leaving room for speculation that such individual differences in SIP might be related to genetic differences in the dopamine signaling system, with special implication of the prefrontal cortex as shown by the elevated c-Fos in HD group. This speculation is based on the integration of this with other studies because no genetic analysis was performed on the rats used in our experiments.

In view of the fact that SIP has been proposed as a behavioral model of drug abuse [13,32,38,50] and psychopathologies such as obsessive–compulsive disorder [15,36,40,42,53,54], a genetic factor related to the dopaminergic system and to SIP acquisition could possibly be involved in vulnerability to drug abuse and/or compulsive behavior. The specific linkage of dopamine D2 receptors to alcoholism and to drug abuse in general [1–3] see [23] for recent review, and the neurochemical differences observed between LD and HD rats, points to HD as a possible vulnerability population to alcohol intake. In line with this, a recent study has suggested that SIP behavior may work additively with genetic susceptibilities for ethanol drinking [13]. In this study, three rat lines selectively bred for high and low ethanol preference were tested for differences in SIP behavior, indicating possible genetic correlations between the selected traits in these lines of rats and behavioral traits indicative of susceptibility to SIP.

In conclusion, our data show the implication of the dopaminergic system in individual differences in SIP. More specifically, we found neuroanatomical divergences in dopamine D1 and D2 receptor binding in different brain areas of the mesocorticolimbic system, and in c-Fos expression in prefrontal cortex, of rats divided into LDs and HDs according to SIP performance. The present data support previously proposed hypotheses about a different dopaminergic function between LD and HD rats [24,25], and points to SIP as a behavioral model for distinguishing populations possibly vulnerable to dopaminergic function disorders.

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