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Chronic treatment with a dopamine uptake blocker changes dopamine and acetylcholine but not glutamate and GABA concentrations in prefrontal cortex, striatum and nucleus accumbens of the awake rat

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Abstract

The present study was aimed to investigate the effects of a chronic treatment with the dopamine uptake blocker nomifensine on the *in vivo* extracellular concentrations of dopamine, acetylcholine, glutamate and GABA in the prefrontal cortex, striatum and nucleus accumbens. Male Wistar rats received intraperitoneal (i.p.) daily injections of nomifensine (10 mg/kg) or saline for 22 days. Microdialysis experiments were performed on days 1, 8, 15 and 22 of treatment to evaluate the effects of the injection of nomifensine or saline. Motor activity of the animals was monitored during microdialysis experiments. Injections of nomifensine increased extracellular concentration of dopamine in striatum and nucleus accumbens, but not in prefrontal cortex. Acetylcholine concentrations in striatum but not in nucleus accumbens were increased by nomifensine on days 15 and 22 of treatment. In prefrontal cortex, nomifensine increased acetylcholine levels without differences among days. No changes were found on glutamate and GABA concentrations in the three areas studied. Injections of nomifensine also increased spontaneous motor activity and stereotyped behaviour without differences among days. These results show that systemic chronic treatment with a dopamine uptake blocker produces differential effects on extracellular concentrations of dopamine and acetylcholine, but not glutamate and GABA, in different areas of the brain.
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Keywords: Nomifensine; Dopamine; Acetylcholine; Glutamate; GABA; Prefrontal cortex; Striatum; Nucleus accumbens; Microdialysis; Motor activity

1. Introduction

Anatomical and biochemical evidence suggest a role for dopamine modulating the activity of other neurotransmitter systems in the prefrontal cortex, striatum and nucleus accumbens. In the prefrontal cortex, dopaminergic afferents arising from the ventral tegmental area (VTA) and cholinergic terminals arising from neurons in the basal forebrain synapse on pyramidal glutamatergic neurons (Houser et al., 1985; Van Eden et al., 1987). Moreover, an interaction between dopamine and acetylcholine in the prefrontal cortex has been reported (Yang and Mogenson, 1990). Dopamine terminals also synapse on GABAergic interneurons (Verney et al., 1990). In the striatum a direct synaptic contact has been shown to exist between dopaminergic terminals from neurons located in the substantia nigra and cholinergic interneurons (Di Chiara et al., 1994; Kubota et al., 1987). In fact, these cholinergic interneurons express dopaminergic receptors (Alcantara et al., 2003; Di Chiara et al., 1994). Also in the striatum, glutamatergic terminals arising from the corticostriatal pathway and dopaminergic terminals synapse on GABAergic interneurons and medium-size spiny GABA projecting neurons (Smith and Bolam, 1990). In the nucleus accumbens, similar to striatum, a synaptic contact between dopaminergic terminals (from VTA) and cholinergic interneurons has been described (Groenewegen et al., 1991). Also the cholinergic interneurons express dopaminergic receptors (Alcantara et al., 2003). In the nucleus accumbens dopaminergic and glutamate afferents (from prefrontal cortex and hippocampus) synapse on GABAergic neurons (Sesack et al., 2003).

Several neurochemical studies have supported the existence of a functional interaction between dopamine and acetylcholine, and also glutamate and GABA in the target areas of the dopaminergic pathways. Thus, systemic administration of both

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direct and indirect dopaminergic agonists increases acetylcholine levels in prefrontal cortex (Acquas et al., 1994; Arnold et al., 2001; Day and Fibiger, 1992). Also dopamine regulates the extracellular concentrations of glutamate and GABA in the prefrontal cortex (Del Arco and Mora, 2002; Grobin and Deutch, 1998; Porras et al., 1997). In both striatum and nucleus accumbens several studies have shown that D1 and D2 dopaminergic receptors modulates acetylcholine release (Acquas et al., 1997; de Belleroche and Gardiner, 1983; Stoof et al., 1992; Wedzony et al., 1988). In agreement with these last studies, dopamine uptake blockers have been shown to change acetylcholine extracellular concentration in striatum and nucleus accumbens (Imperato et al., 1992; Mark et al., 1999; Wedzony et al., 1988; Yamada et al., 2004). Finally, dopamine seems to modulate glutamate and GABA concentrations in striatum and nucleus accumbens (Dalia et al., 1998; de Belleroche and Gardiner, 1983; Kalivas and Duffy, 1997; Porras and Mora, 1995).

In contrast to the above-mentioned reports, few studies have been devoted to the investigation of changes in several neurotransmitter after chronic increases of dopamine as a result of the blockade of its uptake (Jayaram and Steketee, 2005; Williams and Steketee, 2004). This is surprising in view of the reported changes in the dopaminergic systems projecting to the prefrontal cortex, striatum and nucleus accumbens, e.g. changes in the expression of dopamine receptors (Alburges et al., 1993; Goeders and Kuhar, 1987; Martin et al., 1995; Peris et al., 1990; Schmidt-Mutter et al., 1999; Tella et al., 1996; Tsukada et al., 1996; Unterwald et al., 1994), and its relevance to the treatment of psychiatric disorders, particularly depression (Kinney, 1985; Yamada et al., 2004). Moreover, these studies are of interest since drugs of abuse such as cocaine produce a chronic blockade of dopamine uptake. Therefore, it was of interest to investigate the possible changes in the interaction between dopamine and other neurotransmitters after chronic blockade of dopamine uptake.

The aim of the present study was to investigate the effects of a chronic treatment with the dopamine uptake blocker nomifensine (John and Jones, 2006; Orset et al., 2005), on the *in vivo* extracellular concentrations of dopamine, acetylcholine, glutamate and GABA in the prefrontal cortex, striatum and nucleus accumbens. Specifically, we analysed the neurotransmitter extracellular concentrations before and immediately following the daily injection of nomifensine. The effects of nomifensine treatment on the motor activity of the animals were also studied.

2. Experimental procedures

2.1. Animals and surgery

Young (2–4 months) male Wistar rats were housed in individual wire mesh cages, provided with food and water *ad libitum*, and maintained in a temperature-controlled room on a reverse light/dark cycle (lights off 8:00 a.m. to 8:00 p.m.). All *in vivo* experiments, carried out at the Universidad Complutense of Madrid, were conducted during the dark period of the light/dark cycle and following the guidelines of the International Council for Laboratory Animal Science (ICLAS) and the European Union Council Directive (86/609/EEC).

Under Equithesin (2 ml/kg i.p.) anaesthesia, rats were stereotaxically implanted with bilateral guide-cannulae to accommodate microdialysis probes (at the days of the experiments, see below) in prefrontal cortex, striatum or nucleus accumbens (Segovia et al., 1997). When inserted, the tip of the probe was located in: 3.6 mm rostral and 0.9 mm lateral from Bregma and 4.5 mm ventral from dura mater for prefrontal cortex; 0.6 mm rostral and 2.5 mm lateral from Bregma and 7.5 mm ventral from dura mater for striatum; 1.6 mm rostral and 1.6 mm lateral from Bregma and 7.5 mm ventral from dura mater for accumbens (Paxinos and Watson, 1998).

2.2. Drug treatment and microdialysis

Seven to ten days after surgery rats received a daily injection (i.p.) of nomifensine (10 mg/kg) or saline, for 22 days. The dose was selected after performing a dose–response study with the dopamine uptake blocker nomifensine (2, 5 and 10 mg/kg) (data not shown). On days 1, 8, 15 and 22 of treatment microdialysis experiments were performed in the freely moving rat to evaluate the effects of the injections of nomifensine or saline (Segovia et al., 1997). Only one microdialysis experiment was performed in each brain site, left and right sides were used for experiments in different days of treatment. Therefore, each animal was included in two different experimental groups in a pseudorandom manner. Briefly, microdialysis probes (membrane cut off 5000 Da and length 4 mm for prefrontal cortex and striatum, 2 mm for accumbens) were inserted and perfused (2 μl/min) with artificial CSF (pH 7.4) (composition in mM: NaCl, 137; $CaCl_2$, 1.2; KCl, 3; $MgSO_4$, 1; NaH_2PO_4, 0.5; Na_2HPO_4, 2; glucose, 3) containing neostigmine 1 μM. Nomifensine 5 μM was also added for the experiments performed in the prefrontal cortex. Twenty-minute samples were collected 3 h after the insertion of the probe in order to stabilize neurotransmitters levels. The samples were separated in three aliquots to analyse the concentrations of dopamine, acetylcholine, glutamate and GABA. The first three samples (60 min) were used as control, then nomifensine or saline injections were given and the following eight samples (160 min) were collected.

At the end of the experiments the animals were anaesthetised with Equithesin and perfused intracardially with 0.9% saline followed by 10% formalin. Then, the brain was removed and the placement of the microdialysis probe was verified with a cryostat microtome and viewing lens.

2.3. Dopamine analysis

Dopamine content of samples was analysed by reverse-phase HPLC and electrochemical detection (Coulochem II model 5200A, ESA, Chelmsford, MA) using a C18 column (Nova-Pack 4 μm 3.9 mm × 150 mm, Waters, Milford, MA) (Hernández et al., 2003). Mobile phase consisted of 0.1 M acetate–citrate buffer (pH 3.5 adjusted with HCl 1N), 1 mM EDTA, 2.9 mM sodium octyl sulphonate, and 18% methanol. The flow rate was maintained at 1 ml/min. Chromatograms were processed using the Millenium 32 (Waters, Milford, MA) software. The detection limit in our 20 μl samples was 0.15 nM for dopamine.

2.4. Acetylcholine analysis

Acetylcholine content of samples was analysed by cation-exchange HPLC and electrochemical detection (HP1049A, Agilent, Palo Alto, CA) using a microbore analytical column (SepStik 10 μm 530 mm × 1 mm, BAS, West Lafayette, IN) followed by a microbore immobilized enzyme reactor containing acetylcholinesterase and choline oxidase (SepStik 50 mm × 1 mm, BAS, West Lafayette, IN) (Hernández et al., 2003). The mobile phase consisted of 50 mM phosphate buffer, 0.5 mM EDTA, and ProClin® 150 Reagent 5 ml/l (pH 8.5 adjusted with NaOH 1N). The flow rate was maintained at 0.15 ml/min. Chromatograms were processed using the HPChem (Agilent, Palo Alto, CA) software. The detection limit in our 10 μl samples was 5 nM for acetylcholine.

2.5. Glutamate and GABA analysis

The glutamate and GABA content of samples was analysed by reverse-phase HPLC and fluorometric detection (474, Waters, Milford, MA) using a

C18 column (Spherisorb 5 μm 4 mm × 150 mm, Waters, Milford, MA) (Hernández et al., 2003). Precolumn derivatisation of 5 μl samples was performed with an *O*-phthalaldehyde solution. A gradient program of two mobile phases at a flow rate of 1 ml/min was used. Solution A was 95:5 (v/v) mixture of 50 mM sodium acetate buffer (pH 5.67) and methanol, to which 12.5 ml of isopropyl alcohol per liter was added; solution B was a 70:30 (v/v) methanol/water mixture. The excitation filter was set at 340 nm, and the emission filter at 460 nm. Aminoacids were quantified using the Millenium 32 (Waters, Milford, MA) software by the internal standard procedure using 6.25 μM homoserine. The detection limit in our 5 μl samples was 0.05 μM.

2.6. Motor activity

Simultaneously to microdialysis experiments motor activity was recorded by photocell beams (16 infrared light photocells) located at 5 cm from the basement and adapted to the microdialysis cages (27 × 27). Interruptions of the photocell beams were registered automatically by a computer software connected to the activity cages (Cibertec S.A., Madrid, Spain) (Marquez de Prado et al., 2003).

Stereotyped behaviour was measured from 0 to 3 as follows: 0: appearance same as saline treated rats, 1: discontinuous sniffing, increased exploratory activity, 2: continuous sniffing, licking with sporadic activity, 3: licking, heading, without locomotor activity. Animals were observed for 2 min every 20 min after nomifensine injection (Costall et al., 1972; Nakachi et al., 1995; Voikar et al., 1999).

2.7. Chemicals

Nomifensine maleate salt was purchased from Sigma–Aldrich (Madrid, Spain). Nomifensine was dissolved in saline before i.p. administration.

2.8. Statistical analysis

Absolute microdialysis and motor activity data were normalised by subtracting basal levels (average of the three control values) from each post-basal sample. The ''normalised'' data were then used to perform two or three-way ANOVAs and planned comparisons (Snedecor and Cochran, 1989). When appropriate, a repeated measures design was applied. Planned comparisons were performed using the average of ''normalised'' data from samples showing maximal increases. Pearson's coefficient and independence test were also used for the study of correlations between dependent variables and days of treatment.

3. Results

3.1. Effects of chronic treatment with a dopamine uptake blocker on dopamine, acetylcholine, glutamate and GABA extracellular concentrations in the prefrontal cortex

Chronic treatment with nomifensine increased basal dialysate concentrations of dopamine (Table 1). There were differences between days 1 and 22 of treatment ($F_{1,36} = 6.975$; $p = 0.0121$). Moreover, a correlation between dopamine basal levels and days of treatment was found ($r = 0.4250$; $p < 0.05$). In the control group also a correlation in basal levels were found despite not having a significant difference between days of treatment ($r = 0.5077$; $p < 0.05$).

Chronic treatment with nomifensine did not change basal dialysate concentrations of acetylcholine, glutamate and GABA (Tables 1 and 2).

Daily injections of nomifensine produced no changes in dopamine, glutamate and GABA extracellular concentrations (Figs. 1 and 2).

Injection of nomifensine increased acetylcholine extracellular concentration at all days of treatment: day 1 ($F_{1,36} = 40.27$; $p < 0.0001$), 8 ($F_{1,36} = 32.39$; $p < 0.0001$), 15 ($F_{1,36} = 35.51$; $p < 0.0001$) and 22 ($F_{1,36} = 28.74$; $p < 0.0001$) with no differences between days. These increases were significantly different from saline (Fig. 1).

3.2. Effects of chronic dopamine uptake blocker treatment on dopamine, acetylcholine, glutamate and GABA extracellular concentrations in the striatum

Chronic treatment with nomifensine did not change basal dialysate concentrations of dopamine, glutamate and GABA (Tables 1 and 2).

Chronic treatment with nomifensine increased basal dialysate concentrations of acetylcholine. Basal levels on

Table 1
Basal concentrations of dopamine and acetylcholine in different brain areas all days of experiment (1, 8, 15, 22) expressed in nM

Day	Basal dopamine		Basal acetylcholine	
	Saline	Nomifensine	Saline	Nomifensine
Prefrontal cortex				
1	0.47 ± 0.12 (4)	0.74 ± 0.08 (7)	38.34 ± 7.46 (4)	50.15 ± 9.09 (7)
8	0.53 ± 0.09 (5)	0.97 ± 0.15 (6)	41.53 ± 4.74 (4)	44.69 ± 5.67 (6)
15	0.71 ± 0.15 (4)	0.97 ± 0.09 (8)	46.52 ± 7.36 (4)	36.81 ± 7.84 (8)
22	0.92 ± 0.16 (5)	1.19 ± 0.23 (7)**	52.16 ± 20.59 (4)	40.76 ± 2.64 (7)
Striatum				
1	2.15 ± 0.44 (5)	2.06 ± 0.48 (5)	51.52 ± 11.68 (5)	34.92 ± 2.87 (4)
8	1.56 ± 0.32 (4)	2.63 ± 0.63 (8)	38.46 ± 5.31 (4)	23.26 ± 4.33 (6)
15	2.24 ± 0.78 (5)	1.79 ± 0.30 (5)	52.27 ± 5.50 (5)	69.76 ± 8.58 (6)*
22	1.92 ± 0.33 (4)	2.43 ± 0.44 (9)	34.61 ± 11.75 (3)	62.19 ± 7.00 (5)
Accumbens				
1	0.55 ± 0.05 (4)	0.50 ± 0.06 (6)	30.00 ± 5.09 (3)	29.19 ± 5.57 (6)
8	0.38 ± 0.06 (6)	0.57 ± 0.01 (5)	27.56 ± 6.91 (6)	18.69 ± 4.92 (5)
15	0.41 ± 0.05 (5)	0.77 ± 0.13 (5)	23.09 ± 4.95 (4)	19.56 ± 7.40 (4)
22	0.65 ± 0.13 (5)	0.67 ± 0.06 (6)	39.75 ± 9.57 (4)	33.47 ± 6.74 (4)

Data shown as mean ± S.E.M. (*n*) are absolute values of the average of the three basal values. *$p < 0.05$, **$p < 0.02$ vs. day 1 of treatment.

Table 2
Basal concentrations of glutamate and GABA in different brain areas all days of experiment (1, 8, 15, 22) expressed in μM

Day	Basal glutamate		Basal GABA	
	Saline	Nomifensine	Saline	Nomifensine
Prefrontal cortex				
1	1.312 ± 0.439 (4)	1.210 ± 0.248 (7)	0.147 ± 0.052 (4)	0.161 ± 0.054 (7)
8	1.479 ± 0.257 (4)	1.051 ± 0.277 (4)	0.134 ± 0.058 (3)	0.145 ± 0.064 (5)
15	0.994 ± 0.129 (5)	1.216 ± 0.151 (8)	0.063 ± 0.008 (5)	0.128 ± 0.020 (8)
22	1.521 ± 0.256 (4)	1.285 ± 0.227 (8)	0.084 ± 0.017 (5)	0.095 ± 0.014 (8)
Striatum				
1	0.851 ± 0.218 (5)	1.081 ± 0.322 (5)	0.120 ± 0.017 (4)	0.223 ± 0.034 (5)
8	0.699 ± 0.107 (4)	1.745 ± 0.350 (5)	0.153 ± 0.043 (3)	0.405 ± 0.117 (6)
15	0.699 ± 0.201 (4)	1.215 ± 0.127 (9)	0.073 ± 0.008 (4)	0.248 ± 0.036 (9)
22	0.611 ± 0.054 (4)	0.852 ± 0.123 (7)	0.109 ± 0.029 (4)	0.240 ± 0.046 (8)
Accumbens				
1	0.476 ± 0.070 (4)	0.236 ± 0.027 (6)	0.083 ± 0.018 (4)	0.038 ± 0.007 (4)
8	0.352 ± 0.059 (5)	0.443 ± 0.092 (5)	0.088 ± 0.004 (5)	0.062 ± 0.026 (4)
15	0.381 ± 0.108 (5)	0.408 ± 0.081 (5)	0.066 ± 0.023 (5)	0.069 ± 0.025 (6)
22	0.445 ± 0.163 (5)	0.270 ± 0.043 (6)	0.121 ± 0.055 (5)	0.070 ± 0.025 (6)

Data shown as mean ± S.E.M. (*n*) are absolute values of the average of the three basal values.

day 15 were significantly different from day 1 ($F_{1,31} = 5.320$; $p = 0.0279$) (Table 1). Moreover, there was a positive correlation between acetylcholine basal levels and days of treatment ($r = 0.5938$; $p < 0.01$).

Daily injections of nomifensine produced an increase in dialysate concentrations of dopamine: day 1 ($F_{1,37} = 25.94$; $p < 0.0001$); day 8 ($F_{1,37} = 10.78$; $p = 0.0023$), day 15 ($F_{1,37} = 12.24$; $p = 0.0012$) and day 22 ($F_{1,37} = 33.17$; $p < 0.0001$). These increases were significantly different from saline (Fig. 3).

Injection of nomifensine produced an increase in dialysate concentrations of acetylcholine at days 15 ($F_{1,31} = 37.23$; $p < 0.0001$) and 22 ($F_{1,31} = 21.04$; $p < 0.0001$) of treatment that was significantly different from saline (Fig. 3).

Daily injections of nomifensine did not change glutamate and GABA extracellular concentration (Fig. 4).

3.3. Effects of chronic dopamine uptake blocker treatment on dopamine, acetylcholine, glutamate and GABA extracellular concentrations in the nucleus accumbens

Chronic treatment with nomifensine did not change basal dialysate concentrations of dopamine, acetylcholine, glutamate and GABA (Tables 1 and 2).

Daily injections of nomifensine produced an increase in dialysate concentrations of dopamine: day 1 ($F_{1,34} = 49.32$; $p < 0.0001$), day 8 ($F_{1,34} = 12.82$; $p = 0.0011$), day 15 ($F_{1,34} = 37.51$; $p < 0.0001$) and day 22 ($F_{1,34} = 40.86$; $p < 0.0001$). These increases were significantly different from saline (Fig. 5).

Injection of nomifensine did not change acetylcholine, glutamate and GABA extracellular concentration compared to saline (Figs. 5 and 6).

3.4. Effects of chronic dopamine uptake blocker treatment on motor activity

Chronic treatment with nomifensine increased basal motor activity being different from day 1 the days 15 ($F_{1,129} = 6.012$; $p = 0.0155$) and 22 ($F_{1,129} = 4.059$; $p = 0.0460$) of treatment. Also there was a correlation between basal motor activity and days of treatment ($r = 0.2371$; $p < 0.05$) (Table 3).

Injection of nomifensine increased motor activity compared to the effects of saline (Fig. 7A). Nomifensine produced maximal motor activity 20–40 min after nomifensine injection: day 1 ($F_{1,129} = 33.069$; $p < 0.0001$), day 8 ($F_{1,129} = 19.908$; $p < 0.0001$), day 15 ($F_{1,129} = 54.764$; $p < 0.0001$) and day 22 ($F_{1,129} = 15.978$; $p < 0.0001$). The increases of motor activity were not different between days of treatment.

Daily injections of nomifensine produced stereotyped behaviour (Fig. 7B). Nomifensine produced maximal stereotyped behaviour 40–60 min after nomifensine injection (peak values): day 1 ($F_{1,71} = 152.17$; $p < 0.0001$), day 8 ($F_{1,71} = 189.43$; $p < 0.0001$), day 15 ($F_{1,71} = 210.04$; $p < 0.0001$) and day 22 ($F_{1,71} = 216.14$; $p < 0.0001$). There were no differences in the peak stereotyped behaviour score between days of treatment (Fig. 7B).

4. Discussion

The present study was aimed to investigate the effects of chronic treatment (22 days) with the dopamine uptake blocker nomifensine on the extracellular concentrations of dopamine, acetylcholine, glutamate and GABA in the prefrontal cortex, striatum and nucleus accumbens in the awake rat. Also, motor behaviour was studied in this research. Daily nomifensine injections increased dopamine extracellular concentrations in striatum and nucleus accumbens, but not in the prefrontal cortex. These responses were not modified by chronic treatment. On the other hand, nomifensine injections at days 1, 8, 15 and 22 of treatment increased acetylcholine extracellular concentration in the prefrontal cortex but not nucleus accumbens and these effects were not changed by chronic treatment. In striatum nomifensine injections increased acetylcholine extracellular concentration at days 15 and 22, but not at 1 and 8 days of treatment. No changes were found on

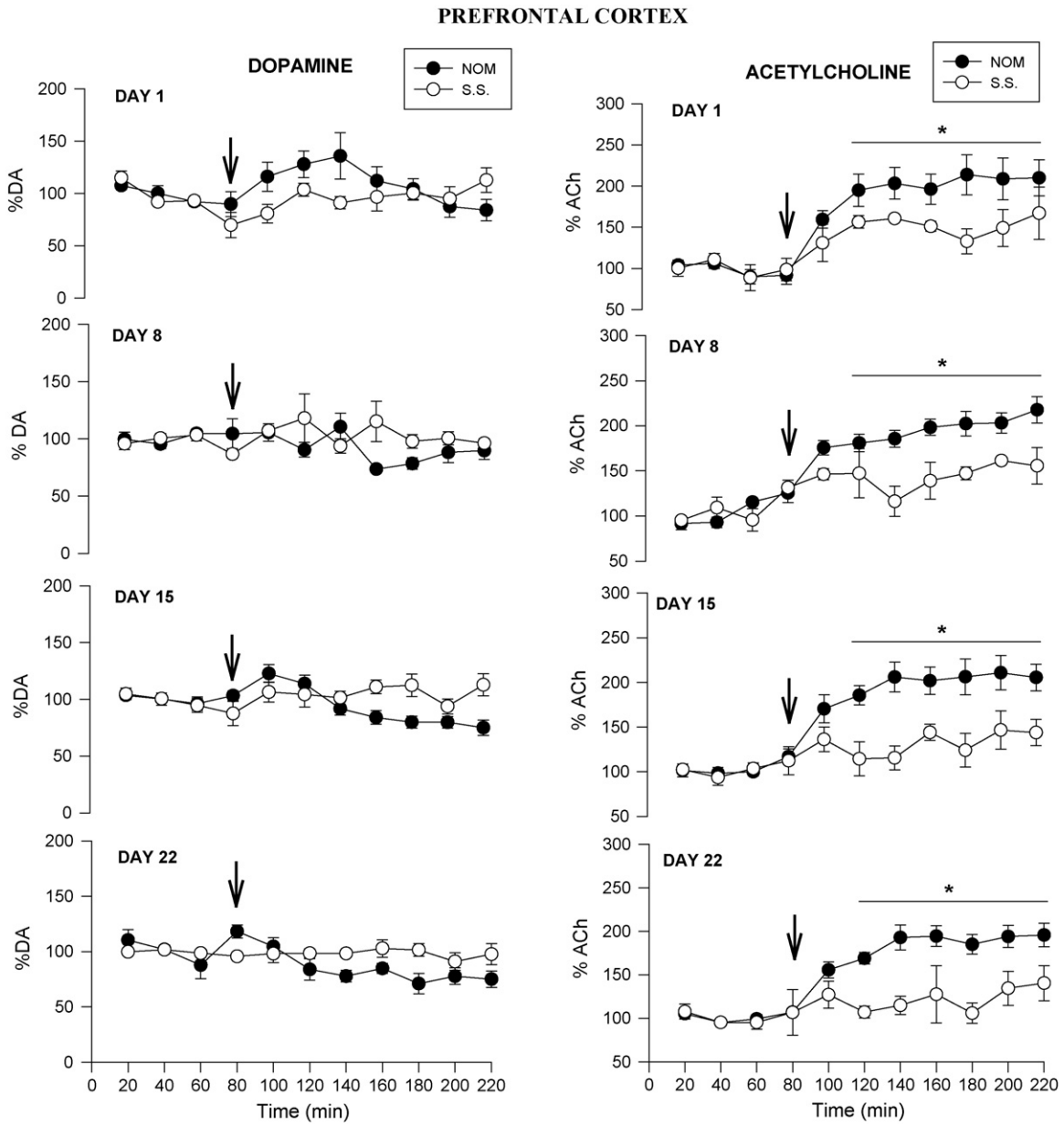

Fig. 1. Effects of nomifensine (NOM 10 mg/kg, i.p., $n = 6–8$) and saline (SS $n = 4–5$) on the extracellular concentrations of dopamine and acetylcholine in the prefrontal cortex. Data (mean ± S.E.M.) are expressed as a percentage of baseline. *$p < 0.05$ (vs. saline; planned comparisons in a three-way ANOVA with repeated measures design).

glutamate and GABA concentrations after nomifensine injections in the three areas under study. These results suggest that chronic treatment with a dopamine uptake blocker differentially changes dopamine and acetylcholine, but not glutamate and GABA, concentrations in the prefrontal cortex, striatum and nucleus accumbens of the awake rat.

4.1. Dopamine

The increase of extracellular concentrations of dopamine in striatum and nucleus accumbens after an acute injection of nomifensine has been previously reported (Butcher et al., 1991; Carboni et al., 1989; Kuczenski and Segal, 1992; Nakachi et al., 1995). Our results are also in agreement with those showing that dopamine uptake inhibitors have smaller effects on extracellular dopamine levels in the prefrontal cortex relative to the nucleus accumbens or striatum (Tzschentke, 2001). The reported lower density of dopamine transporters described in this area of the brain compared to striatum or nucleus accumbens would underlie the differential effects of dopamine uptake inhibitors (Tzschentke, 2001). In fact, diffusion rather than reuptake seems to be the more predominant mechanism to eliminate dopamine from the synaptic cleft in the prefrontal cortex (Garris and Wightman, 1994; Tzschentke, 2001). Lastly, we cannot rule out the possibility that the presence of nomifensine in the perfusion medium of the experiments performed in the prefrontal cortex may be occluding a small effect of nomifensine on dopamine concentrations.

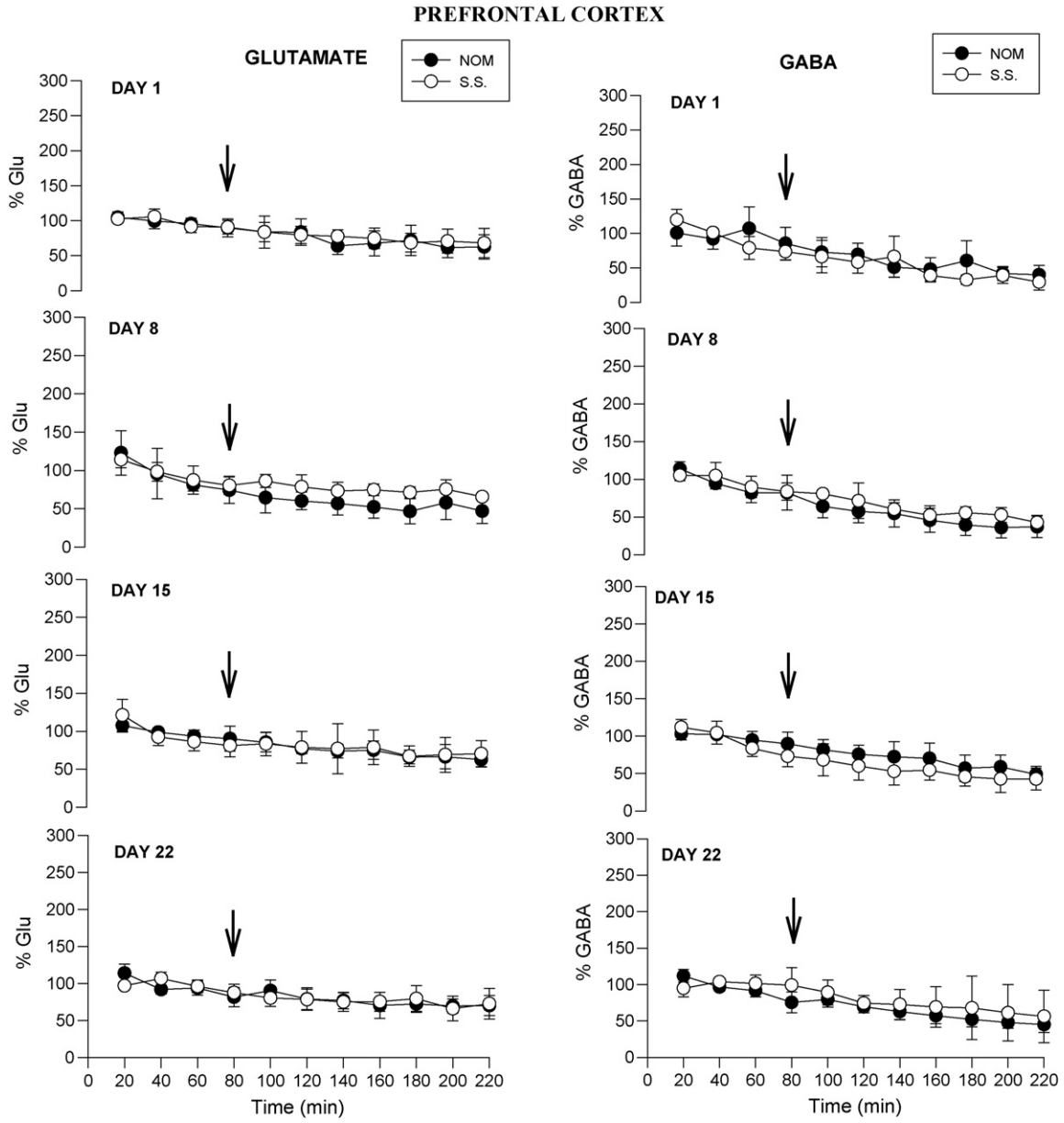

Fig. 2. Effects of nomifensine (NOM 10 mg/kg, i.p., $n = 4–8$) and saline (SS $n = 4–5$) on the extracellular concentrations of glutamate and GABA in the prefrontal cortex. Data (mean ± S.E.M.) are expressed as a percentage of baseline.

In striatum and nucleus accumbens several reports have described a number of changes after chronic treatment with nomifensine, e.g. changes in the expression of dopamine receptors (Lee and Tang, 1982; Martin et al., 1995). However, no changes have been reported in the number of dopamine transporters after chronic treatment with this same drug (Kula and Baldessarini, 1991; Tella et al., 1997). These last reports are in agreement with our own results in which we show that chronic injections of nomifensine did not change the increases of dopamine. Interestingly, it has been reported a potentiation of the increases of dopamine in the nucleus accumbens after short-term chronic treatment with cocaine (Kalivas and Duffy, 1990), which is in agreement with the enhanced expression of dopamine transporters (Alburges et al., 1993; Tella et al., 1996; see also Kula and Baldessarini, 1991). These later findings points to a differential effect of chronic treatment with cocaine and nomifensine (Tella et al., 1996).

4.2. Acetylcholine

Nomifensine increased acetylcholine extracellular concentrations in the prefrontal cortex at all days of treatment. However, these increases were not modified by the chronic treatment. These results are in agreement with previous reports showing that systemic administration of dopamine uptake blockers and dopamine agonists increases acetylcholine levels in the prefrontal cortex (Acquas et al., 1994; Arnold et al., 2001; Day and Fibiger, 1992). In relation to these findings, several reports have suggested the possibility that subcortical GABA systems could play a role in modulating the release of

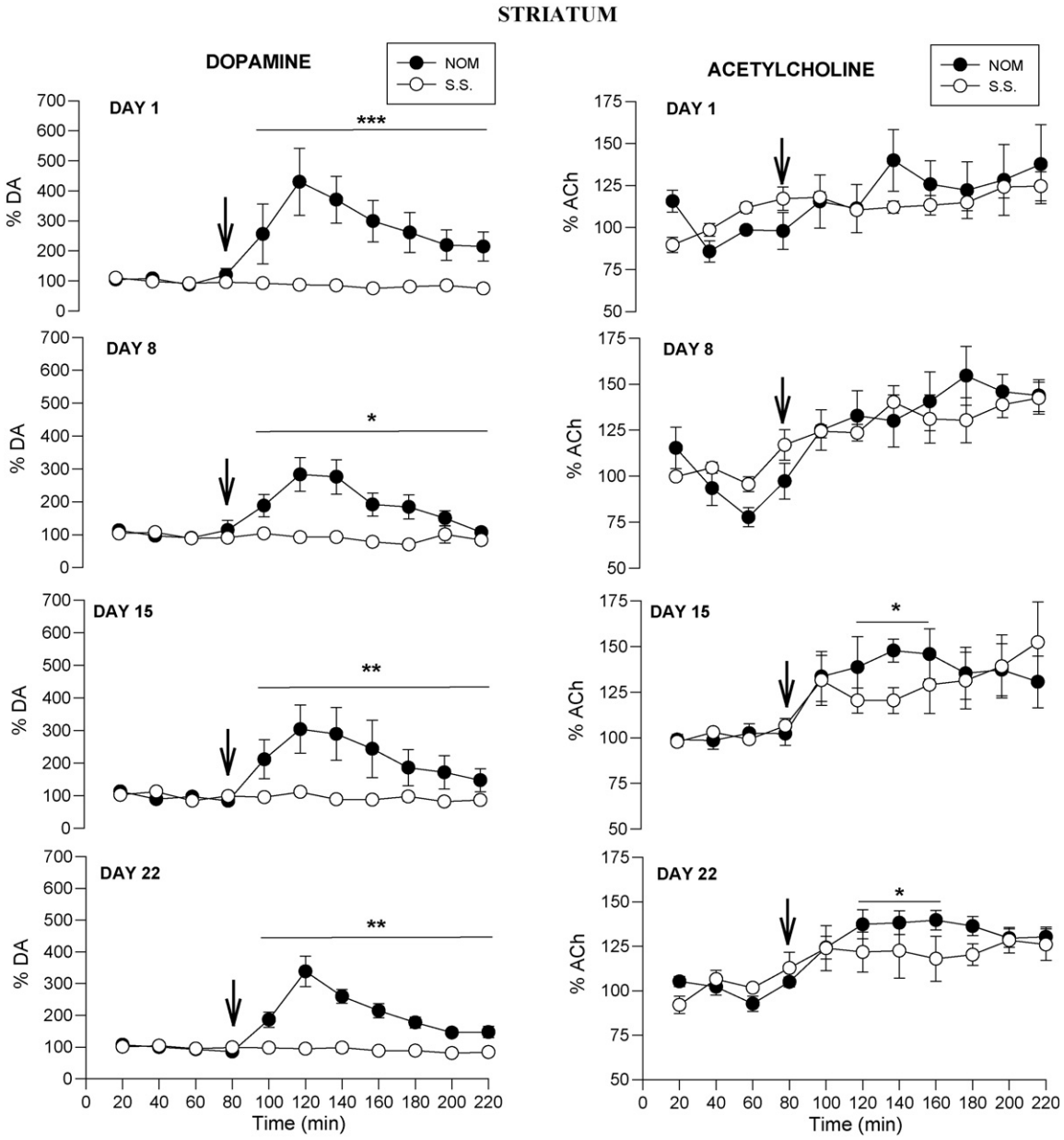

Fig. 3. Effects of nomifensine (NOM 10 mg/kg, i.p., $n = 4–9$) and saline (SS $n = 4–5$) on the extracellular concentrations of dopamine and acetylcholine in the striatum. Data (mean ± S.E.M.) are expressed as a percentage of baseline. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (vs. saline; planned comparisons in a three-way ANOVA with repeated measures design).

acetylcholine in the prefrontal cortex. Briefly, dopamine could inhibit the GABAergic projections from nucleus accumbens to the basal forebrain (Heimer et al., 1997; Zaborszky and Cullinan, 1992), which in turn would release the activity of cholinergic neurons projecting to the prefrontal cortex, resulting in an increased acetylcholine concentration in this area of the brain (Bourdelais and Kalivas, 1992; Yang and Mogenson, 1989). This suggestion, however, has been recently challenged since local perfusion of dopaminergic antagonists in the nucleus accumbens do not completely block the increases in cortical acetylcholine produced by amphetamine (Arnold et al., 2000). Therefore, other mechanisms should be involved in the increases of acetylcholine in prefrontal cortex evoked by nomifensine. In this regard, it has been suggested that the glutamatergic projection from pedunculopontine nucleus to basal forebrain (Zaborszky and Cullinan, 1992) could play a role in regulating cortical acetylcholine release (Fadel et al., 2001).

In contrast to the findings reported here for the prefrontal cortex, injections of nomifensine increased extracellular acetylcholine concentration in striatum after 15 days of treatment (Hernandez et al., 2006). The dopaminergic control of the release of acetylcholine in striatum and nucleus accumbens seems to be mediated, at least in part, by D2 inhibitory and D1 excitatory receptors. In particular acetylcholine release in the striatum is inhibited by local D2 receptors and is stimulated by extra-striatal D1 receptors (i.e., located in substantia nigra pars reticulata, thalamus and/or cortex)

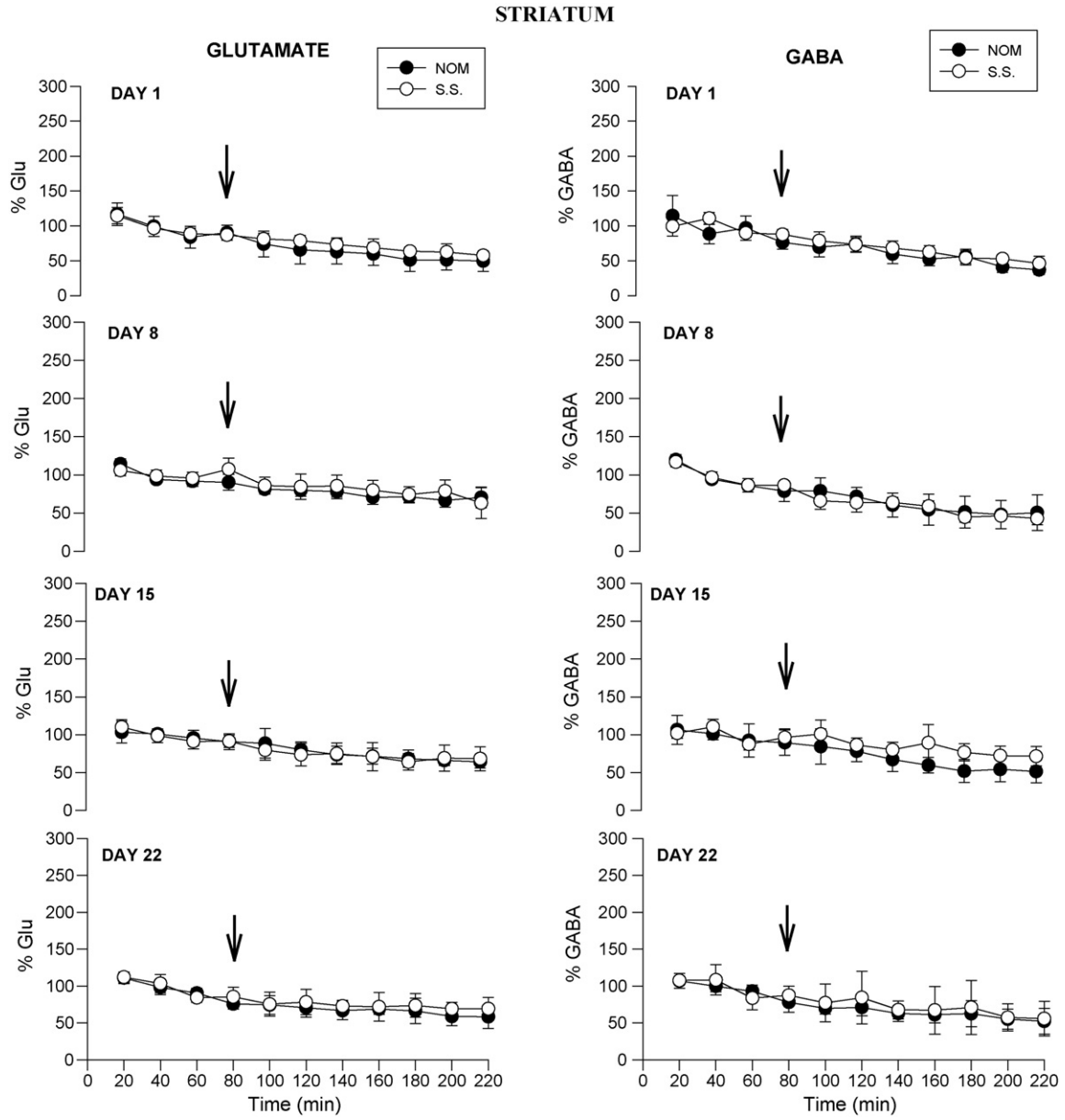

Fig. 4. Effects of nomifensine (NOM 10 mg/kg, i.p., $n = 5–9$) and saline (SS $n = 3–5$) on the extracellular concentrations of glutamate and GABA in the striatum. Data (mean ± S.E.M.) are expressed as a percentage of baseline.

(Abercrombie and DeBoer, 1997; Acquas et al., 1997; de Belleroche and Gardiner, 1983; Ikarashi et al., 1997; Stoof et al., 1992), although a minor excitatory action of local D1 receptors on acetylcholine release has also been described (Ikarashi et al., 1997). Likewise, acetylcholine release in the nucleus accumbens seem to be under the control of local inhibitory D2 receptors (de Belleroche and Gardiner, 1983; Stoof et al., 1992; Wedzony et al., 1988) and local excitatory D1 receptors (Consolo et al., 1999; Keys and Mark, 1998). It is possible that chronic treatment with nomifensine could produce a shift in the dopaminergic receptor balance modulating acetylcholine release in the striatum and nucleus accumbens. In fact, a number of changes in the expression of D1 and D2 receptors in the brain have been shown to occur after chronic treatment with the uptake blockers nomifensine and cocaine (Alburges et al., 1993; Goeders and Kuhar, 1987; Martin et al., 1995; Peris et al., 1990; Schmidt-Mutter et al., 1999; Tella et al., 1996; Tsukada et al., 1996; Unterwald et al., 1994). Thus, increases in D1 receptor expression have been reported in the striatum, nucleus accumbens and substantia nigra (Alburges et al., 1993; Schmidt-Mutter et al., 1999; Tella et al., 1996; Unterwald et al., 1994; but see Cheetham et al., 1995; Hilakivi et al., 1995; Tsukada et al., 1996). Regarding D2 receptors, an increase of their expression has also described in the nucleus accumbens (Goeders and Kuhar, 1987; Peris et al., 1990). The effects of nomifensine or cocaine on D2 expression in striatum is more controversial since increases, decreases and also no changes have been reported (Alburges et al., 1993; Hilakivi et al., 1995; Martin et al., 1995; Schmidt-Mutter et al., 1999; Tsukada et al., 1996). This variability of the results seems to be

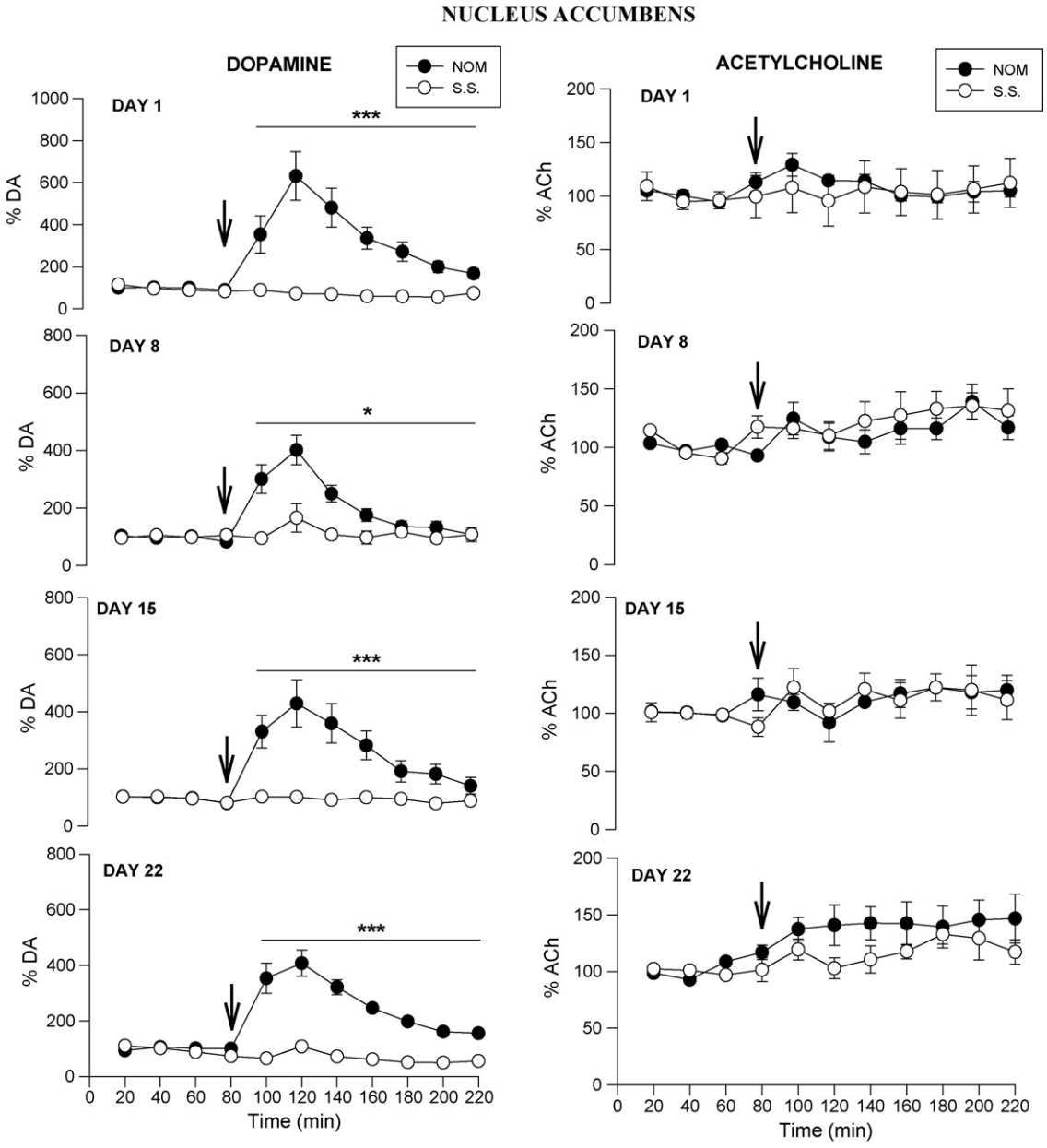

Fig. 5. Effects of nomifensine (NOM 10 mg/kg, i.p., $n = 4$–6) and saline (SS $n = 3$–6) on the extracellular concentrations of dopamine and acetylcholine in the nucleus accumbens. Data (mean ± S.E.M.) are expressed as a percentage of baseline. $^{*}p < 0.05$, $^{***}p < 0.001$ (vs. saline; planned comparisons in a three-way ANOVA with repeated measures design).

related with the use of different administration protocols and treatment durations. Therefore, further research is needed to elucidate the exact contribution of the changes in D1 and D2 receptor expression to the results obtained in the present study.

Table 3
Basal motor activity in the different days of treatment in the nomifensine and control group

Day	Saline	Nomifensine
1	428 ± 53 (14)	396 ± 50 (18)
8	605 ± 79 (13)	513 ± 66 (18)
15	650 ± 71 (13)	596 ± 57 (26)**
22	558 ± 81 (13)	565 ± 46 (23)*

Data shown as mean ± S.E.M. (n) are absolute values of the average of the three basal values. $^{*}p < 0.05$, $^{**}p < 0.02$ vs. day 1 of treatment.

4.3. Glutamate and GABA

To our knowledge there are no previous reports on the effects of systemic injections of nomifensine on the extracellular concentrations of glutamate and GABA in the brain. We report here that injections of nomifensine did not change the extracellular concentrations of glutamate and GABA in any of the areas of the brain studied. These results are in contrast with several other studies showing that dopamine modulates the levels of these amino acids in the prefrontal cortex, striatum and nucleus accumbens (Dalia et al., 1998; de Belleroche and

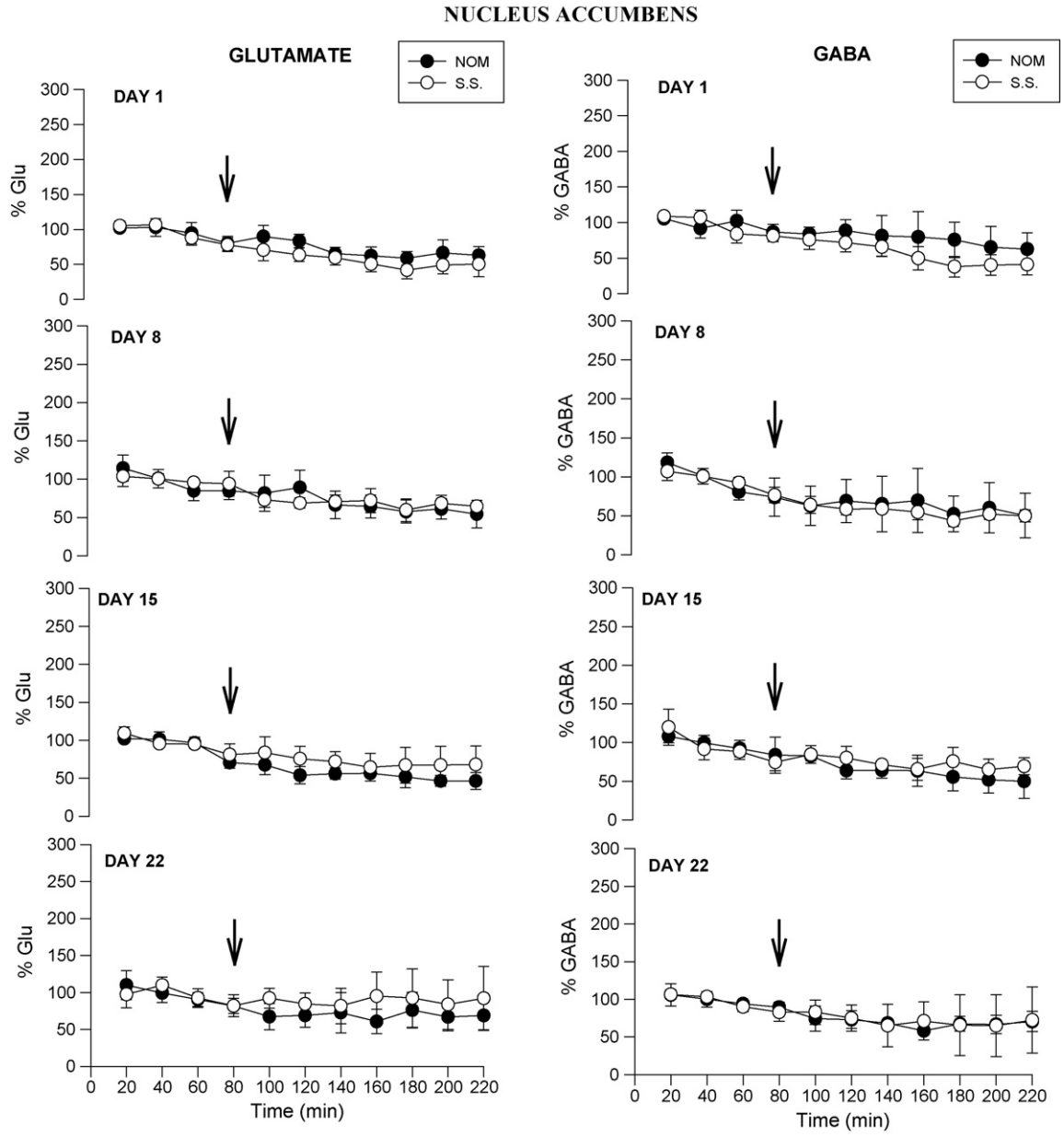

Fig. 6. Effects of nomifensine (NOM 10 mg/kg, i.p., $n = 4–6$) and saline (SS $n = 4–5$) on the extracellular concentrations of glutamate and GABA in the nucleus accumbens. Data (mean ± S.E.M.) are expressed as a percentage of baseline.

Gardiner, 1983; Grobin and Deutch, 1998; Kalivas and Duffy, 1997; Porras et al., 1997; Porras and Mora, 1995). Moreover, previous studies have also shown that injections of cocaine produced increases of glutamate and GABA concentrations in the prefrontal cortex, although these increases were only found in cocaine-sensitised rats (Jayaram and Steketee, 2005; Williams and Steketee, 2004). We have recently proposed that extracellular concentrations of glutamate and GABA, as monitored by microdialysis, are a reflection of the activity of the neuron-astrocyte network (Del Arco et al., 2003). In line with this hypothesis, we suggest here that chronic treatment with nomifensine may modulate this interaction so as to render no changes of glutamate and GABA in the areas under study. Further research is needed to clarify the mechanics through which blockade of dopamine uptake could influence the extracellular concentrations of glutamate and GABA.

4.4. Motor activity

The effects of chronic treatment with nomifensine on motor activity were also investigated in the present study. After each daily injection of nomifensine, animals showed an increase in locomotor activity and stereotyped behaviour (Garris et al., 2003; Nakachi et al., 1995; Stanford et al., 2002). However, chronic treatment did not modify these motor responses. Some reports indicated a relationship between the increases of dopamine in basal ganglia and the increase in motor activity after the systemic administration of a dopamine uptake blocker.

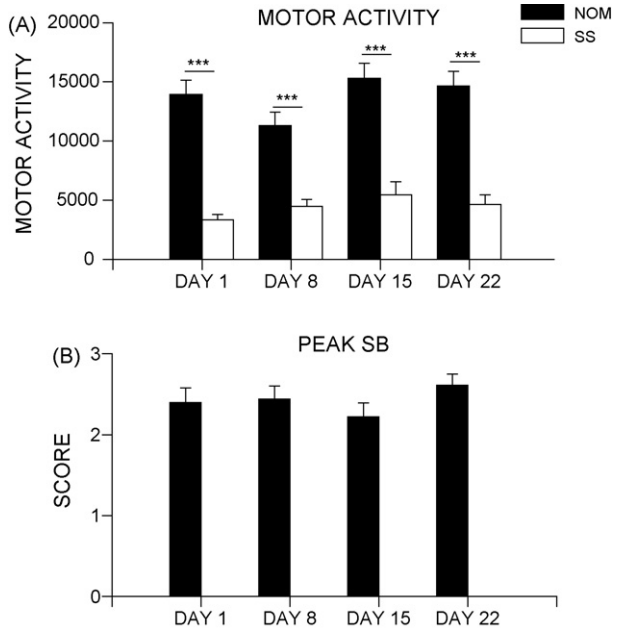

Fig. 7. Effects of nomifensine (NOM 10 mg/kg, i.p., $n = 18–26$) and saline (SS $n = 13–14$) on motor activity (A) and stereotyped behaviour (SB) (B). (A) Data (mean ± S.E.M.) are expressed as the cumulative increase in absolute values. $***p < 0.001$ (vs. saline values); planned comparisons in a three-way ANOVA with repeated measures design). (B) Data (mean ± S.E.M.) are expressed as the average of the score of peak stereotyped behaviour (peak SB) (40–60 min after nomifensine injection).

In fact, it has been suggested that after administration of dopamine uptake blockers, increases of dopamine found in the nucleus accumbens are related to the locomotor activity; meanwhile the increases of dopamine in the striatum are related to the stereotyped behaviour (al-Khatib et al., 1995; Kelly et al., 1975; Nakachi et al., 1995; Sharp et al., 1987). This would be in agreement with our results from daily injections of nomifensine. Also, our findings in which the increases of dopamine induced by nomifensine in the striatum and nucleus accumbens were not modified as a result of the chronic treatment may explain the lack of changes in the motor responses along treatment. These results contrast with the behavioural sensitisation shown by animals chronically treated with cocaine (Jayaram and Steketee, 2005; Peris et al., 1990; Unterwald et al., 1994; Williams and Steketee, 2004). However, to our knowledge there are no studies reporting sensitisation to long-term treatment with nomifensine. Therefore, our results would provide a further understanding of the different behavioural consequences of chronic treatment with the uptake blockers nomifensine and cocaine (Tella et al., 1997).

5. Conclusions

It has been previously suggested the possible existence of at least two distinct classes of dopamine uptake blockers. One class of blockers (cocaine) would lead to up-regulation of dopamine transporters, whereas the treatment with another class (nomifensine) does not alter transporter levels (Tella et al., 1997). The differential changes in neurotransmitter release and of expression of dopamine receptors found after chronic treatment with nomifensine or cocaine (reviewed in Section 4) would support this suggestion. These differences in neuroadaptive changes produced after long-term treatment would underlie differences in the behavioural responses of these two classes of dopamine uptake blockers.

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