Selective enhancement of mesocortical dopaminergic transmission by noradrenergic drugs: therapeutic opportunities in schizophrenia

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Abstract

The superior efficacy of atypical vs. classical antipsychotic drugs to treat negative symptoms and cognitive deficits in schizophrenia appears related to their ability to enhance mesocortical dopamine (DA) function. Given that noradrenergic (NE) transmission contributes to cortical DA output, we assessed the ability of NE-targeting drugs to modulate DA release in medial prefrontal cortex (mPFC) and nucleus accumbens (NAc), with the aim of selectively increasing mesocortical DA. Extracellular DA was measured using brain microdialysis in rat mPFC and NAc after local/systemic drug administration, electrical stimulation and selective brain lesions. Local GBR12909 [a selective DA transporter (DAT) inhibitor] administration increased DA output more in NAc than in mPFC whereas reboxetine [a selective NE transporter (NET) inhibitor] had an opposite regional profile. DA levels increased comparably in both regions of control rats after local nomifensine (DAT + NET inhibitor) infusion, but this effect was much lower in PFC of NE-lesioned rats (DSP-4) and in NAc of 6-OHDA-lesioned rats. Electrical stimulation of the locus coeruleus preferentially enhanced DA output in mPFC. Consistently, the administration of reboxetine + RX821002 (an α₂-adrenoceptor antagonist) dramatically enhanced DA output in mPFC (but not NAc). This effect also occurred when reboxetine + RX821002 were co-administered with haloperidol or clozapine. The preferential contribution of the NE system to PFC DA allows selective enhancement of DA transmission by simultaneously blocking NET and α₂-adrenoceptors, thus preventing the autoreceptor-mediated negative feedback on NE activity. Our results highlight the importance of NET and α₂-adrenoceptors as targets for treating negative/cognitive symptoms in schizophrenia and related psychiatric disorders.

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Introduction

Mesocortical and mesolimbic dopamine (DA) systems play a crucial role in many psychiatric disorders including schizophrenia (Carlsson, 1978). A general enhancement of brain dopaminergic neurotransmission in schizophrenia was suggested by pharmacological evidence (Creese et al., 1976; Seeman & Lee, 1975). However, current views indicate a hyperactivity of subcortical DA transmission together with a hypoactive mesocortical system (Abi-Dargham et al., 2000; Akil et al., 1999; Laruelle et al., 1996; Lewis & Lieberman, 2000; Weinberger et al., 1988).

The overall efficacy of classical (DA D₂ receptor antagonists) and atypical antipsychotics (APDs, preferential 5-HT₂A/₂C vs. DA D₂ receptor antagonists) to treat positive symptoms is similar (Lieberman et al., 2005). In contrast, some APDs, and particularly clozapine, are superior to classical APDs for the treatment of negative symptoms and cognitive impairment (Kane et al., 1988; Keefe et al., 2006; Leucht et al., 2009; Meltzer & McGurk, 1999). This clinical feature has been related to the ability of atypical (but not classical)
APDs to increase DA release in the mesocortical pathway (Diaz-Mataix et al. 2005; Ichikawa et al. 2001; Kuroki et al. 1999; Rollema et al. 1997; Westerink et al. 2001). Indeed, an optimal prefrontal DA function is crucial for working memory and executive functions (Castner et al. 2000; Floresco & Magyar, 2006; Goldman-Rakic et al. 2000; Robbins & Arnsten, 2009; Vijayaraghavan et al. 2007; Williams & Goldman-Rakic, 1995).

A key step determining the intensity and duration of synaptic DA signalling is the reuptake of the released transmitter into nerve terminals through high-affinity plasma membrane transporters. Previous studies indicate a lower density of DA transporter (DAT) in PFC compared to striatum (Letchworth et al. 2000; Marshall et al. 1990; Sesack et al. 1998). Conversely, the PFC contains a higher density of noradrenaline (NE) transporter (NET) (Miner et al. 2003; Schroeter et al. 2000) compared to NAc. In fact, NE axons may contribute to the removal of DA from the extracellular brain space, since NET shows a similar affinity for NE and DA (Raiteri et al. 1990, 2006; Mazei et al. 1990; Marshall et al. 1997; Westerink et al. 1999; Rollema et al. 1997; Westerink et al. 1999).

Here we evaluated simultaneously the contribution of noradrenergic transmission to DA reuptake and release in both mesocortical and mesolimbic pathways. As a result, we report on a marked and selective enhancement of mesocortical DA transmission by combining the NET inhibitor reboxetine and the α2-adrenoceptor antagonist RX821002.

**Drugs and reagents**

Desipramine hydrochloride, GBR12909 dihydrochloride, haloperidol, nomifensine maleate, N-(2-chloro-ethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4), 6-hydroxydopamine hydrochloride (6-OHDA) and RX821002 hydrochloride were purchased from Sigma (Spain). Clozapine, fluoxetine hydrochloride and reboxetine mesylate were obtained from Tocris (UK). Drugs were dissolved in artificial cerebrospinal fluid [aCSF (mm): NaCl, 125; KCl, 2.5; CaCl2, 2.52; MgCl2, 1.18] and distilled water for local and systemic administration (pH adjusted to 6–7), respectively. Clozapine was dissolved in few drops of glacial acetic acid and diluted with saline. DSP-4, 6-OHDA and GBR12909 solutions were prepared prior to use. 6-OHDA was dissolved in water containing 0.1% ascorbic acid. All reagents used were of analytical grade and obtained from Merck (Germany).

**Microdialysis procedures**

Microdialysis experiments were conducted as previously described (Bortolozzi et al. 2005; Diaz-Mataix et al. 2005). Briefly, concentric dialysis probes were implanted under pentobarbital anaesthesia (60 mg/kg i.p.) at the following brain coordinates (in mm): mPFC, AP +3.2, L −0.8, DV −6.0, 4-mm membrane length; or NAc, AP +1.6, L −1.1, DV −8.0, 1.5-mm membrane length (Paxinos & Watson, 1998). The probe in NAc samples included core and shell subdivisions (Fig. 1b). Groups of rats were implanted with two probes ipsilaterally in mPFC (as above) and NAc (AP +1.6, L −3.9, DV −7.5, with a lateral 20° angle). Experiments were performed in freely moving rats ~20 h after surgery except those involving the electrical stimulation of LC (see below). Probes were perfused with aCSF pumped at 1.5 µl/min. After an initial 30-min stabilization period, four baseline samples were collected (20 min/fraction) before local or systemic drug administration. Control groups were perfused with aCSF or injected with vehicle.

To examine the effects of electrical stimulation of LC on DA release, a stimulating electrode was implanted in LC at AP −2.0 (from lambda; nose down 15° from horizontal plane), L −1.2, DV −7.2 (Mateo et al. 1998). The bipolar stimulating electrode consisted of two stainless-steel enamel-coated wires (California Fine Wire, USA) with a diameter of 150 µm and in-vitro impedances of 10–30 kΩ. Additionally, two microdialysis probes were implanted in mPFC and NAc (as above). On the following day, rats were anaesthetized with chloral hydrate (400 mg/kg i.p. followed by supplementary doses of 50–70 mg/kg, i.p.). Ten-min

**Materials and methods**

**Animals**

Male Wistar rats (250–320 g, Iffa-Credo, France) were maintained on a 12-h light/dark cycle (lights on 07:00 hours), room temperature 22±2 °C, with food and water available ad libitum. Animal care followed European Union regulations (O. J. of E. C. L358/1 18/12/1986) and was approved by the Institutional Animal Care and Use Committee of the School of Medicine, University of Barcelona.
dialysate fractions were collected (flow rate 3.0 \( \mu l/\) min) and body temperature was maintained at 37 \( ^{\circ}C \) with a heating pad. LC was phasically stimulated for 20 min after four basal fractions: 0.1-ms pulses delivered at 20 Hz for 250 ms every 1 s (average frequency 5 Hz) at 0.7 mA for 20 min using a Grass stimulation unit S-48 (Devoto et al. 2005; Florin-Lechner et al. 1996).

Brain dialysate fractions were collected on microvials containing 5 \( \mu l \) of 0 M HClO4 and rapidly injected into the HPLC equipment as described previously (Diaz-Mataix et al. 2005). DA was detected amperometrically (+0.7 V) (Hewlett-Packard 1049, USA) with a limit of detection of 2–3 fmol/sample. At the end of the experiments, animals were killed by an anaesthetic overdose. Brains were quickly removed and frozen on dry ice before sectioning (40 \( \mu m \)) with a cryostat (HM500-Om Microm, Germany). Coronal brain sections were stained with Neutral Red to verify the correct placement of probes and electrodes (Fig. 1).

**Brain lesions**

To examine the relative contribution of noradrenergic and dopaminergic systems to the release of DA in mPFC and NAc, we performed specific lesions: 
(a) treatment with the NE neurotoxin DSP-4, and 
(b) lesion of ventral tegmental area (VTA) DA system with 6-OHDA. To lesion NE neurons, DSP-4 (40 mg/kg i.p.) was administered 60 min after the injection of fluoxetine (10 mg/kg i.p.) and GBR12909 (20 mg/kg s.c.), to protect serotonin (5-HT) and DA neurons, respectively (Bortolozzi & Artigas, 2003; Dailly et al. 2006; Fritschy & Grzanna, 1989). Microdialysis experiments were performed 5 d after DSP-4 administration in awake rats implanted with a single probe in mPFC or NAc.

For 6-OHDA lesions, rats were pre-treated 60 min before with fluoxetine (10 mg/kg i.p.) and desipramine (25 mg/kg s.c.) to protect 5-HT and NE neurons, respectively (Robinson & Whishaw, 1988; Tseng et al. 2005). Rats were unilaterally injected with 6-OHDA (total dose 8 \( \mu g/1 \) \( \mu l \)) in two locations within the VTA [AP -5.2, L -2.2 (10\( ^{\circ} \)), DV -7.8; AP -5.8, L -1.9 (10\( ^{\circ} \)), DV -7.7]. The injection rate was 1 \( \mu l/min \), followed by a 3-min pause before slowly withdrawing the infusion cannula. Microdialysis experiments were performed 8 d later in rats implanted with two microdialysis probes, one in mPFC (or NAc) ipsilateral to the lesioned VTA and the other one in the contralateral mPFC (or NAc), used as a control [mPFC: AP +3.2, L ±1.5 (10\( ^{\circ} \)), DV -5.7; NAc: AP +1.6, L ±3.9 (20\( ^{\circ} \)), DV -7.5].

The efficacy of DSP-4 and 6-OHDA to lesion NE and DA systems, respectively, was assessed by analysis of NE and DA in brain tissue by HPLC-ED (Adell et al. 1989; Bortolozzi & Artigas, 2003). The effect of lesions was examined in brain areas containing a substantial innervation of NE (PFC, for DSP-4 lesion) and DA (NAc, for 6-OHDA lesion). At the end of the microdialysis experiments, rats were killed and their brains were quickly removed and placed over a cold plate. In these animals, probe location was assessed by visual inspection with a low power magnification microscope. Brains were sectioned at 1-mm-wide coronal sections and PFC (for DSP-4 lesion) and NAc (for 6-OHDA lesion) were carefully dissected out. Only rats with more than 90% depletions in NE (DSP-4) or DA (6-OHDA) lesion were included.

**Statistical analysis**

Microdialysis results are expressed as fmol/30-\( \mu l \) fraction and shown as percentages of baseline. Area under the curve (AUC) of selected time-periods were also used. Statistical analysis was performed using one- or two-way ANOVA of AUC or
DA values (repeated measures) followed by Newman–Keuls post-hoc test. Basal DA dialysate levels and tissue monoamine contents were compared using Student’s t test. Data are expressed as means ± S.E.M. The significance level was set at $p < 0.05$.

**Results**

**Local effect of DAT and NET inhibitors on DA output in mPFC and NAc**

Perfusion of aCSF did not significantly alter DA output in mPFC and NAc of awake rats. The mean baseline concentrations of DA dialysate samples from mPFC and NAc (single probe experiments) were $19 ± 1$ fmol/fraction ($n = 33$) and $19 ± 3$ fmol/fraction ($n = 27$), respectively.

Local infusion of the DAT + NET inhibitor nomifensine (1, 10 and 30 $\mu$M) increased extracellular DA in both areas in a concentration-dependent manner (Fig. 2a). The maximal DA increase ($AUC_{6–16}$) was $660 ± 52\%$ in mPFC and $527 ± 130\%$ in NAc at 30 $\mu$M ($n = 5$ each). Two-way ANOVA of AUCs revealed a significant effect of concentration ($F_{3,33} = 27.13$, $p < 0.00001$) and non-significant effects of region and concentration × region interaction. Post-hoc test (Newman–Keuls) revealed significant differences among all tested concentrations.

Unlike nomifensine, significant regional differences were found for the selective DAT (GBR12909) and NET (reboxetine) inhibitors after local administration. Local GBR12909 infusion markedly increased DA output in NAc and evoked a minor increase in mPFC (Fig. 2b). Mean DA elevations at 30, 100 and 300 $\mu$M, expressed as percentage of baseline, were respectively: (a) mPFC ($n = 6$): $146 ± 11$, $150 ± 24$ and $122 ± 28$ and (b) NAc ($n = 4$): $382 ± 75$, $390 ± 91$ and $287 ± 80$. Two-way ANOVA of AUCs showed a significant effect of the drug concentration ($F_{3,33} = 6.39$, $p < 0.01$), region ($F_{1,32} = 27.92$, $p < 0.00001$) and concentration × region interaction ($F_{3,32} = 3.19$, $p < 0.05$). Post-hoc test (Newman–Keuls) revealed significant differences between NAc and mPFC at concentrations of 30 and 100 $\mu$M and a marginal difference ($p = 0.052$) at 300 $\mu$M.

Local reboxetine application elevated extracellular DA in mPFC but not in NAc (Fig. 2c). Reboxetine effects at 1, 10 and 30 $\mu$M, expressed as percentage of baseline, were respectively: (a) mPFC ($n = 6$): $267 ± 19$, $278 ± 21$, and $282 ± 33$ and (b) NAc ($n = 4$): $129 ± 21$, $117 ± 28$ and $90 ± 19$. Two-way ANOVA showed a significant effect of concentration ($F_{3,32} = 8.50$, $p < 0.001$), region ($F_{1,32} = 60.07$, $p < 0.0001$) and concentration ×
Effects of selective brain lesions on the modulation of DA output in mPFC and NAc induced by nomifensine

We further explored the effect of nomifensine in the mPFC and NAc of rats underlying specific lesions with (a) the NE neurotoxin DSP-4 and (b) unilateral lesion of VTA DA system with 6-OHDA, respectively.

Rats pretreated with DSP-4 showed a 90% depletion of tissue NE level (85 ± 8 vs. 894 ± 92 pmol/g of wet tissue in PFC, p < 0.00001) without a significant change in tissue DA in this area. DSP-4-lesioned rats had higher basal values (fmol/fraction) of dialysate DA in mPFC (37 ± 8, n = 6) and NAc (31 ± 6, n = 6), compared to their respective controls in mPFC (15 ± 2, n = 5) and NAc (24 ± 7, n = 5), yet only the DA increase in mPFC reached statistical significance (p < 0.05) (Fig. 3c).

Local infusion of nomifensine (30 µM) by reverse dialysis increased mPFC DA output in control rats to 660 ± 52% of baseline, but to a much lower extent (180 ± 11% of baseline) in mPFC of DSP-4-pretreated rats (Fig. 3a). However, DSP-4 pretreatment did not alter the ability of nomifensine to increase extracellular DA in NAc (DA increase of 528 ± 130% and 682 ± 176% in control and DSP-4-pretreated rats, respectively). Two-way ANOVA revealed a significant effect of lesion × region interaction (F_{1,18} = 7.48, p < 0.05). Post-hoc t test revealed that the effect of nomifensine in mPFC of DSP-4-lesioned rats was significantly different to the other groups.

The unilateral dopaminergic VTA lesion by 6-OHDA was assessed in the same rats comparing lesion side (ipsilateral to 6-OHDA application) with control side (contralateral to 6-OHDA application). Tissue DA and NE levels were significantly decreased to 93% and 71%, respectively in NAc (492 ± 174 vs. 7226 ± 2272 pmol/g of DA in wet tissue and 298 ± 53 vs. 1019 ± 1173 pmol/g of NE in wet tissue, p < 0.01). Basal DA values (fmol/fraction) in the control and lesioned sides were: (a) control mPFC: 12 ± 4, (b) lesioned mPFC: 9 ± 3, (c) control NAc: 11 ± 4 and, (d) lesioned NAc: 9 ± 2 (n = 5–6). Non-significant differences of DA output were found between control and lesioned sides.

Simultaneous local nomifensine (30 µM) infusion by reverse dialysis in both control and lesioned mPFC increased DA output to 660 ± 72% (control mPFC) and 405 ± 47% (lesioned mPFC) of baseline (Fig. 3b). On the other hand, nomifensine (30 µM) perfusion enhanced DA output to 798 ± 194% of baseline in control NAc and to 284 ± 69% in lesioned NAc. Two-way ANOVA revealed a significant effect of lesion (F_{1,12} = 11.03, p < 0.01) and non-significant effects of region and lesion × region interaction. Post-hoc Newman–Keuls
test indicated that only DA values in lesioned NAc were statistically different from control NAc.

**Electrical stimulation of LC differentially increases DA output in mPFC and NAc**

We then assessed the effect of electrical stimulation of LC on DA output in mPFC and NAc of the same rats. Mean DA basal values (fmol/10-min fraction) were $15\pm3$ ($n=18$) and $17\pm3$ ($n=17$) in mPFC and NAc, respectively.

Burst LC stimulation ($n=5$) significantly elevated extracellular DA in mPFC and NAc compared to animals with misplaced electrodes, including pericoeruleus area ($n=9-10$) and sham control rats ($n=3$, no current was passed through the LC electrode) (Fig. 4). The DA output in mPFC showed a sharp rise during the stimulation period which declined rapidly. Maximal DA increase was $244\pm44\%$ of baseline. Two-way ANOVA revealed significant effects of the treatment ($F_{2,14}=6.17$, $p<0.05$), time ($F_{9,168}=8.94$, $p<0.0001$) and treatment $\times$ time interaction ($F_{18,168}=3.78$, $p<0.0001$). Burst LC stimulation induced a modest and slow increase of DA release in NAc ($153\pm20\%$ of baseline). Two-way ANOVA indicated a significant effect of time ($F_{9,128}=2.13$, $p<0.05$) and treatment $\times$ time interaction ($F_{18,128}=1.89$, $p<0.05$) and non-significant effects of stimulation and time. Post-hoc Newman–Keuls test indicated that DA output in mPFC during LC stimulation was significantly greater than in control rats.

Finally, two-way ANOVA of DA output of the stimulated groups revealed significant effects of time ($F_{9,12}=11.67$, $p<0.00001$) and time $\times$ region interaction ($F_{9,12}=2.39$, $p<0.05$). Post-hoc Newman–Keuls test indicated that the DA output in the mPFC during LC stimulation was significantly greater than in NAc.

**Selective enhancement of cortical DA output by noradrenergic drugs in APD pre-treated rats**

Overall, the above results suggest that (1) the extracellular DA concentration is distinctly regulated in mPFC and NAc, and (2) NE terminals markedly contribute to the control of mPFC DA (but not of NAc DA) either by co-releasing DA and/or taking up DA via NET.

We next studied the feasibility of selectively increasing mesocortical DA transmission through NE-acting drugs. We conducted two sets of experiments in rats implanted with two microdialysis probes (mPFC and NAc). Baseline DA concentrations in mPFC and NAc (double-probe experiments) were $9\pm1$ fmol/fraction ($n=69$) and $9\pm2$ fmol/fraction ($n=62$), respectively.

In the first set of experiments, the selective NET inhibitor reboxetine (30 μM) was locally applied by reverse dialysis in mPFC and NAc followed by the systemic administration of the selective $\alpha_2$-adrenoceptor antagonist RX821002 (1 mg/kg i.p.) 2 h later, to disinhibit the autoreceptor-mediated negative feedback on NE neuron activity.
extracellular DA (AUCs) obtained in mPFC and NAc during local reboxetine application plus its combination with RX821002. Two-way ANOVA revealed a significant effect of treatment \((F_{2,18} = 79.18, p < 0.0001)\) and region \((F_{1,18} = 50.40, p < 0.0001)\) as well as a significant treatment \(\times\) region interaction \((F_{2,18} = 20.74, p < 0.0001)\).

In the second set of experiments, both drugs were given systemically (reboxetine, 3 mg/kg i.p.; RX821002, 1 mg/kg s.c.). The effect of the reboxetine + RX821002 combination was examined alone and in rats pretreated with APD drugs: haloperidol (classical, 0.1 mg/kg s.c.) and clozapine (APD, 3 mg/kg s.c.). The whole set of data are shown in Fig. 6 and were analysed using two-way ANOVA with treatment and time as main factors (main effects are shown in Table 1). The AUC data of relevant fractions \((12-16)\) for each combination treatment were also calculated and analysed by two-way ANOVA with treatment and region as main factors (Fig. 7).

**Vehicle + reboxetine + RX821002**
Vehicle injections did not alter DA output in mPFC or NAc. The administration of either reboxetine or RX821002 alone moderately enhanced DA output, yet their combined administration dramatically increased DA levels in mPFC \((869 \pm 139\% \text{ of baseline}, n = 5)\) but not in NAc \((188 \pm 33\%, n = 4)\) (Fig. 6a,b; see Table 1 for detailed statistical analysis). Post-hoc \(t\) tests (Newman–Keuls) revealed a significant difference of the reboxetine + RX821002 treatment vs. the rest of experimental groups in mPFC. No significant differences were found for DA values in NAc.

Two-way ANOVA of AUC data (Fig. 7a) revealed a significant effect of treatment \((F_{3,39} = 26.6, p < 0.00001)\), region \((F_{1,39} = 21.0, p < 0.0001)\) and region \(\times\) treatment interaction \((F_{3,39} = 14.9, p < 0.00001)\).

**Clozapine + reboxetine + RX821002**
The systemic administration of clozapine elevated DA output to \(241 \pm 27\% \text{ of baseline in mPFC} (n = 4, \text{ data are } AUC_{4-16})\). In NAc, systemic clozapine administration did not significantly alter DA output \((99 \pm 13\%, n = 4)\).

The combination of clozapine + reboxetine + RX821002 markedly increased DA output to \(781 \pm 123\% \text{ of baseline in mPFC} (n = 6)\). This effect was significantly greater than that of clozapine alone and that of clozapine + reboxetine (Newman–Keuls test post-ANOVA) (Fig. 6b, Table 1). In NAc, the administration of reboxetine + RX821002 increased the effect of clozapine to \(335 \pm 127\% \text{ of baseline} (n = 5)\) (Fig. 6d, Table 1). No significant differences between treatments were found in NAc.

When comparing AUC data within regions (Fig. 6b), two-way ANOVA revealed a significant effect of treatment \((F_{3,30} = 11.9, p < 0.0001)\), region \((F_{1,30} = 10.5, p < 0.005)\) and a marginal significance of region \(\times\) treatment interaction \((F_{3,30} = 2.5, p = 0.078)\).

**Haloperidol + reboxetine + RX821002**
The systemic administration of haloperidol, given alone, did not alter the DA output in mPFC \((116 \pm 12\%, n = 9, AUC_{4-16})\), while in NAc it significantly increased DA levels to \(162 \pm 20\% \text{ of baseline} (n = 7)\).

The combination of haloperidol + reboxetine + RX821002 induced a large elevation of DA levels in mPFC to \(1375 \pm 275\% \text{ of baseline} (n = 6)\). This effect was significantly greater than that of haloperidol alone or that of the combined administration of reboxetine + RX821002 (post-hoc Newman–Keuls; Fig. 6c, Table 1). In NAc, the co-administration of haloperidol + reboxetine + RX821002 increased DA levels to \(238 \pm 43\% \text{ of baseline} (n = 5)\) (Fig. 6f, Table 1). No significant differences between treatments were found for DA values in this region.

Two-way ANOVA of AUC data (Fig. 7c) revealed a significant effect of treatment \((F_{3,39} = 18.2, p < 0.00001)\), region \((F_{1,39} = 19.5, p < 0.0001)\) and region \(\times\) treatment interaction \((F_{3,39} = 14.2, p < 0.00001)\).
Discussion

The results of the present study confirm and extend previous observations on a differential regulation of DA release in mPFC and NAc. We systematically compared the effect of agents modulating extracellular DA in both pathways. The data support a significant contribution of noradrenergic neurotransmission to DA release in mPFC compared to NAc. The different mechanisms involved in the control of the active (extracellular) DA fraction in both areas offer new therapeutic opportunities to treat non-psychotic symptoms in schizophrenia, associated with a reduced cortical dopaminergic function. Hence, we demonstrate that noradrenergic drugs dramatically and selectively enhance mesocortical DA, using a strategy previously shown to potentiate the effects of reuptake blockers on serotonergic (Artigas et al. 1996) and noradrenergic systems (Mateo et al. 1998).

Relative contribution of NE neurotransmission to DA output in mPFC and NAc

The effects of DAT and/or NET inhibitors agree with previous observations indicating that NET inhibitors increase extracellular NE and DA in PFC, but not in...
Table 1. Statistical analyses of the combination treatment of NE-targeting drugs, reboxetine and RX821002, on dopamine release in vehicle, clozapine and haloperidol pre-treated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>mPFC</th>
<th>NAc</th>
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<tr>
<td></td>
<td>Effect</td>
<td>F</td>
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<tr>
<td>Vehicle</td>
<td></td>
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<tr>
<td>Veh + Veh</td>
<td>93±19  (6)</td>
<td>T&lt;sub&gt;3.15&lt;/sub&gt;=19.0</td>
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<tr>
<td>Reb + Veh</td>
<td>195±38 (5)</td>
<td>t&lt;sub&gt;12,24&lt;/sub&gt;=33.6</td>
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<tr>
<td>Veh + RX</td>
<td>234±42 (4)</td>
<td>T × t&lt;sub&gt;12,24&lt;/sub&gt;=19.0</td>
</tr>
<tr>
<td>Reb + RX</td>
<td>869±139* (6)</td>
<td></td>
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<tr>
<td>Clozapine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veh + Veh</td>
<td>197±30 (4)</td>
<td>T&lt;sub&gt;3.15&lt;/sub&gt;=5.0</td>
</tr>
<tr>
<td>Reb + Veh</td>
<td>180±46 (4)</td>
<td>t&lt;sub&gt;12,24&lt;/sub&gt;=24.2</td>
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<tr>
<td>Veh + RX</td>
<td>348±67 (5)</td>
<td>T × t&lt;sub&gt;12,24&lt;/sub&gt;=10.3</td>
</tr>
<tr>
<td>Reb + RX</td>
<td>761±123* (4)</td>
<td></td>
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<tr>
<td>Haloperidol</td>
<td></td>
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<tr>
<td>Veh + Veh</td>
<td>120±16 (6)</td>
<td>T&lt;sub&gt;3.15&lt;/sub&gt;=18.8</td>
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<tr>
<td>Reb + Veh</td>
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<td>T × t&lt;sub&gt;12,128&lt;/sub&gt;=16.0</td>
</tr>
<tr>
<td>Reb + RX</td>
<td>1375±275* (9)</td>
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</table>

Data (effect) are given as percentage of baseline in each experimental group (AUC<sub>T<sub>-10</sub></sub>). Number of animals in each group is given in parentheses.

*Significantly different to all other treatments.
+ Significantly different to all treatments except for Veh + RX.

Data have been analysed using two-way ANOVA with treatment (T) and time (t) as main factors.

**Noradrenergic contribution to mesocortical DA**

Trans-synaptically to reach NET sites, as observed for DA itself (Sesack et al. 1998) (see Fig. 8a).

Furthermore, DSP-4 (Fig. 3a) almost abolished the effect of nomifensine on DA output in mPFC, but not in NAc, further supporting a preferential contribution of NE fibres to mPFC DA release. We used standard lesion procedures to extensively damage DA and NE neurons/fibres. Thus, similarly to previous studies (Bortolozzi & Artigas, 2003; Fritschy & Grzanna, 1989; Robinson & Whishaw, 1988; Tseng et al. 2005) DSP-4 and 6-OHDA depleted tissue NE and DA concentrations by >90%, respectively. The clear-cut mPFC–NAc difference of DSP-4 on nomifensine’s effect may reflect the aforementioned differences on NE axon densities innervating both brain structures and also their ability to release DA. Moreover, a preferential LC sensitivity to the DSP-4 lesion cannot be discounted (Dailly et al. 2006; Grzanna et al. 1989; Jonsson et al. 1981). A limitation of the present study is the lack of immunohistochemical or autoradiographic analysis of lesions, which, as in other studies (Grzanna et al. 1989;
Vos et al. (1999; Szot et al. 2010) would have permitted a detailed assessment of lesion effects on NE and DA axons. Conversely, local nomifensine increased DA output less in NAc than in mPFC of the unilaterally 6-OHDA-lesioned rats (Fig. 3b), compared to the contralateral (unlesioned) side, respectively, suggesting that most DA output in NAc arises from VTA DA fibres. Even with the prior protection of NE fibres by desipramine prior to 6-OHDA application, a marked reduction of tissue NE was found in the ipsilateral NAc (71% for NE vs. 93% for DA), possibly due to toxin diffusion to the neighbouring median forebrain bundle. Previous studies have shown the inability of desipramine to fully protect NE axons from 6-OHDA lesions (Harden et al. 1998; King & Finlay, 1995).

Although DSP-4 and 6-OHDA markedly affected the nomifensine-induced rise in extracellular DA, baseline levels were almost unaltered, with the exception in mPFC of DSP-4 pretreated rats. This is consistent with previous literature indicating compensatory changes to maintain extracellular monoamine concentrations despite marked differences in tissue concentrations (Abercrombie & Zigmond, 1989; Robinson et al. 1994; Romero et al. 1998; Zigmond et al. 1990; see however Devoto et al. 2008).

The enhancement of DA output in mPFC (244% of baseline) after electrical stimulation of LC agrees with previous observations on a potential co-release of DA from LC NE fibres in PFC (Devoto et al. 2001, 2005; Devoto & Flore, 2006). Since rats in the present study were implanted with two probes (in mPFC and NAc), we were able to compare the effect of LC stimulation on DA output in mPFC and NAc of the same animals. LC stimulation also moderately increased (153% of baseline) the DA output in NAc although with a blunted time-course, an effect that may arise from $\alpha_1$-adrenergic stimulation of VTA DA neurons following LC stimulation (Grenhoff & Svensson, 1993). Further, $\alpha_1$-adrenoceptor blockade abolishes the behavioural effect produced by the stimulation of VTA DA neurons (Auclair et al. 2002, 2004; Darraçq et al. 1998). Although an $\alpha_1$-adrenergic stimulation of mesocortical DA neurons cannot be excluded, the larger DA increase in mPFC, its temporal association with LC stimulation and the lack of effect when the stimulating electrodes were placed outside the LC supports a noradrenergic origin of the DA release in mPFC.

Thus, the present results suggest that the extracellular DA concentration in NAc mainly arise from VTA DA fibres whereas that in mPFC has a dual contribution, from the VTA and from LC NE fibres. Since DA is an intermediate metabolite in the synthesis of NE in noradrenergic neurons, the co-release of DA and NE may reflect a deficient activity of dopamine-β-hydroxylase in cortical noradrenergic axons, a possibility that deserves further investigation. This region-specific noradrenergic contribution to DA release...
release allows the selective modulation of mesocortical DA function.

**Selective enhancement of DA release in mPFC: association with APD treatments**

Many studies implicate prefrontal catecholamine function in cognition (Arnsten & Li, 2005; Goldman-Rakic et al., 2000; Robbins & Roberts, 2007; Sara, 2009). The present and previous observations show striking similarities between the factors governing DA and NE release in mPFC. Current views indicate a hypoactive mesocortical DA pathway in schizophrenia that may underlie negative and/or cognitive symptoms and APDs increase mPFC DA output (see Introduction). This has been considered a useful pharmacological feature accounting for the clinical superiority of some APDs in non-psychotic symptoms (Kane et al., 1988; Keene et al., 2006; Leucht et al., 2009; Meltzer & McGurk, 1999). Actually, blockade of DA D_{2} receptors is effective in treating positive symptoms, possibly related to a subcortical DA hyperactivity (Laruelle et al., 1996), but not negative/cognitive symptoms. Conversely, DA D_{2} receptor blockade induces negative symptoms in healthy individuals (Artaloytia et al., 2006).

Previous reports indicate that reuptake blockade in serotonergic and noradrenergic neurons induces a very marked increase of the respective neurotransmitter in the vicinity of cell bodies in the raphe nuclei (Adell & Artigas, 1991; Bel & Artigas, 1992) and LC (Grandoso et al., 2004; Mateo et al., 1998). The excess neurotransmitter in this area activates their respective autoreceptors (5-HT_{1A} and α_{2}-adrenoceptors), which leads to a reduced neuronal activity and terminal monoamine release. Autoreceptor blockade enables the recovery of cell firing and terminal release, thus permitting the full pharmacological effect of reuptake blockade (Artigas et al., 1996; Grandoso et al., 2004; Invernizzi & Garattini, 2004; Mateo et al., 1998; Romero & Artigas, 1997). Given the marked involvement of noradrenergic fibres in the uptake/co-release of DA in mPFC, we used this strategy to selectively enhance cortical DA function (Fig. 8).

α_{2}-adrenergic antagonists and NE reuptake inhibitors, acting mainly on NE neurons, increase moderately
but selectively prefrontal DA output (Devoto et al. 2004; Gresch et al. 1995; Hertel et al. 1999a, b; Liner et al. 2001; Millan et al. 2000; Swanson et al. 2006; Valentini et al. 2004; Wadenberg et al. 2007). However, our results show for the first time that α2-adrenoceptor blockade markedly potentiates the effect of NET inhibitors on DA output in mPFC (as also observed for cortical NE output; Swanson et al. 2006) but not in NAc (Figs 5–8).

The increase in cortical DA output also occurred when reboxetine + RX821002 were administered in combination with classical (haloperidol – lacking appreciable affinity for α2-adrenoceptor) and APDs (clozapine – with antagonist properties at α2-adrenoceptors). These drug combinations did not elevate DA output in NAc or produce even a small effect compared to the marked elevation seen in mPFC. The doses used (3 mg/kg for clozapine, 0.1 mg/kg for haloperidol) are close to their ED50 values for occupation of their primary receptor targets (5-HT2, haloperidol) are close to their ED50 values for occupation of their primary receptor targets (5-HT2A/3C and D2, respectively) (Schotte et al. 1993). Further, the dose of clozapine is in the lower range of those shown to enhance cortical DA release (Diaz-Mataix et al. 2005; Ichikawa et al. 2001; Rollema et al. 1997).

Clozapine increased DA output in mPFC, an effect mediated by cortical 5-HT1A receptor activation (Bortolozzi et al. 2010; Diaz-Mataix et al. 2005) although α2-adrenoceptor blockade has also been suggested (Ashby & Wang, 1996; Svensson, 2003). However, since the elevation of DA output produced by reboxetine + RX821002 was much larger than that of reboxetine + clozapine, it seems reasonable to assume that clozapine antagonizes α2-adrenoceptors much less than RX821002.

Consistent with previous reports, haloperidol produced a moderate DA increase in NAc but not in mPFC (Kuroki et al. 1999; Li et al. 1998). However, the effect of haloperidol + reboxetine + RX821002 was more marked than that of the latter two drugs, possibly due to blockade of DA D2 receptors by haloperidol, which would remove the D2-mediated negative feedback on DA release following the large increase produced by reboxetine + RX821002. The lower occupancy of DA D2 receptors produced by clozapine probably accounts for the lower enhancement of mPFC DA output when combined with reboxetine + RX821002.

**Therapeutic implications**

Overall, the above results indicate that (1) extracellular DA concentration is distinctly regulated in mPFC and NAc, (2) NE terminals largely contribute to the control of DA output in mPFC (but not NAc) by co-releasing DA and taking up DA via NET, and (3) a marked and selective enhancement of DA function in mPFC is feasible through the combined administration of NET blockers and α2-adrenoceptor antagonists. The latter effect occurs when these drugs are administered alone or in combination with haloperidol or clozapine. This opens the way to perform clinical trials in which reboxetine or other NET blockers, used as antidepressants, can be combined with α2-adrenoceptor antagonists in order to test their clinical efficacy on negative symptoms and/or cognitive dysfunction in schizophrenia and other psychiatric disorders.

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**Statement of Interest**

None.

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