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The putative antidepressant DOV 216,303, a triple reuptake inhibitor, increases monoamine release in the prefrontal cortex of olfactory bulbectomized rats

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ABSTRACT

The first line of antidepressant treatment nowadays are selective serotonin reuptake inhibitors. Although they are relatively safe to use, selective serotonin reuptake inhibitors (SSRIs) can induce severe side effects. New promising antidepressants may be the triple monoamine reuptake inhibitors, which not only enhance serotonin and norepinephrine neurotransmission, but also increase brain dopamine levels. Recently it has been shown that one of the triple reuptake inhibitors, DOV 216,303 has antidepressant-like effects in the olfactory bulbectomy (OBX) model of depression, but the alterations in monoaminergic neurotransmission in these animals are still unknown. In the present study we investigated not only the effect of acute, but also chronic treatment of DOV 216,303 in OBX rats on monoamine and metabolite levels. The main results are decreased baseline dopamine levels in the prefrontal cortex one day after OBX, while 38 days after OBX no difference could be observed in monoamine levels after vehicle treatment. Treatment with DOV 216,303 leads to increased extracellular levels of serotonin and norepinephrine neurotransmission, but also increased dopamine levels in OBX animals as well as their controls. This increase could be observed after one single administration, but also after chronic treatment. However, a DOV 216,303 challenge in chronically treated animals resulted in lower monoamine concentrations than the same challenge in untreated animals. More research is needed to investigate this seemingly hyporesponsivity to chronic DOV 216,303 treatment.

1. Introduction

Major depressive disorder is one of the most prevalent psychiatric disorders. Its core symptoms are depressed mood and loss of interest or pleasure in activities that were once enjoyed (i.e., anhedonia) (Torpey and Klein, 2008). Selective serotonin reuptake inhibitors (SSRIs) are the most frequently prescribed antidepressants, because they are relatively effective and safe to use. Nevertheless, SSRIs can induce adverse side effects, including sexual dysfunction (Waldinger et al., 1998), agitation (Henry and Demotes-Mainard, 2006), weight gain and sleeplessness (Croom et al., 2009), causing patients to withdraw from treatment (Demyttenaere and Jaspers, 2008).

Although the exact mechanisms underlying depression remain unclear, an important theory explaining the symptoms of depression, already for more than 30 years, is the monoamine hypothesis (Schildkraut, 1965). This hypothesis proposes that a deficiency of central monoamines is crucial for depression. Despite the dysfunction of mesolimbic dopaminergic system in depression, causing anhedonia, research on norepinephrine- and serotonin-containing circuits has largely overshadowed the role of dopamine in depression. (Dunlop and Nemeroff, 2007; Kinney et al., 2000; Nestler and Carlezon, 2006). Therefore the development of new broad spectrum antidepressants that also increase brain dopamine levels is an attractive strategy (Skolnick et al., 2006; Skolnick et al., 2003). One of these new putative antidepressants is the triple monoamine reuptake inhibitor (TRI) DOV 216,303. Previously it has been shown that DOV 216,303 has antidepressant-like effects in the forced swim test (both rats and mice) and the mouse tail suspension test (Skolnick et al., 2003) as well as in humans (Skolnick et al., 2006). Recently, in our lab it was demonstrated that two weeks of treatment with DOV 216,303 had antidepressant-like behavioural effects, without showing sexual side effects, in the olfactory bulbectomy (OBX) model of depression (Breuer et al., 2008).

In the OBX rat model, the olfactory bulbs are removed resulting in neurochemical changes resembling those of depressed patients (Song and Leonard, 2005), namely decreased striatal dopamine (Masini et al., 2004) and decreased serotonin levels in the hippocampus and

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basolateral amygdala (van der Stelt et al., 2005). Removal of the bulbs also leads to hyperactivity in a novel stressful environment such as an open field. This hyperactivity can be reversed by chronic, but not acute, antidepressant treatment (Kelly et al., 1997; Mar et al., 2000; van Riezen and Leonard, 1990), lasting up to 10 weeks after cessation of treatment (Breuer et al., 2007). Although DOV 216,303 showed antidepressant-like effects in the OBX animal model of depression, it is not known how monoaminergic neurotransmission is altered in these animals after acute and chronic DOV 216,303 treatment.

Therefore, in the present study we investigated the effect of acute and chronic treatment of DOV 216,303 in olfactory bulbectomized rats on monoamine levels (serotonin, norepinephrine and dopamine) and their metabolites in the medial prefrontal cortex using in vivo microdialysis in freely moving rats. In this study we used the only dose of DOV 216,303 (20 mg/kg) shown to be effective in reducing behavioural hyperactivity in the OBX animal model (Breuer et al., 2008). The aim of this study was to determine the contribution of the different monoaminergic systems in OBX rats after acute and chronic treatment with a triple reuptake inhibitor. The prefrontal cortex is innervated by serotonergic, noradrenergic and dopaminergic fibers and plays a major role in major depressive disorder (Koenigs and Grafman, 2009).

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (Harlan, Zeist the Netherlands) weighing between 290 and 350 g at time of OBX or Sham surgery were socially housed, two or four per cage on a 12 h/12 h light/dark cycle with lights on at 6:00 h and off at 18:00 h. Food and water were available ad libitum. Animals had one week to acclimate to their environment and were subjected to an open field test before receiving surgery. After microdialysis probe implantation animals were housed individually until the end of the experiment. For Experiment I (acute drug treatment), 25 animals were used and 36 animals for Experiment II (chronic drug treatment).

The care and use of laboratory animals and all the experimental procedures were in accordance with the governmental guidelines and approved by the Ethical Committee for Animal Research of the Faculties of Pharmaceutical Sciences, Chemistry and Biology at Utrecht University, the Netherlands.

2.2. Olfactory bulbectomy

For the olfactory bulbectomy (OBX) surgery animals were anesthetized by inhalation of isoflurane gas (2–3%), mixed with nitrous oxide and oxygen and placed in a stereotaxic instrument (Kopf, David Kopf Instruments). Lidocaine hydrochloride (2%) + adrenaline (0.001%) were applied in the incision as a local anesthetic. Two burr holes with a diameter of 2 mm were drilled bilaterally, 8 mm anterior to bregma and 2 mm from the midline of the frontal bone overlying the olfactory bulbs. The bulbs were aspirated with a blunt hypodermic needle attached to a vacuum pump. Burr holes were paced with haemostatic sponge to prevent blood loss. Sham operated animals underwent the same procedure except that their olfactory bulbs were not removed. All incisions were closed with use of 4-0 vicryl suture material and animals received Rimadyl (5 mg/kg, subcutaneously) for pain relief.

2.3. Microdialysis probe implantation

For Experiment I, a cuprofane microdialysis probe (MAB 4.7.2 CU) was implanted in the prefrontal cortex (PFC), immediately after OBX/Sham surgery. The coordinates of the PFC were tooth bar set at −3.3, A: +3.2 mm, ML: +0.8 mm, and DV: −4.0 mm from bregma and skull. Probes were anchored in place with three screws and dental cement on the skull. For Experiment II, the probe was implanted on day 16 of DOV 216,303 treatment, the experimental set-up for Experiment II is shown in Fig. 1.

2.4. Microdialysis experiment

There was no significant difference in weight between the different groups at time of the microdialysis day, weights of the animals in the different groups ranged from 365 till 410 g.

The day after implantation, microdialysis experiments were performed in conscious freely moving animals. The system was perfused with Ringer solution (147 mM NaCl, 2.3 mM KCl, 2.3 mM $CaCl_2$, and 1 mM $MgCl_2$) with use of a KdScientific Pump 220 series (USA) at constant flow rate of 1 ml/min. Animals were connected to a dual channel swivel (type 375/D/22QM) which allowed them to move relatively unrestricted. During microdialysis, the pump rate was set at 0.09 ml/h. Two hours after connection of the animals to the system, ten 30-minute samples were manually collected in vials containing 15 µl of 0.1 M acetic acid and frozen at −80 °C until analysis with HPLC. After two hours of baseline samples DOV 216,303 (synthesized by Sepracor Inc., Marlborough, USA) (20 mg/kg, 2 ml/kg, i.p.) or vehicle (0.9% NaCl) was administered intraperitoneally to the animals. At the end of the microdialysis test day all animals were sacrificed and their brains were removed and examined to verify complete olfactory bulb ablation and probe placement accuracy.

2.5. HPLC analysis

Microdialysis samples were stored at −80 °C until analysis. Neurotransmitters, norepinephrine, dopamine and serotonin and the metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were detected simultaneously by HPLC with electrochemical detection using an Alexys 100 LC-EC system (Antec Leyden, The Netherlands) (Korte-Bouws et al., 1996; Verhagen et al., 2009). The system consisted of two pumps, one autosampler with a 10 port injection valve, two columns and two detector cells. Column 1 (ALF 105 C18 1 × 50 × mm, 3 µm particle size) in combination with detector cell 1, separated and detected dopamine and serotonin. Column 2 (ALF 115 C18 1 × 150 mm, 3 µm particle size) in combination with detector cell 2, separated and detected norepinephrine and the metabolites. The mobile phase for column 1 consisted of 50 mM phosphoric acid, 8 mM KCl, 0.1 mM EDTA (pH 6.0), 12% Methanol and 500 mg/l 1-Octanesulfonic acid, sodium salt (OSA). The mobile phase for column 2 consisted of 50 mM phosphoric acid, 50 mM citric acid, 8 mM KCl, 0.1 mM EDTA (pH 3.2), 10% methanol and 500 mg/l OSA. Both mobile phases were pumped at 50 µl/min. Samples were kept at 8 °C during analysis. From each microdialysis sample 5 µl was injected simultaneously onto each column. The neurotransmitters were detected electrochemically using µVT-03 flow cells (Antec Leyden, The Netherlands) with glassy carbon working electrodes. Potential

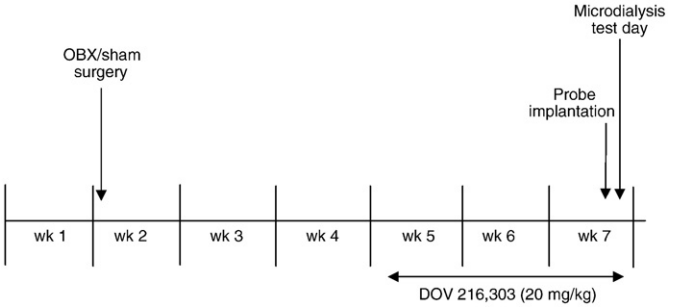

Fig. 1. Design of Experiment II with OBX and microdialysis during chronic drug treatment.

settings were for dopamine and serotonin +0.30 V versus Ag/AgCl and for norepinephrine and metabolites +0.59 V versus Ag/AgCl. The columns and detector cells were kept at 35 °C in a column oven. The chromatogram was recorded and analyzed using the Alexys data system (Antec Leyden, The Netherlands). The limit of detection was 0.03 nM (S/N ratio 3:1).

2.6. Drug treatment

In Experiment I, animals received only one injection during the microdialysis day with either DOV 216,303 (+/−1-(3,4-dichlorophenyl)-3-azabicyclo-[3.1.0]hexane hydrochloride) (20 mg/kg, i.p.) or vehicle (0.9% NaCl). For Experiment II, drug treatment started 3 weeks after OBX/Sham surgery. Animals received one injection per day for 17 days, with the last injection on the microdialysis test day. OBX and Sham animals were randomly assigned to DOV 216,303 (20 mg/kg, i.p.) or vehicle (0.9% NaCl) treatment groups ($n=9$ per group).

2.7. Histology

All the animals were sacrificed at the end of the microdialysis test day. For the acute treatment experiment brains were fixed in 4% paraformaldehyde and later on transferred to a 30% sucrose solution and after three days frozen slices of 60 µm were made and stained with a cresyl violet staining for probe track verification. For the chronic treatment experiment brains were quickly frozen in isopentane and stored at −80 °C, verification of probe track was done. Data of animals were discarded if olfactory bulbs were not completely ablated, or if the microdialysis probe was not in the PFC.

2.8. Statistical analysis

The area under the curve of the monoamine response was calculated using the trapezoid algorithm. To detect significant differences in area under the curve data analysis of variance (one-way ANOVA) was used.

Data from Experiment I as well as Experiment II, were analyzed by two-way ANOVA with the between group factors 'drug' (2 levels: vehicle and DOV 216,303) and 'lesion' (OBX and Sham). Subsequent repeated analysis of variance (RM-ANOVA) was performed for the OBX and Sham-treated group with time as 'within' and drug treatment as 'between' factor. The level of significance was set a priori at $P<0.05$. In case the ANOVAs were significant they were followed by post-hoc multiple comparisons with the use of Tukey's test. Data are given as mean ± S.E.M.

3. Results

3.1. Baseline monoamine levels

No animals were excluded because all olfactory bulb ablations were complete. In total six animals from Experiment II were excluded from the analysis based on incorrect microdialysis probe placement. In two animals the microdialysis sampling during the experiment stopped and therefore these incomplete data were excluded from analysis. For Experiment I, the number of animals used in each treatment group was Sham-vehicle ($n=7$), OBX-vehicle ($n=6$), Sham-DOV 216,303 ($n=6$), and OBX-DOV 216,303 ($n=6$). For Experiment II, the number of animals used in each treatment group was Sham-vehicle ($n=5$), OBX-vehicle ($n=7$), Sham-DOV 216,303 ($n=9$), and OBX-DOV 216,303 ($n=7$).

In Experiment I, a microdialysis experiment was performed to measure monoamine and metabolite levels in the prefrontal cortex, one day after removal of the olfactory bulbs.

Baseline dopamine levels in the prefrontal cortex were significantly lower one day after OBX when compared to Sham operated animals ($F_{1,21}=8.8$, $P=0.007$), while baseline levels of norepinephrine, serotonin or metabolites were not affected (Fig. 2). In Experiment II, a microdialysis study was carried out after chronic treatment with DOV 216,303. After 17 days of treatment with DOV 216,303, only significantly increased baseline serotonin levels ($F_{1,22}=5.7$, $P=0.026$) were found in both OBX and Sham-treated animals. A trend was observed for increased dopamine baseline levels in the DOV 216,303 treated groups ($F_{1,22}=3.2$, $P=0.085$) (Table 1). The concentrations of norepinephrine and the metabolites DOPAC, HVA and 5-HIAA were not significantly altered by surgery or treatment after 17 days (Table 1).

3.2. Experiment I: acute drug treatment

After a single dose of DOV 216,303, significant increases in dopamine, norepinephrine and serotonin levels were measured in both OBX and Sham animals. The change in dopamine, norepinephrine and serotonin levels over time were dependent of the group animals were assigned to, for dopamine ($F_{18,126}=27.8$, $P<0.001$), for norepinephrine ($F_{18,126}=24.2$, $P<0.001$), and for serotonin ($F_{18,120}=19.5$, $P<0.001$). Apart from this time × group interaction there was also a highly significant effect of time, for dopamine ($F_{6,126}=78.2$, $P<0.001$), for norepinephrine ($F_{6,126}=74.6$, $P<0.001$), for serotonin ($F_{6,120}=64.1$, $P<0.001$) and group, for dopamine ($F_{3,21}=34.6$, $P<0.001$), norepinephrine ($F_{3,21}=12.3$, $P<0.001$) and serotonin ($F_{3,20}=39.2$, $P<0.001$). Furthermore, dopamine levels of Sham animals were significantly higher than those of DOV 216,303 treated OBX rats (Fig. 3A, B and C). These results are also represented by a higher area under the curve ($F_{1,21}=225$, $P<0.001$) (Fig. 4) and a significant increase in absolute concentrations of all monoamines is shown in DOV 216,303 treated Sham and OBX animals. This effect was stronger in Sham animals than in OBX rats ($F_{1,21}=28.8$, $P<0.001$). Acute DOV 216,303 treatment also significantly decreased the area under the curve of DOPAC ($F_{1,21}=19.6$, $P<0.001$) and the area under the curve of 5-HIAA ($F_{1,21}=17.8$, $P<0.001$). These differences were independent of surgery.

Sixty minutes after a challenge with DOV 216,303 on the microdialysis day DOPAC levels of DOV 216,303 treated OBX animals were significantly lower than those of vehicle treated Sham animals. From 90 till 180 min after treatment DOPAC levels of both DOV

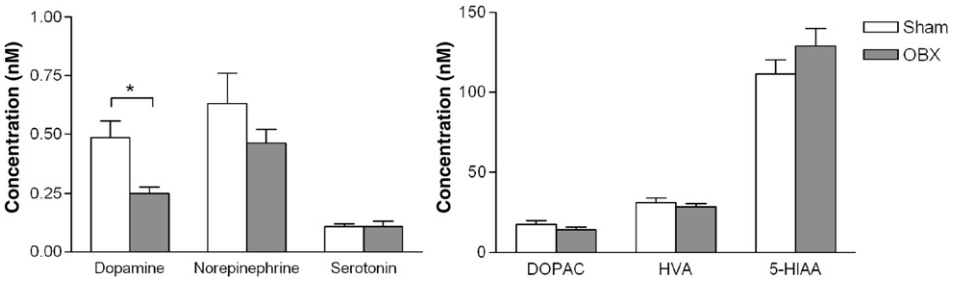

Fig. 2. Baseline levels of monoamines and metabolites in the prefrontal cortex one day after OBX/Sham surgery. *$P<0.01$, significant difference between OBX and Sham.

Table 1
Baseline extracellular monoamine and metabolite concentrations after long term treatment with DOV 216,303 or vehicle. Data represented as mean ± S.E.M.

	Sham-vehicle (nM)	Sham-DOV 216,303 (nM)	OBX-vehicle (nM)	OBX-DOV 216,303 (nM)
Dopamine	0.171 ± 0.064	0.382 ± 0.082	0.176 ± 0.053	0.237 ± 0.075
Norepinephrine	0.341 ± 0.065	0.351 ± 0.07	0.242 ± 0.05	0.338 ± 0.093
Serotonin	0.09 ± 0.021	0.128 ± 0.012[a]	0.061 ± 0.012	0.120 ± 0.025[a]
DOPAC	21.53 ± 9.54	25.53 ± 5.98	18.07 ± 5.97	18.58 ± 4.20
HVA	31.98 ± 12.4	44.39 ± 7.82	27.38 ± 7.02	35.45 ± 7.55
5-HIAA	115.34 ± 24.12	153.45 ± 12.88	104.14 ± 14.97	119.80 ± 17.66

[a] $P<0.05$ compared to vehicle treated corresponding controls.

216,303 treated groups are significantly lower than those of both vehicle treated groups. No significant interaction between surgery and treatment was found for norepinephrine, serotonin and DOPAC, HVA and 5-HIAA. A summary of the statistics for the area under the curve data for Experiment I is represented in Table 2.

3.3. Experiment II: chronic drug treatment

An injection with DOV 216,303 in chronically treated OBX and Sham animals significantly increased dopamine, norepinephrine and serotonin (Fig. 3D, E and F) in the prefrontal cortex and significantly decreased DOPAC levels in both OBX and Sham animals (Table 3). Dopamine, norepinephrine and serotonin levels altered over time, for dopamine ($F_{6,132}=23.2$, $P<0.001$), for norepinephrine ($F_{6,120}=24.2$, $P<0.001$) and for serotonin ($F_{6,132}=26.9$, $P<0.001$). This effect was dependent on the group animals were in, for dopamine ($F_{18,132}=7.3$, $P<0.001$), norepinephrine ($F_{18,120}=9.6$, $P<0.001$) and serotonin ($F_{18,132}=6.7$, $P<0.001$). There was also an overall difference between groups, dopamine ($F_{3,22}=12.9$, $P<0.001$), norepinephrine ($F_{3,20}=8.9$, $P<0.001$) and serotonin ($F_{3,22}=24.1$, $P<0.001$). There was no effect of surgery or an interaction between surgery and treatment for all monoamines and metabolites. An injection of DOV 216,303 in animals chronically treated with DOV 216,303 resulted in a significantly higher area under the curve of dopamine, norepinephrine and serotonin. DOV 216,303 treatment also significantly decreased the area under the curve for DOPAC (Fig. 5, Table 3), while no significant alterations were observed on HVA and 5-HIAA concentration. The differences were independent of the surgery. No significant interactions between surgery and treatment were found for all monoamines and metabolites. A summary of the statistics for the area under the curve data for Experiment II is given in Table 3.

4. Discussion

The present study demonstrates that one day after olfactory bulbectomy (OBX), baseline dopamine levels are significantly

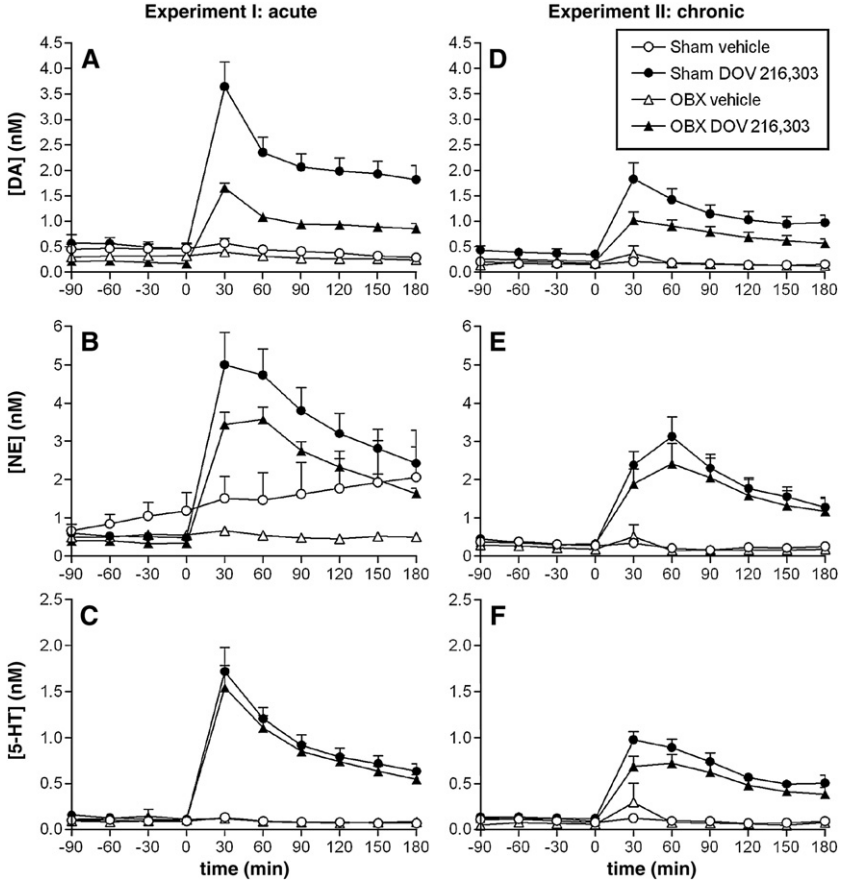

Fig. 3. Monoamine levels in the prefrontal cortex after acute (A, B and C) or chronic (D, E and F) treatment with vehicle or DOV 216,303 in OBX and Sham animals. Time points −90 till 0 min represent baseline measurements. At $t=0$ min a single injection was given with either DOV 216,303 or vehicle. [DA] is concentration dopamine, [NE] is concentration norepinephrine and [5-HT] is concentration serotonin.

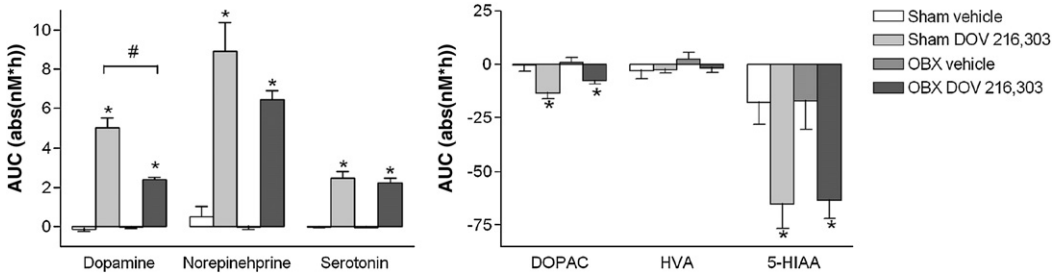

Fig. 4. Area under the curve (AUC) (absolute concentrations) of monoamines and metabolites after acute treatment with DOV 216,303 or vehicle in OBX and Sham animals. *$P<0.001$ compared to vehicle treated controls, #$P<0.001$ effect of surgery.

decreased in the prefrontal cortex of OBX animals as compared to Sham operated controls, while levels of norepinephrine, serotonin and metabolites remain unaltered. This difference in dopamine concentration was no longer present 38 days after OBX surgery, nor could any difference be observed in baseline norepinephrine, serotonin or metabolite levels between OBX and Sham at that time point. Another finding of this study is that chronic treatment with the triple reuptake inhibitor DOV 216,303 increases extracellular concentrations of dopamine, norepinephrine and serotonin in the medial prefrontal cortex of both OBX and Sham animals and significantly increased extracellular baseline serotonin concentrations. A single injection of DOV 216,303 one day after OBX results in elevated extracellular levels of dopamine, norepinephrine and serotonin in the medial prefrontal cortex of OBX and Sham animals, only for dopamine this increase was significantly higher in Sham animals than OBX animals. An injection of DOV 216,303 in chronically treated DOV 216,303-animals also resulted in an increase in extracellular dopamine, norepinephrine and serotonin. These monoamine concentrations, however, do not reach the same levels as for the acute treatment as can be seen in Fig. 3.

The findings that there were no differences in baseline extracellular monoamine or metabolite levels between OBX and Sham animals 38 days after OBX surgery are in contrast with previous microdialysis studies in the dorsal hippocampus and basolateral amygdala. One of these studies demonstrated decreased extracellular concentrations of serotonin, but not of dopamine two weeks after surgery, lasting up for five months for the dorsal hippocampus (van der Stelt et al., 2005). Another study showed elevated dopamine levels in the ventral striatum and dorsal striatum two weeks after OBX (Masini et al., 2004). Most studies examined extracellular monoamine release two weeks after OBX, arguing that at that time point behavioural changes that characterize OBX emerge. However, changes in basal locomotor activity, body temperature, heart rate and heart rate variability already occur immediately after OBX (Vinkers et al., 2009). This study is to our knowledge the first study analyzing extracellular monoamines levels one day after olfactory OBX. The fact that OBX acutely leads to decreases in dopamine is consistent with the finding that OBX leads to anhedonia measured with intracranial self stimulation (ICSS) (Slattery et al., 2007). OBX leads to increased ICSS thresholds up to seven days after surgery. After seven days, thresholds went back to baseline, which is consistent with our findings that 38 days after OBX no difference in dopamine could be detected anymore, assuming that lower dopamine levels are reflected in higher ICSS thresholds and an anhedonic state of the animal.

Chronic treatment (17 days) with the triple reuptake inhibitor DOV 216,303 increases extracellular levels of dopamine, norepinephrine and serotonin in the medial prefrontal cortex of OBX as well as Sham animals. This is in line with the antidepressant-like behavioural effects of DOV 216,303 in the OBX animal model of depression (Breuer et al., 2008). It fits the profile of the compound, because DOV 216,303 is a potent reuptake blocker of the dopamine transporter (DAT), norepinephrine transporter (NET) and serotonin transporter (SERT). Chronic DOV 216,303 treatment also increased baseline extracellular serotonin concentrations in both OBX and Sham animals, whereas the baseline levels of the other monoamines and metabolites remained unaffected.

It is important to realize that DOV 216,303 is a racemate, and its enantiomers DOV 21,947 [(+)-1-(3,4-dichlorophenyl)-3-azabicyclo-[3.1.0]hexane hydrochloride] and DOV 102,677 [(−)-1-(3,4-dichlorophenyl)-3-azabicyclo-[3.1.0]hexane hydrochloride]) have been characterized (Skolnick et al., 2003). The optically active compound DOV 21,947 is almost twice as biologically active in the Porsolt swim test than DOV 216,303 [(±)-1-(3,4-dichlorophenyl)-3-azabicyclo-[3.1.0]hexane hydrochloride] (Skolnick et al., 2006; Skolnick et al., 2003). In line with our microdialysis study, it has been shown previously that acute treatment with DOV 102,677 (20 mg/kg, i.p.) increases extracellular levels of dopamine, norepinephrine and serotonin in the prefrontal cortex, and also increases extracellular levels of dopamine and serotonin in the rat nucleus accumbens (Popik et al., 2006). DOV 102,677 (20 mg/kg, p.o.) was the minimal effective dose in the Porsolt swim test (Popik et al., 2006).

By blocking the SERT, DOV 216,303 causes an acute increase in serotonin levels which in turn act on the somatodendritic 5-HT_{1A} autoreceptors, inhibiting a further release of serotonin. Long term blockade of the serotonin transporter causes downregulation of the 5-HT_{1A} autoreceptor, thereby disinhibiting serotonin release and causing sustained elevated extracellular serotonin levels (Blier and de Montigny, 1994). Moreover, serotonin release in the prefrontal

Table 2
Summary statistics of area under the curve (AUC) of monoamine response after acute DOV 216,303 treatment.

	Surgery	Treatment	Surgery × Treatment
Dopamine	$F_{1,21}=24.2$[a]	$F_{1,21}=225$[a]	$F_{1,21}=28.8$[a]
Norepinephrine	$F_{1,21}=3.6$	$F_{1,21}=87.4$[a]	$F_{1,21}=1.6$
Serotonin	$F_{1,21}=0,4$	$F_{1,21}=152$[a]	$F_{1,21}=0.4$
DOPAC	$F_{1,21}=2.0$	$F_{1,21}=19,6$[a]	$F_{1,21}=0.84$
HVA	$F_{1,21}=1.0$	$F_{1,21}=0.39$	$F_{1,21}=0.59$
5-HIAA	$F_{1,21}=0.01$	$F_{1,21}=17.8$[a]	$F_{1,21}=0.003$

[a] $P\leq0.001$.

Table 3
Summary statistics of area under the curve (AUC) of monoamine response after chronic DOV 216,303 treatment.

	Surgery	Treatment	Surgery × Treatment
Dopamine	$F_{1,22}=1.9$	$F_{1,22}=41.2$[a]	$F_{1,22}=2.4$
Norepinephrine	$F_{1,19}=0.11$	$F_{1,19}=14.9$[a]	$F_{1,19}=0.004$
Serotonin	$F_{1,22}=0,4$	$F_{1,22}=76$[a]	$F_{1,22}=2.59$
DOPAC	$F_{1,21}=3$	$F_{1,21}=15.9$[a]	$F_{1,21}=0.11$
HVA	$F_{1,21}=2.39$	$F_{1,21}=4.4$[b]	$F_{1,21}=0.20$
5-HIAA	$F_{1,22}=0.55$	$F_{1,22}=5.8$[b]	$F_{1,22}=0.69$

[a] $P\leq0.001$.
[b] $P<0.05$.

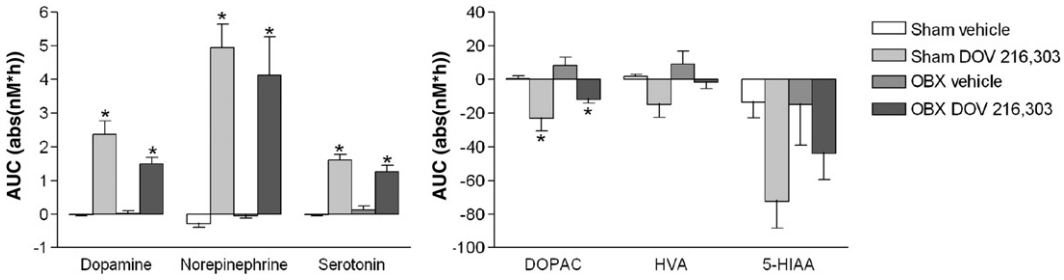

Fig. 5. Area under the curve (AUC) (absolute concentrations) of monoamines and metabolites 17 days after chronic treatment with DOV 216,303 or vehicle in OBX and Sham animals. $^{*}P \leq 0.001$ compared to vehicle treated control.

cortex is under the influence of postsynaptic 5-HT_{1A} receptors (Casanovas et al., 1999). These postsynaptic 5-HT_{1A} receptors directly influence serotonergic activity of the dorsal raphe nuclei projecting to the prefrontal cortex and have been functionally localized in the medial prefrontal cortex (Martin-Ruiz and Ugedo, 2001). Systemic administration of $5HT_{1A}$ receptor agonists resulted in a greater reduction in 5-HT release in the frontal cortex than in the dorsal raphe nucleus (Casanovas et al., 1997). We cannot exclude the involvement of postsynaptic 5-HT_{1A} receptors in the elevated serotonin levels found after chronic DOV 216,303 administration.

In this study we also looked at the effect of a DOV 216,303 challenge on monoamine release in the prefrontal cortex in long term DOV 216,303-treated animals as well as one day after OBX surgery. As expected, a single injection of the triple reuptake inhibitor one day after OBX results in an elevation of extracellular dopamine, norepinephrine and serotonin in the prefrontal cortex of OBX as well as Sham animals. However, only the increase in dopamine was significantly higher for Sham animals when compared to the OBX group. This can be explained by the finding that OBX animals had lower dopamine levels one day after OBX, suggesting that less dopamine was available. A DOV 216,303-challenge in chronically treated DOV 216,303-animals also resulted in an increase in extracellular dopamine, norepinephrine and serotonin. However, as can be seen in Fig. 3 the monoamine concentrations in these groups do not reach the same levels as for the acute treatment. This difference in response can be explained by a developed tolerance of the system to DOV 216,303 after chronic use. However, we cannot exclude the possibility of interference with surgery the day before and therefore cannot appropriately compare these two groups, because of differences in experimental set-up. Therefore further experiments are needed to explain this difference in concentrations. Recently, antidepressant effects of DOV 216,303 were observed in the OBX model (Breuer et al., 2008). The elevated monoamine levels found in our microdialysis study can be a possible explanation for this antidepressant effect. However, since a single DOV 216,303 administration elicits less monoamine release in the chronically treated group compared to the acutely treated group, it cannot be excluded that, on the long term it is necessary to have DOV 216,303 systemically available to elicit a behavioural effect.

In summary, this study showed that one day after OBX, dopamine concentration levels in the prefrontal cortex are significantly decreased in OBX animals, which can be a possible explanation for the anhedonic state of the OBX animals (Slattery et al., 2007). A single administration of the triple reuptake inhibitor DOV 216,303 in these animals resulted in a significant increase in all three monoamines, however, for dopamine, this increase was less in OBX than Sham animals. Chronic treatment with DOV 216,303 leads to increased baseline levels of serotonin in the prefrontal cortex, partly explaining the antidepressant effects found in previous studies (Breuer et al., 2008). A challenge with DOV 216,303 elicits elevated levels of serotonin, norepinephrine and dopamine concentrations in OBX and Sham animals. This increased release profile seemed to be lower in the chronically treated DOV 216,303 group compared to an acute injection, but more research is needed to investigate this seemingly hyporesponsivity to chronic DOV 216,303 treatment.

References

Blier, P., de Montigny, C., 1994. Current advances and trends in the treatment of depression. Trends Pharmacol. Sci. 15, 220–226.

Breuer, M.E., Groenink, L., Oosting, R.S., Westenberg, H.G., Olivier, B., 2007. Long-term behavioral changes after cessation of chronic antidepressant treatment in olfactory bulbectomized rats. Biol. Psychiatry 61, 990–995.

Breuer, M.E., Chan, J.S., Oosting, R.S., Groenink, L., Korte, S.M., Campbell, U., Schreiber, R., Hanania, T., Snoeren, E.M., Waldinger, M., Olivier, B., 2008. The triple monoaminergic reuptake inhibitor DOV 216, 303 has antidepressant effects in the rat olfactory bulbectomy model and lacks sexual side effects. Eur. Neuropsychopharmacol. 18, 908–916.

Casanovas, J.M., Lesourd, M., Artigas, F., 1997. The effect of the selective 5-HT1A agonists alnespirone (S-20499) and 8-OH-DPAT on extracellular 5-hydroxytryptamine in different regions of rat brain. Br. J. Pharmacol. 122, 733–741.

Casanovas, J.M., Hervas, I., Artigas, F., 1999. Postsynaptic 5-HT1A receptors control 5-HT release in the rat medial prefrontal cortex. Neuroreport 10, 1441–1445.

Croom, K.F., Perry, C.M., Plosker, G.L., 2009. Mirtazapine: a review of its use in major depression and other psychiatric disorders. CNS Drugs 23, 427–452.

Demyttenaere, K., Jaspers, L., 2008. Review: bupropion and SSRI-induced side effects. J. Psychopharmacol. 22, 792–804.

Dunlop, B.W., Nemeroff, C.B., 2007. The role of dopamine in the pathophysiology of depression. Arch. Gen. Psychiatry 64, 327–337.

Henry, C., Demotes-Mainard, J., 2006. SSRIs, suicide and violent behavior: is there a need for a better definition of the depressive state? Curr. Drug Saf. 1, 59–62.

Kelly, J.P., Wrynn, A.S., Leonard, B.E., 1997. The olfactory bulbectomized rat as a model of depression: an update. Pharmacol. Ther. 74, 299–316.

Kinney, G.G., Taber, M.T., Gribkoff, V.K., 2000. The augmentation hypothesis for improvement of antidepressant therapy: is pindolol a suitable candidate for testing the ability of 5HT1A receptor antagonists to enhance SSRI efficacy and onset latency? Mol. Neurobiol. 21, 137–152.

Koenigs, M., Grafman, J., 2009. The functional neuroanatomy of depression: distinct roles for ventromedial and dorsolateral prefrontal cortex. Behav. Brain. Res. 201, 239–243.

Korte-Bouws, G.A., Korte, S.M., De Kloet, E.R., Bohus, B., 1996. Blockade of corticosterone synthesis reduces serotonin turnover in the dorsal hippocampus of the rat as measured by microdialysis. J. Neuroendocrinol. 8, 877–881.

Mar, A., Spreekmeester, E., Rochford, J., 2000. Antidepressants preferentially enhance habituation to novelty in the olfactory bulbectomized rat. Psychopharmacology (Berl.) 150, 52–60.

Martin-Ruiz, R., Ugedo, L., 2001. Electrophysiological evidence for postsynaptic 5-HT (1A) receptor control of dorsal raphe 5-HT neurones. Neuropharmacology 41, 72–78.

Masini, C.V., Holmes, P.V., Freeman, K.G., Maki, A.C., Edwards, G.L., 2004. Dopamine overflow is increased in olfactory bulbectomized rats: an in vivo microdialysis study. Physiol. Behav. 81, 111–119.

Nestler, E.J., Carlezon Jr., W.A., 2006. The mesolimbic dopamine reward circuit in depression. Biol. Psychiatry 59, 1151–1159.

Popik, P., Krawczyk, M., Golembiowska, K., Nowak, G., Janowsky, A., Skolnick, P., Lippa, A., Basile, A.S., 2006. Pharmacological profile of the "triple" monoamine neurotransmitter uptake inhibitor, DOV 102, 677. Cell. Mol. Neurobiol. 26, 857–873.

Schildkraut, J.J., 1965. The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am. J. Psychiatry 122, 509–522.

Skolnick, P., Popik, P., Janowsky, A., Beer, B., Lippa, A.S., 2003. Antidepressant-like actions of DOV 21, 947: a "triple" reuptake inhibitor. Eur. J. Pharmacol. 461, 99–104.

Skolnick, P., Krieter, P., Tizzano, J., Basile, A., Popik, P., Czobor, P., Lippa, A., 2006. Preclinical and clinical pharmacology of DOV 216, 303, a "triple" reuptake inhibitor. CNS Drug Rev. 12, 123–134.

Slattery, D.A., Markou, A., Cryan, J.F., 2007. Evaluation of reward processes in an animal model of depression. Psychopharmacology (Berl) 190, 555–568.

Song, C., Leonard, B.E., 2005. The olfactory bulbectomised rat as a model of depression. Neurosci. Biobehav. Rev. 29, 627–647.
Torpey, D.C., Klein, D.N., 2008. Chronic depression: update on classification and treatment. Curr. Psychiatry Rep. 10, 458–464.
van der Stelt, H.M., Breuer, M.E., Olivier, B., Westenberg, H.G., 2005. Permanent deficits in serotonergic functioning of olfactory bulbectomized rats: an in vivo microdialysis study. Biol. Psychiatry 57, 1061–1067.
van Riezen, H., Leonard, B.E., 1990. Effects of psychotropic drugs on the behavior and neurochemistry of olfactory bulbectomized rats. Pharmacol. Ther. 47, 21–34.
Verhagen, L.A., Luijendijk, M.C., Korte-Bouws, G.A., Korte, S.M., Adan, R.A., 2009. Dopamine and serotonin release in the nucleus accumbens during starvation-induced hyperactivity. Eur. Neuropsychopharmacol. 19, 309–316.
Vinkers, C.H., Breuer, M.E., Westphal, K.G., Korte, S.M., Oosting, R.S., Olivier, B., Groenink, L., 2009. Olfactory bulbectomy induces rapid and stable changes in basal and stress-induced locomotor activity, heart rate and body temperature responses in the home cage. Neuroscience 159, 39–46.
Waldinger, M.D., Berendsen, H.H., Blok, B.F., Olivier, B., Holstege, G., 1998. Premature ejaculation and serotonergic antidepressants-induced delayed ejaculation: the involvement of the serotonergic system. Behav. Brain. Res. 92, 111–118.