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Original article

# Chronic antidepressant-like effect of EMD386088, a partial 5-HT$_6$ receptor agonist, in olfactory bulbectomy model may be connected with BDNF and/or CREB signalling pathway

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ABSTRACT

*Background:* The removal of the olfactory bulbs has been attributed to behavioral changes and neuroplasticity manifesting themselves among others like increases in brain neurotrophin expression and neurogenesis. Earlier data presented that EMD386088, a 5-HT$_6$ receptor partial agonist, exerts antidepressant-like properties after chronic administration in olfactory bulbectomy (OB) model as was it compared with amitriptyline (AMI). The aim of this study was to compare acute and chronic biochemical effects of EMD386088, administered in its antidepressant active (2.5 mg/kg) and non-active (1.25 mg/kg) doses, found in the open field test in OB rats, with those of AMI (10 mg/kg). The levels of 5-HT$_6$ receptor protein and selected neurotrophins in prefrontal cortex (PFC) and hippocampus (Hp) of rats have been examined.

*Methods:* 5-HT$_6$ receptor protein and selected neurotrophins: brain-derived neurotrophic factor (BDNF), cAMP-response element binding protein (CREB), the product of the immediate early gene c-*fos* (cFos) protein levels were assessed using a Western blot analysis in PFC and Hp of bulbectomized rats after acute or chronic (14-day) EMD386088 or AMI intraperitoneal (*ip*) treatment.

*Results:* The acute treatment with EMD386088 caused significant increases in CREB and BDNF protein levels in PFC, and an increase in BDNF in Hp of OB rats, while AMI injection decreased CREB and did not change BDNF levels. After the chronic administration of EMD386088, the increasing levels of BDNF and CREB were still observed in PFC and Hp.

*Conclusions:* The antidepressant-like effect of EMD386088 may be associated with the neuroplasticity activation in PFC and Hp in rats.



## Introduction

The 5-HT$_6$ receptors are positively linked to adenylate cyclase (AC) *via* Gαs protein [1–3]. Later studies have also revealed several alternative intracellular signalling pathways e.g. coupling to $G_{\alpha o}/G_i$ protein, regulation of $Ca^{2+}$ currents *via* G-protein, or coupling to Fyn tyrosine kinase and the mTOR pathway [4–6]. They are almost exclusively located in central nervous system, beyond 5-HT neurons [7], situated postsynaptically either on GABAergic or glutamatergic neurons, as well as on selected populations of GABAergic interneurons in central nervous system (CNS) with high distribution in limbic areas [7–10]. Hence 5-HT$_6$ receptor ligands exert their pharmacological activity by modulation of different neurotransmission systems. Moreover, high 5-HT$_6$ receptor affinity was demonstrated for some antidepressant (*e.g.* amitriptyline, mianserin) and antipsychotic drugs (*e.g.* clozapine) [11]. Thus, the 5-HT$_6$ receptor seems to be an interesting target for new strategies in the search for therapeutic agents treating mood disorders. An additional advantages of such substances would be the possibility of a relative lack of peripheral side effects [12].

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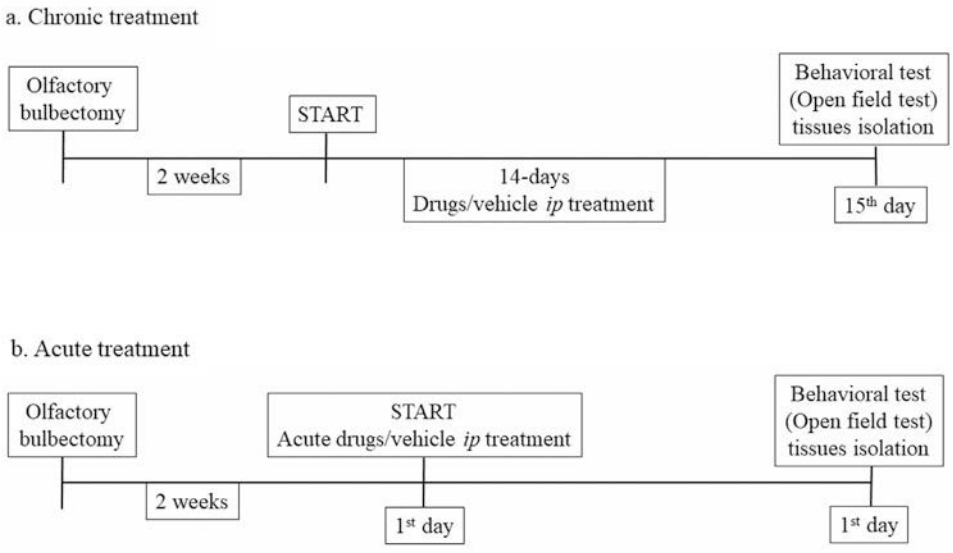
In various animal models of mood disorders 5-$HT_6$ receptor agonists produce antidepressant-like activity [13–19]. One of them, EMD386088, a 5-$HT_6$ receptor partial agonist [20,21], has been shown to evoke antidepressant-like properties in rats after its intraperitoneal (*ip*) acute, sub-chronic and chronic injections [18]. We previously demonstrated that EMD386088 exerts an antidepressant-like effect after acute (at a dose of 5 mg/kg) and sub-chronic treatments (the drug was administered three times in 24 h at a dose of 2.5 mg/kg) in the forced swim test (FST) in rats. These schemes of EMD386088 administration significantly decreased immobility and increased rats' climbing or swimming behavior [18]. For the olfactory bulbectomy (OB) model the doses of 2.5 mg/kg (active) and 1.25 mg/kg (non-active) have been chosen from those earlier experiments as a continuation of studies determining the antidepressant-like effect of EMD386088. In OB model, EMD386088 was administered acutely and chronically once daily for 14 days. The obtained behavioral data showed that chronic, but not acute, administration of EMD386088 (2.5 mg/kg) produced antidepressant-like activity, significantly improving the learning deficit in OB-rats without affecting performance in sham-operated (SH) animals, studied in the passive avoidance test, and reducing OB-related rats' locomotor hyperactivity. The dose of 1.25 mg/kg of EMD386088 given once and repeatedly was inactive [18]. Moreover, the obtained neurochemical data showed that antidepressant-like activity of EMD386088 observed in FST may be connected with the activation of dopaminergic system, but not with noradrenergic or serotonergic ones. The mechanism of the above-mentioned effect is probably connected with a significant affinity of EMD386088 for dopamine transporter (DAT) as it has been showed in *in vitro* assay. The importance of dopaminergic system activation in antidepressant-like activity of EMD386088 seems to be confirmed by behavioral data demonstrating abolishment of EMD386088 effect by the preferential $D_1$- and $D_2$-like receptor antagonists (SCH23390 and sulpiride, respectively) [19]. Furthermore, we have also demonstrated that activation of 5-$HT_6$ receptor by EMD386088 may facilitate antidepressant-like activity of some antidepressants, whose mechanism of action is connected with dopaminergic and noradrenergic transmission but not with serotonergic one [22].

Some researchers have proven that antidepressant treatment enhances neuroplasticity, and can reverse deficits occurred during the acute phase of depression [23]. Further, an acute increase in the amount of synaptic monoamines caused by antidepressants is believed to produce secondary neuroplastic transitions that involve transcriptional and translational changes mediating molecular and cellular plasticity [24,25]. Moreover, the chronic administration of antidepressant drugs (*e.g.* selective serotonin reuptake inhibitors, selective noradrenaline reuptake inhibitors, monoamine oxidase inhibitors, or tianeptine) as well as electroconvulsive therapy cause the up-regulation of the brain-derived neurotrophic factor (BDNF) and the expression of the cAMP response element binding protein (CREB) [26–28]. This effect has been validated in human *post-mortem* tissues and connected with antidepressant-like effects occurred in animal models of depression [23]. These observations have led to the hypothesis that alternations in functional and structural plasticity are required for the response to antidepressant treatment [26]. An ideal antidepressant treatment should reverse, observed in depressed patients, the atrophy of the hippocampus (Hp) as well as the prefrontal cortex (PFC) by a stimulation of the neurotrophic mechanisms [23].

In the line with literature data [29–31] we hypothesized the possible role of neurotrophins in antidepressant activity of EMD386088 observed after its chronic administration in OB model [18]. The present study was designed as a continuation of our previously described behavioral procedure [18] in order to confirm that hypothesis. in this part of the experiment we exploited rats' brain structures PFC and Hp obtained after tests performed in OB model [18]. Using a Western blot analysis, we examined the expression levels of BDNF, total CREB, cFos (the product of immediate early gene c-*fos*), and 5-$HT_6$ receptor proteins in PFC and Hp of rats receiving acutely and chronically (14-day) EMD386088 at doses of 1.25 and 2.5 mg/kg. The obtained results were compared with amitriptyline (AMI) as a reference antidepressant drug with a significant affinity (pKi = 7.14 ± 0.01 nM) for 5-$HT_6$ receptors [32], administered in the same schedule to the SH and OB rats.




**Scheme 1.** Schedule of the experimental procedure.

## Materials and methods

### Drugs

The drugs used: 5-chloro-2-methyl-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-indole hydrochloride (EMD386088, synthesized by Adamed (Pieńków, Poland)), amitriptyline (AMI, Sigma Aldrich, Germany), dissolved in distilled water immediately before administration. EMD386088 and AMI as well as vehicle (0.9% sodium chloride, NaCl) were administered *ip* in a volume of 2 ml/kg, 60 min before acute experiment and once daily during 14 days. The doses of drugs used in experiment: antidepressant-like active doses: 2.5 mg/kg of EMD386088 and 10 mg/kg of AMI; non active antidepressant-like dose of EMD386088: 1.25 mg/kg, according to previously described behavioral data [18].

### Experimental design

This experiment was designed as a biochemical continuation of behavioral tests performed and described previously [18].

Briefly, the behavioral experiments were performed on male Wistar rats (250–300 g, each group consisted of 6–8 animals) purchased from Charles River Laboratories (Germany). The animals were housed for a period of 6 days in standard rats' cages (dimensions 26.5 × 15 × 42 cm) in an environmentally controlled room (ambient temperature 21 ± 2 °C; relative humidity 50–60%; 12:12 light:dark cycle, lights on at 8:00), in groups of four rats. Standard laboratory food (LSM-B) and filtered water were freely available. All animals were used only once. All the experimental procedures were approved by the IV Local Ethics Commission in Warsaw and were in accordance with the 1996 NIH Guide for the Care and Use of Laboratory Animals. The all experiment was carried out according to Scheme 1. The OB procedure and behavioral methods were done and described previously [18]. In the present procedure we used tissues collected from control animals and rats given chronic and acute treatment of investigated drugs.

### Tissue collection

24 h after the last dose of injected compounds (day 15th) for chronic administration and 60 min after acute injection the behavioral experiments were carried out and on average 1 h after them the animals were sacrificed and tissues (PFC and Hp) were rapidly dissected out from the rats' brains, on an ice-cold glass, than received PFC and Hp were frozen in dry ice and stored at −80 °C until required.

### Western blot analysis

PFC and Hp were collected and homogenized using T-PER mammalian protein extraction reagent (Thermo Fisher Scientific,

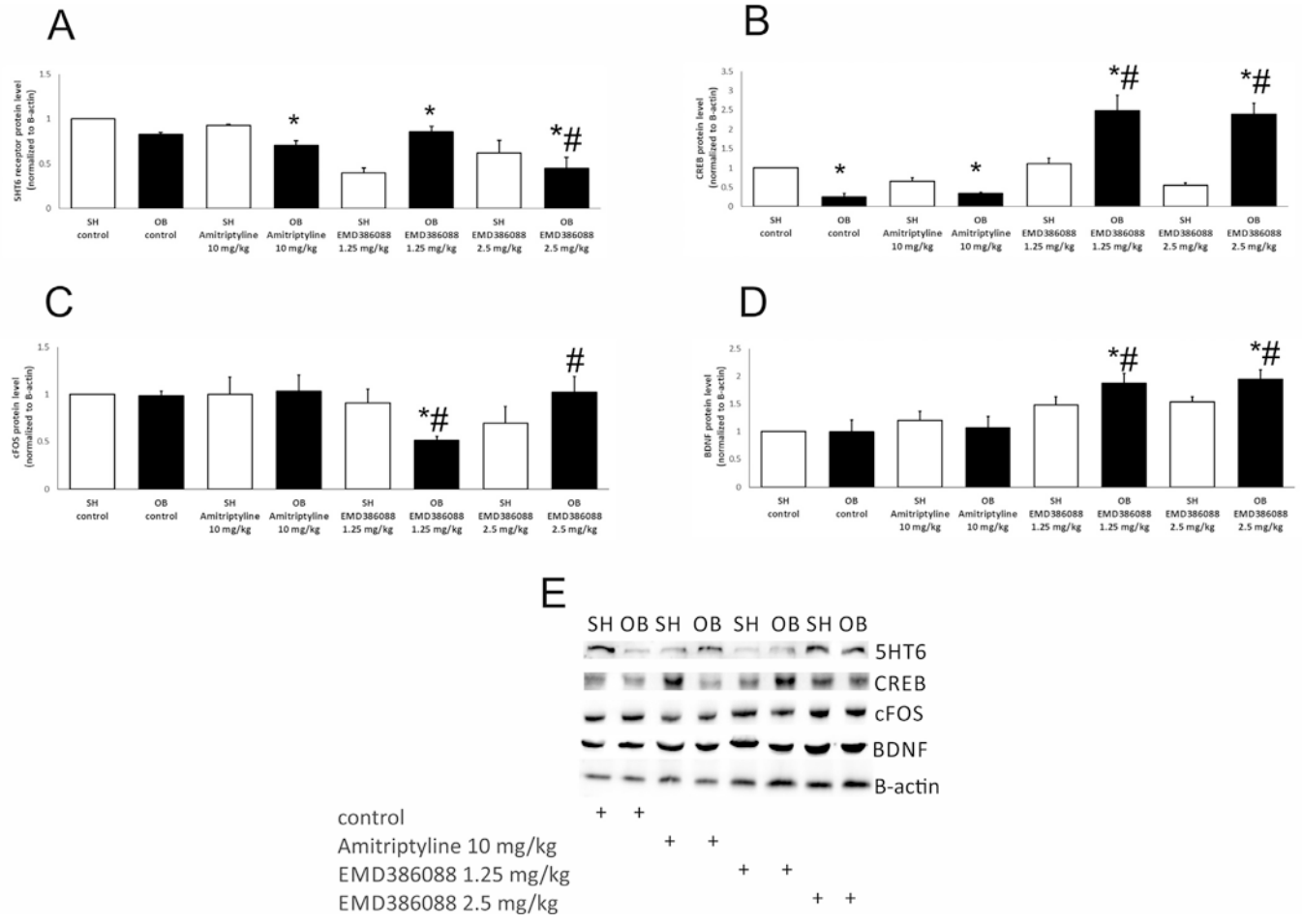



**Fig. 1.** Effects of acute treatment of EMD386088 and amitriptyline in OB rats on (a) 5-$HT_6$ receptor, (b) CREB, (c) cFos and (d) BDNF protein levels in the rats PFC and (e) the Western Blot graphs. Data were obtained from four to five independent experiments. Values are presented as means ±SD. Data are expressed as in relation to β-actin. The data were analysed by two-way ANOVA. $^{*}p < 0.001$ relative to sham respective group, # $p < 0.001$ relative to OB control.

Waltham, MA, USA) with protease inhibitor cocktail set III (Calbiochem, Merck, Germany) as well as phosphatase inhibitors (Cayman Chemical, Ann Arbor, MI, USA). Protein concentrations were determined using the Bradford reaction. Forty μg of the total protein were subjected to 10% SDS-polyacrylamide gel electrophoresis as described previously [33]. The following primary antibodies were used: anti-cAMP response element binding protein (total CREB, 37 kDa), anti-cFos (62 kDa), anti-brain derived neutrophic factor (BDNF, 15 kDa) (Thermo Fisher Scientific), anti-serotonin receptor 6 (5HT-6, 47 kDa) (Santa Cruz Biotechnology, Dallas, TX, USA) diluted 1:500 and anti-β-actin diluted 1:1000. The secondary antibody was anti rabbit IgG (HRP) diluted 1:2000 (Thermo Fisher Scientific). Proteins were detected using the Clarity Western ECL Luminol Substrate (Bio-Rad, Hercules, CA, USA). Chemi Doc Camera with Image Lab 5.2.1 software (Bio-Rad) was used to quantify the integrated optical density of the bands. The ratio of the optical density of protein of interest to the optical density of β-actin was used to calculated results, which were expressed as fold changes of SH rat's samples (control).

*Statistical analysis*

For each analysed effect in tissues obtained from for EMD386088, AMI and vehicle treated treatment in SH-operated or OB rats, the data were obtained from four to five independent behavioral experiments. Values obtained from two-way ANOVA are presented as means±SD. Dependent variables were proteins level, exploratory variables were depression and EMD386088 or AMI treatment. Three null hypothesis were tested: H1- that the means of the dependent variable were equal for different treatment, H2–the means of the dependent variable were equal for SH or OB rats, and H3–that is no interaction. Calculations were performed using STATISTICA 13.1 software (StatSoft Inc., Tulsa, OK, USA) at a significance level of $p \leq 0.05$.

## Results

*Effects of acute treatment of EMD386088 and AMI in OB rats on 5-HT$_6$ receptor, CREB, cFos and BDNF protein levels in the rat PFC*

OB procedure caused significant ($p < 0.001$) decrease in 5-HT$_6$ receptor level in AMI and EMD386088 2.5 mg/kg treated groups *vs.* respective SH group, but opposite effect was observed in OB group treated with EMD386088 at the dose of 2.5 mg/kg *vs.* SH group. Treatment of EMD386088 1.25 mg/kg lead to restoration of level of 5-HT$_6$ receptors *vs.* SH group, while injection of EMD386088 at the dose of 2.5 mg/kg caused significant decrease in 5-HT$_6$ receptors level *vs.* OB vehicle group (Fig. 1a.). The two-way ANOVA analysis revealed significant effect of OB [$F(1,32) = 14.999$; $p < 0.001$] and treatment on 5HT$_6$ protein level [$F(3,32) = 652.45$; $p < 0.00001$] and for interaction [$F(3,32) = 576.25$; $p < 0.00001$] (Fig. 1a).

OB procedure significantly decreased CREB protein level in PFC of rats after acute treatment of vehicle or AMI (Fig. 1b), while acute treatment of EMD386088 (at the doses of 1.25 and 2.5 mg/kg)

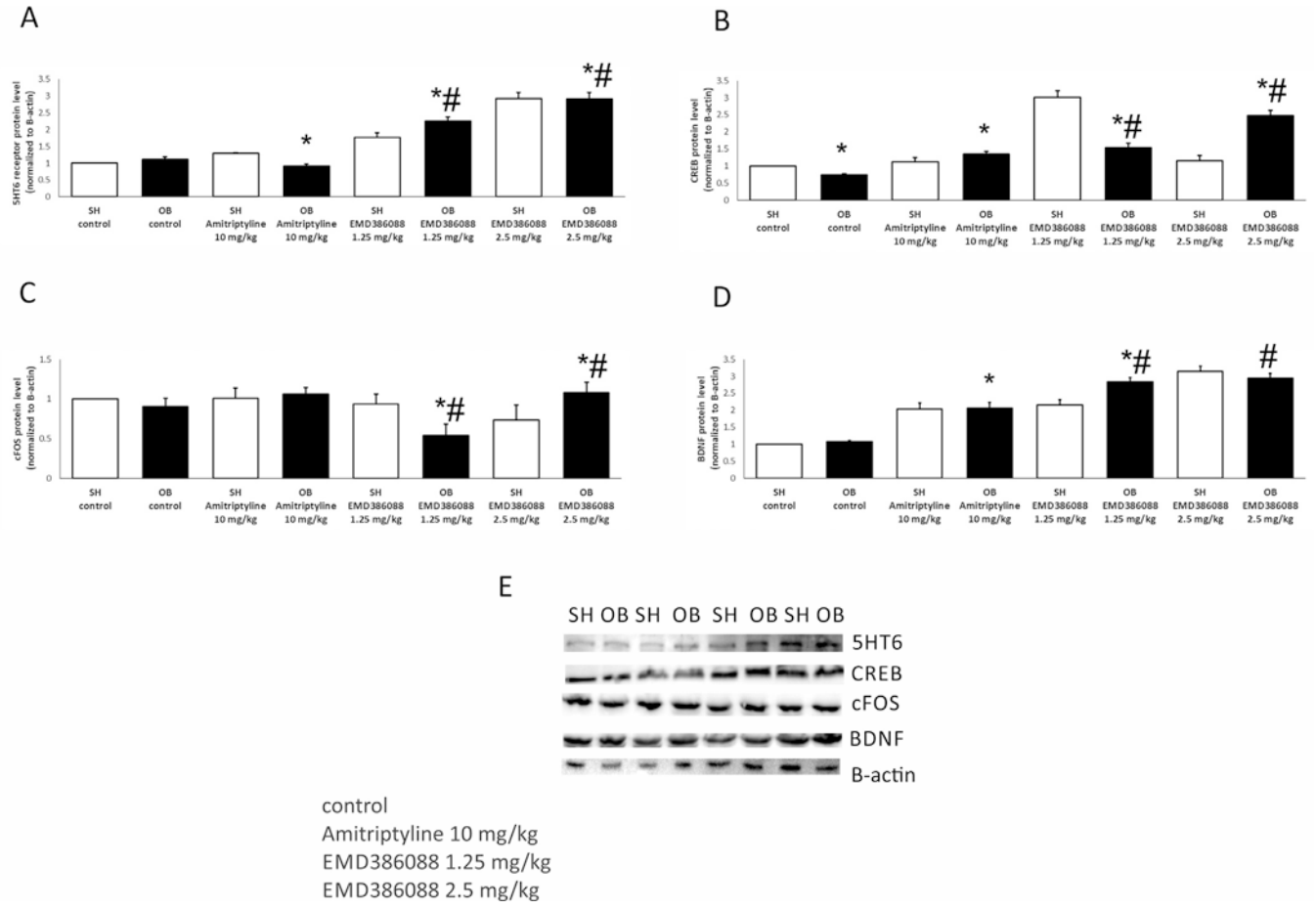



**Fig. 2.** Effects of chronic treatment of EMD386088 and amitriptyline in OB rats on (a) 5-HT$_6$ receptor, (b) CREB, (c) cFos and (d) BDNF protein levels in the rats PFC and (e) the Western Blot graphs. Data were obtained from four to five independent experiments. Values are presented as means ±SD. Data are expressed as in relation to β-actin. The data were analysed by two-way ANOVA. *$p < 0.001$ relative to sham respective group l, # $p < 0.001$ relative to OB control.

caused a significant increase in CREB level in OB rats *vs*. respective SH groups (Fig. 1b). The treatment of EMD386088 (1.25 mg/kg and 2.5 mg/kg) significantly reversed decrease in CREB level caused by OB procedure (Fig. 1b). The two-way ANOVA showed a significant effect of OB compared to SH operated rats on CREB amount in PFC [$F(1,32) = 267.58$; $p < 0.00001$], a significant effect of treatment of OB [$F(3,32) = 377.92$; $p < 0.000001$], and significant interaction [$F(3,32) = 373.88$; $p < 0.00001$] (Fig. 1b).

There was no observed a difference between SH and OB rats in cFos protein level for vehicle or AMI acute injected rats in PFC (Fig. 1 c). However, OB procedure in treatment of EMD386088 at a dose of 1.25 mg/kg caused significant decrease in cFos protein level in PFC. Acute *ip* injection of 2.5 mg/kg EMD386088 in OB rats resulted in restoration of cFos protein level in PFC, comparable with OB control rats (Fig. 1c). The two-way ANOVA showed significant effect on cFos level in PFC of OB when compared to SH operated rats [$F(1,32) = 44.57$; $p < 0.00001$], significant effect of treatment in OB PFC [$F(3,32) = 414.07$; $p < 0.000001$], and significant interaction [$F(3,32) = 464.46$; $p < 0.00001$] (Fig. 1c).

OB procedure did not change BDNF protein level in PFC *vs.* SH groups of rats for vehicle or AMI acute injected animals. Acute treatment with EMD386088 at the doses of 1.25 and 2.5 mg/kg in OB rats significantly increased BDNF protein level in PFC (Fig. 1d). The two-way ANOVA showed significant effect of OB on BDNF protein in PFC in comparison to SH operated rats [$F(1,32) = 83.71$; $p < 0.00001$], significant effect of treatment in OB PFC [$F(3,32) = 100.43$; $p < 0.000001$], and significant interaction [$F(3,32) = 137.47$; $p < 0.00001$] (Fig. 1c).

*Effects of chronic treatment of EMD386088 and AMI in OB rats on 5-HT$_6$ receptor, CREB, cFos and BDNF protein levels in the rat PFC*

After chronic (14-day) administration of AMI (10 mg/kg) no changes were observed in 5-HT$_6$ receptor protein level in OB rats, while chronic treatment with EMD386088 (1.25 and 2.5 mg/kg *ip*) significantly and dose-dependently increased the level of 5-HT$_6$ receptor *vs.* vehicle groups (Fig. 2a). The two-way ANOVA showed significant effect in OB compared to SH operated rats on 5-HT$_6$ receptor level in PFC [$F(1,32) = 75.42$; $p < 0.00001$], a significant effect of treatment in OB PFC [$F(3,32) = 551,32$; $p < 0.000001$], and significant interaction [$F(3,32) = 790.49$; $p < 0.000001$] (Fig. 2a).

OB procedure slightly increased level of CREB protein in PFC of OB rats treated with AMI and EMD386088 2.5 mg/kg and decreased in OB control group (Fig. 2 b). For EMD386088 treatment, at the dose of 1.25 mg/kg in PFC of SH rats, CREB protein level increased almost three fold *vs.* SH control, while at the dose of 2.5 mg/kg significant increasing of CREB protein was observed only in PFC of OB rats. EMD386088 in both doses reversed changes induced by OB in PFC of rats (Fig. 2b). The two-way ANOVA showed significant effect of OB on CREB level in PFC when compared to SH operated rats [$F(1,32) = 48.41$; $p < 0.00001$], significant effect of treatment in OB PFC [$F(3,32) = 774.05$;

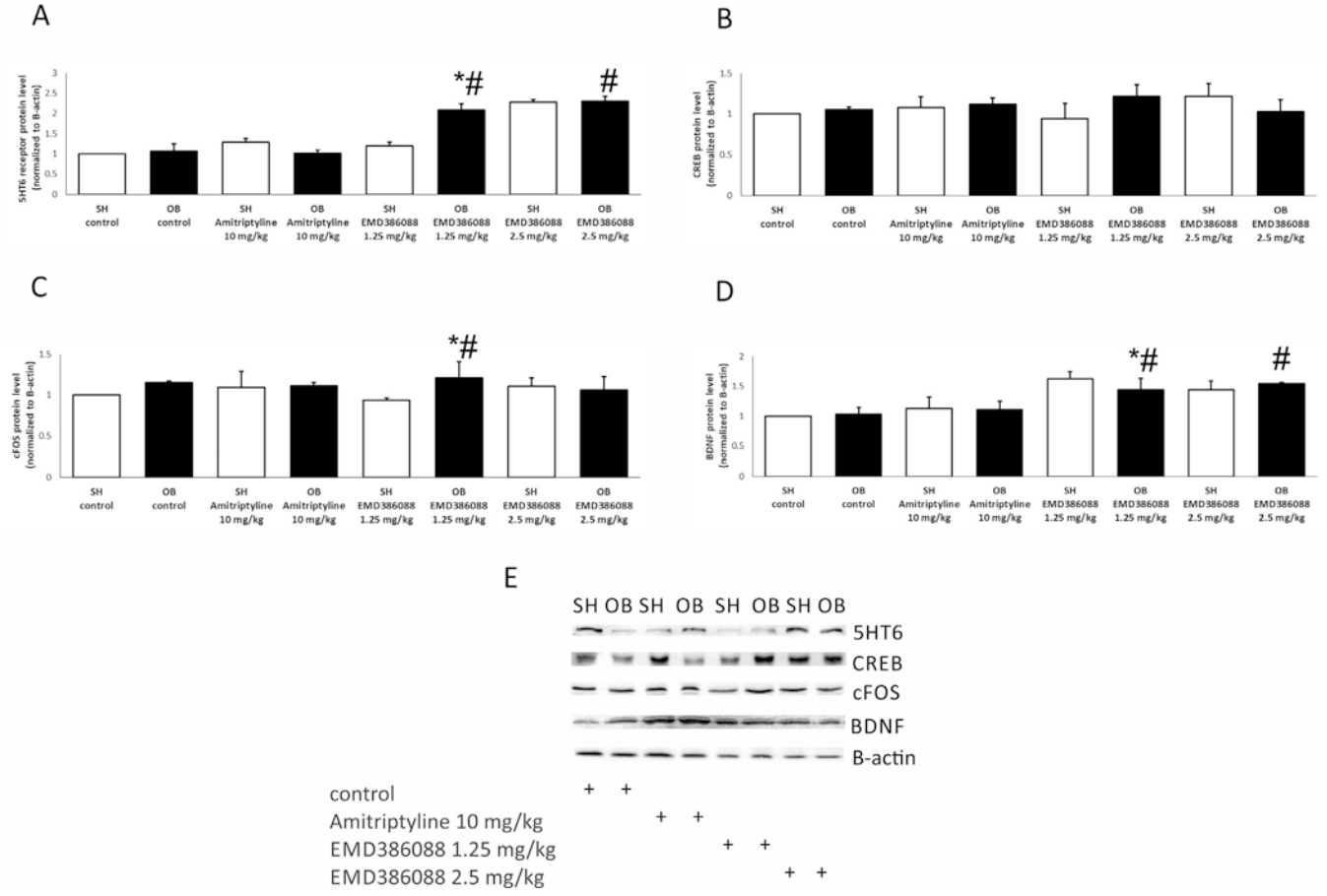



**Fig. 3.** Effects of acute treatment of EMD386088 and amitriptyline in OB rats on (a) 5-HT$_6$ receptor, (b) CREB, (c) cFos and (d) BDNF protein levels in the rats Hp and (e) the Western Blot graphs. Data were obtained from four to five independent experiments. Values are presented as means ±SD. Data are expressed as in relation to β-actin. The data were analysed by two-way ANOVA. *$p < 0.001$ relative to sham respective group, # $p < 0.001$ relative to OB control.

$p < 0.000001$], and significant interaction [$F(3,32) = 683.88$; $p < 0.000001$] (Fig. 2b).

OB procedure has different influence on cFos protein level in PFC of rats after chronic administration of investigated compounds. The lowest cFos protein level was observed for OB rats treated with EMD386088 at the dose of 1.25 mg/kg, while EMD386088 2.5 mg/kg treatment in the OB animals led to restoration level of cFos (Fig. 2c). The two-way ANOVA showed significant effect of OB on cFos amount in PFC when compared to SH operated rats [$F(1,32) = 20.16$; $p < 0.001$], significant effect of treatment in OB PFC [$F(3,32) = 565.62$; $p < 0.000001$], and significant interaction [$F(3,32) = 883.45$; $p < 0.0001$] (Fig. 2c).

OB procedure did not change the level of BDNF protein in PFC in all group except animals treated with EMD386088 (1.25 mg/kg and 2.5 mg/kg) where significant ($p < 0.001$) increase in BDNF was observed (Fig. 2d). The two-way ANOVA showed significant effect of OB on BDNF level in PFC when compared to SH operated rats [$F(1,32) = 29.92$; $p < 0.001$], significant effect of treatment in OB PFC [$F(3,32) = 334.14$; $p < 0.000001$], and significant interaction [$F(3,31) = 160.08$ $p < 0.0001$] (Fig. 2d).

*Effects of acute treatment of EMD386088 and AMI in OB rats on 5-HT$_6$ receptor, CREB, cFos and BDNF protein levels in the rat Hp*

OB procedure did not change the level of 5HT$_6$ receptor after acute treatment of vehicle, AMI or EMD386088 at the dose of 2.5 mg/kg. After injection of EMD386088 at the dose of 1.25 mg/kg OB procedure induced significant increase in 5-HT$_6$ receptor amount *vs.* SH group treated with EMD386088 1.25 mg/kg (Fig. 3a). The two-way ANOVA showed significant effect in OB Hp in comparison to SH operated rats [$F(1,31) = 137.08$; $p < 0.00001$], significant effect of treatment in OB Hp rats [$F(3,31) = 771.88$; $p < 0.000001$], and significant interaction [$F(3,31) = 152.26$; $p < 0.0001$] (Fig. 3a).

After acute treatment of EMD386088, AMI or vehicle, slight, and not statistical significant decrease in CREB protein level in SH Hp in rats was observed only in group treated with EMD386088 at the dose of 1.25 mg/kg (Fig. 3 b). The two-way ANOVA showed no significant effects of OB on CREB amount in Hp when compared to SH operated rats [$F(1,31) = 1.79$; *NS*], significant effect of treatment in OB Hp rats [$F(3,31) = 2.72$; *NS*], and significant interaction [$F(3,32) = 14.03$; $p < 0.01$] (Fig. 3b).

OB procedure increased level of cFos protein in Hp after acute injection only in group treated with EMD386088 1.25 mg/kg. The two-way ANOVA showed significant effect in OB Hp compared to SH operated rats [$F(1,31) = 19.94$; $p < 0.0001$], no significant effect of treatment in OB Hp rats [$F(3,31) = 2.54$; *NS*], and no significant interaction [$F(3,32) = 1.03$; *NS*] (Fig. 3c).

OB procedure did not change the level of BDNF protein in Hp of rats after acute injection of vehicle or AMI. Administration of EMD386088 (1.25 and 2.5 mg/kg) increased the level of BDNF protein in OB as well as in SH-operated rats (Fig. 3d). The two-way

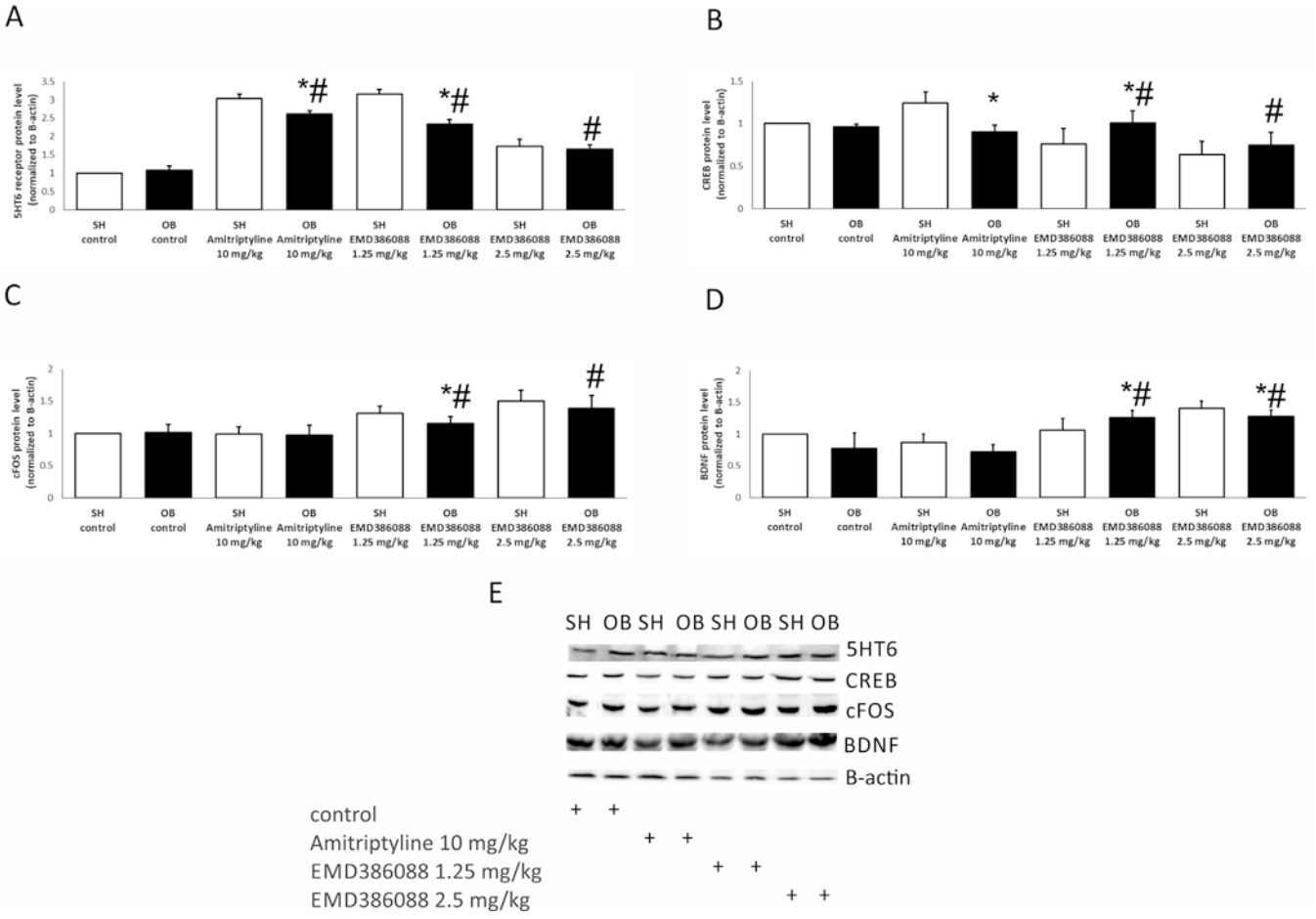



**Fig. 4.** Effects of chronic treatment of EMD386088 and amitriptyline in OB rats on (a) 5-HT$_6$ receptor, (b) CREB, (c) cFos and (d) BDNF protein levels in the rats Hp and (e) the Western Blot graphs. Data were obtained from four to five independent experiments. Values are presented as means ±SD. Data are expressed as in relation to β-actin. The data were analysed by two-way ANOVA. *$p < 0.001$ relative to sham respective group, # $p < 0.001$ relative to OB control.

ANOVA showed significant effect in OB Hp compared to SH operated rats [$F(1,31) = 4.58$; $p < 0.05$], significant effect of treatment in OB Hp rats [$F(3,31) = 111.99$; $p < 0.00001$], and significant interaction [$F(3,31) = 66.84$; $p < 0.0001$] (Fig. 3d).

*Effects of chronic treatment of EMD386088 and AMI in OB rats on 5-HT$_6$ receptor, CREB, cFos and BDNF protein levels in the rat Hp*

OB procedure significantly decreased Hp 5-HT$_6$ receptor level in AMI or EMD386088 1.25 mg/kg treated rats *vs.* respective SH-operated rats (Fig. 4a). There was no significant effect of OB procedure *vs.* SH operated rats in control, vehicle treated, group, and EMD386088 2.5 mg/kg (Fig. 4a). Chronic administration of AMI or EMD386088 led to increase in 5-HT$_6$ receptor protein level in SH and OB groups *vs.* vehicle group. The two-way ANOVA showed significant effect in OB Hp compared to SH operated rats [$F(1,31) = 36.34$; $p < 0.0001$], significant effect of treatment in OB Hp rats [$F(3,31) = 55.63$; $p < 0.00001$], and significant interaction [$F(3,31) = 35.22$; $p < 0.001$] (Fig. 4a).

In OB rats' Hp after chronic treatment of AMI a significant decrease in CREB *vs.* SH-operated AMI rats was observed (Fig. 4b). After EMD386088 treatment, there was observed significant increase in CREB level in Hp of OB *vs.* respective SH rats. The two-way ANOVA showed no significant effect on the amount of this protein in OB Hp compared to SH operated rats [$F(1,31) = 0.19$; *NS*], significant effect of treatment in OB Hp rats [$F(3,31) = 248.90$; $p < 0.00001$], and significant interaction [$F(3,31) = 154.60$; $p < 0.00001$] (Fig. 4b).

OB procedure did not change cFos and BDNF levels in rats' Hp *vs.* SH-operated animals treated with AMI or vehicle, while EMD386088 treatment significantly increased cFos level (Fig. 4c) as well as BDNF protein level. The two-way ANOVA for cFos assessment showed: no significant effect in OB Hp compared to SH operated rats [$F(1,31) = 0.75$; *NS*], significant effect of treatment in OB Hp rats [$F(3,31) = 49.44$; $p < 0.0001$], and significant interaction [$F(3,31) = 6.38$; $p < 0.01$] (Fig. 4c). For BDNF amount study showed: no significant effect in OB Hp compared to SH operated rats [$F(1,31) = 3.53$; *NS*], significant effect of treatment in OB Hp rats [$F(3,31) = 102.48$; $p < 0.0001$], and significant interaction [$F(3,31) = 13.02$; $p < 0.0001$] (Fig. 4d).

## Discussion

In the present study, we demonstrated for the first time that previously described the antidepressant-like activity of the partial 5-HT$_6$ receptor agonist EMD386088, observed after its chronic treatment (14 days) to OB rats [18], may be connected with the increase in the neurotrophin levels detected in the Hp and the PFC. OB model was considered to be an effective tool to screen antidepressant-like activity for the past 40 years in rodents [34]. This is a well-documented and validated method that leads to depression-like behavioral deficits, is recommended as predictive validity method [35] and postulated as a more accurate model of depression with comorbid anxiety [36]. Though, last investigations showed that OB model is not good enough to detect antidepressant-like activity of some antidepressants such as fluoxetine [37]. We used OB model in our earlier study based on large amount of literature data indicating that this procedure can induce a dysfunction of the cortical-hippocampal-amygdala circuits, manifesting as specific behavioral changes (*e.g.* hyperlocomotion, food seeking, avoidance) as well as neuroendocrine and immune alterations Moreover, above-mentioned changes seem to be correlated with the dysfunctions observed in depressed human beings [34,36,38,39]. The behavioral changes observed in the rat OB model have been connected with the neuronal reorganization as well as neuroplasticity following by the olfactory bulbs removal [40,41]. These alterations were mainly associated with: cognitive deficits, reduced social interaction, and exploration of a new environment are connected with the neuronal degeneration in the OB rats, while the impaired structural plasticity of the Hp has been linked to the emotional and spatial memory deficits [38]. The chronic antidepressant drug treatments as well as acute deep brain stimulation of the infralimbic PFC in OB rodents [36] increase the neurotrophin expression and neurogenesis [13,25,42]. Hence, according to literature data [29,35,42–44], we investigated the level of neurotrophins such as CREB and BDNF as well as the product of the immediate early gene c-*fos* and 5-HT$_6$ receptor protein in selected brain structures (PFC and Hp) in the OB rats. The OB procedure reduced the CREB expression in the PFC, but not in Hp. No effects has been found in the BDNF, cFos or 5-HT$_6$ receptor expression in OB *vs.* SH animals. Furthermore, these results are not in agreement with several previous data, showing alterations of CREB and BDNF in structures tested [36,43,45]. The reason of this discrepancy is hard to explain, but it could be due to the different method of drug delivery, age and species of animals, or time interval between the last dose of a drug tested and tissue collection as well as detection model used [26]. The aim of our experiment was also to compare the effects of EMD386088 administered in active (2.5 mg/kg) and non-active (1.25 mg/kg) antidepressant doses with a reference antidepressant drug AMI (10 mg/kg). AMI was chosen as an antidepressant compound widely investigated in different animal models of depression as well as the drug used in depressive patients. Moreover, it is believed that part of AMI antidepressant mechanism of action may be connected with its antagonistic properties for 5-HT$_6$ receptors [3]. Thus, it was interesting to compare obtained results for EMD386088, a partial agonist of 5-HT$_6$ receptors with the drug that possesses opposite properties for 5-HT$_6$ receptors, especially in a situation where large literature data demonstrate antidepressant-like activity for both agonists and antagonists of these receptors [5,13,15,17,46–51]. The acute treatment with EMD386088 (1.25 and 2.5 mg/kg) caused significant and dose-dependent increases in the CREB and BDNF protein levels in the PFC, and an increase in BDNF in the Hp of the OB rats. The acute AMI injection decreased CREB and did not change the BDNF level. The antidepressant-like activity in previously carried out behavioral tests in OB rats in case of acute administration of AMI as well as EMD386088 was not observed [18]. de Foubert et al. [25,42] have shown that the acute and sub-chronic (4-day) treatments with another 5-HT$_6$ receptor agonist LY586713 lead to stimulatory activity on the hippocampal BDNF expression. The 5-HT$_6$ receptors are coupled to G-protein which stimulating adenylate cyclase; therefore, their activation is followed by an increase in cyclic AMP (cAMP) [2,3]. The activation of cAMP leads to the activation of CREB which is a transcription component for the BDNF gene and plays an essential role in the neuroplasticity theory of depression [23,38]. Hence, the observed increase in the BDNF level might be directly linked to the acute activation of the 5-HT$_6$ receptors after the single dose of EMD386088 and was similar to the effects observed after an acute treatment with LY586713 [25]. A comparison of the changes induced by the acute administration of EMD386088 in PFC and Hp revealed some noticeable differences. The increase in BDNF was observed particularly in the PFC after the acute treatment with EMD386088 and was dose-dependent. The elevated level of BDNF in the PFC corresponded with an increased level of CREB in this region of the brain in the OB rats. In the Hp, we did not observe any increase in the CREB level after a single injection of AMI or EMD386088, while a high increase in the BDNF level in OB rats treated with EMD386088 was observed. The positive effects of 5-HT$_6$ receptor agonists, *i.e.* EMD386088 in our investigations and LY586713 in [25], were different than those obtained for antidepressants. A single administration of different

antidepressants (*e.g.* fluoxetine, mianserin, sertraline, desipramine and included AMI (this study)) does not increase the BDNF expression [28,52]. The increase in the BDNF protein after the acute treatment with 5-$HT_6$ receptor agonists may be connected in an indirect glutamatergic manner. The localization of the 5-$HT_6$ receptors in the granule cells in the Hp and their stimulation evoke excitatory transmission, which is attributed to increase in the BDNF mRNA expression, as in case of the effects produced by an acute administration of compound LY451646 considered as the glutamate AMPA receptor potentiator [53,54]. Differences in the protein level of the 5-$HT_6$ receptors between the rats' PFC and Hp were also observed after the acute treatment with EMD386088. In PFC, the protein level of the 5-$HT_6$ receptors after administration of EMD386088 (2.5 mg/kg) decreased in the SH-operated and the OB rats *versus* the vehicle-treated animals. In the case of a lower dose of EMD386088 (1.25 mg/kg), the observed increase in 5-$HT_6$ receptor protein level of the OB rats *versus* the SH rats, was comparable to that in the vehicle-treated animals. A significant and more potent increase in the 5-$HT_6$ receptor protein level was detected after acute treatment with EMD386088 in the Hp. After a chronic administration of EMD386088, there is still observed, a cortical increase in the 5-$HT_6$ receptor protein level in the OB and the SH rats, while there were no differences in groups treated with AMI or a vehicle. The effects observed in the Hp were the opposite; the highest increase in the $5HT_6$ receptors was observed in the case of AMI and 1.25 mg/kg of EMD386088, and a lower, but still significant, increase was measured for 2.5 mg/kg of EMD386088. The differences in the SH and the OB animals were not observed. Thus, the different effects on the protein level of the $5HT_6$ receptors after the AMI *versus* EMD386088 treatment may be connected to their different affinities to these receptors as well as the different intrinsic activity. EMD386088 is a partial 5-$HT_6$ receptor agonist [20,21], while AMI possesses antagonistic properties for these receptors [3].

After the chronic administration of EMD386088, the increases in the BDNF and CREB levels were still observed in the PFC and the Hp. These results were different than those obtained by de Foubert et al. [42], wherein the chronic administration of LY586713 led to a rapid agonist-induced desensitization of the 5-$HT_6$ receptors, which had no effect of the BDNF mediated by the activation of an elevated level of CREB. However, our investigations were in line with previous microdialysis studies with another 5-$HT_6$ receptor agonist WAY-208466, where the tolerance for the enhanced cortical GABA levels following both acute and chronic (14-day) treatment did not develop [55]. The results of the CREB and BDNF protein levels obtained for 14-day treatment with EMD386088 were in line with the activity of the clinically used antidepressants. The decrease in the neurotrophins in hippocampal and cortical regions in depressed patients and in the animal models as well as the ability of antidepressants (acting through different mechanisms) to induce neurogenesis and regulate plasticity in adult brains is well-characterized [24,26,56–59]. Previously, several researchers reported that increased BDNF signalling is essential for an appropriate response to antidepressants in the animal models of depression [26,59]. Hence, the observed antidepressant-like effect of EMD386088 may be caused by the changes in the BDNF levels, particularly in PFC. Moreover, some researchers have shown the possible connection between OB procedure and dysfunction in dopaminergic system in CNS. The dysfunction in reward system (mesolimbic dopaminergic system is thought as a major component of reward system) was observed in OB animals, while stimulation of dopaminergic function improved OB rodents behavior (*e.g.* anhedonia) related with reward system [60]. Moreover, it is well-known that dopaminergic system modulates cognitive functions in the PFC and the Hp. In OB animals the memory-related impairment was observed, which was attenuated, at least in part, by the stimulation of dopaminergic system by aripiprazole [43]. This mechanism of action of aripiprazole was connected with its agonistic properties for $D_1$-like receptors. The researchers have shown increased phosphorylate CREB and cFos protein levels *via* $D_1$-like receptor stimulation and improved cognitive deficits in OB animals after aripiprazole treatment. This positive effect was attenuated by co-administration of aripiprazole with $D_1$-like receptor antagonist SCH23390. Hence, the authors concluded that modulation of dopaminergic system might be connected with alleviating of cognitive deficits in depression [43]. Previously we demonstrated dopaminergic activity of EMD386088 [19]. We might suppose that observed antidepressant-like properties of EMD386088 in behavioral tests as well as increasing of CREB level in PFC may be related to its dopaminergic activity. This requires further research using chronic administration of EMD386088 and neurochemical assays related to dopamine system activity.

In our study, we also examined the effect of the OB-procedure and treatment (acute and chronic) on the cFos level in both regions studied. cFos is a very useful approach for mapping the distribution of neurons, which may be elicited by different physiological and pharmacological signals [61]. There could be observed a low level of cFos transcription under basal conditions and its excitation upon a wide range of transcriptional stimulation [62]. Further, cFos induction reflects the functional activity of neurons, but "normal" neuronal activity does not produce the induction of cFos. The increase in the cFos expression may be induced by neurotrophic factors (particularly rapid CREB phosphorylation precedes cFos), neurotransmitters, depolarization, and an increase in the $Ca^{2+}$ influx or elevation of intracellular $Ca^{2+}$ [58,62]. Hence, determination of the cFos protein level seems to be interesting and helpful in explaining biochemical changes in rats' brain after acute and chronic administrations of a 5-$HT_6$ receptor partial agonist. EMD386088 at a single dose of 1.25 mg/kg, significantly decreased cFos level in PFC OB rats, but the injection of 2.5 mg/kg of EMD386088 caused restoration to the basal control level of cFos. Anyway similar findings were obtained after chronic EMD386088. In Hp, a slight, but significant, increase in the cFos level was detected in OB rats after the acute injection of 1.25 mg/kg of EMD386088; the dose of 2.5 mg/kg of EMD386088 did not change the cFos level. A similar increase in cFos mRNA expression was measured after systemic acute administration of EMDT, another 5-$HT_6$ receptor agonist, at antidepressant-like active doses in some mouse brain structures, *i.e.* striatum and cerebral cortex [13]. After the acute and chronic injections of AMI, any changes in the cFos level in both brain regions were observed. Moreover, the OB procedure did not evoke any significant alterations. Thus far, only a few studies have been focused on the examination of the cFos level after the single administration of some antidepressants (*e.g.* fluoxetine or desipramine) [58,61,63]. In these studies, the researchers reported different cFos expressions in various PFC regions, *e.g.* administration of fluoxetine caused an increase in cFos expression in the prelimbic cortex and the ventrolateral PFC, while in the cingulate cortex any changes were not observed [58]. Furthermore, in another study [61], an increase in the cFos expression after the acute injection of fluoxetine was measured; however, statistical significance was obtained in the nucleus accumbens shell only.

In conclusion, the acute and chronic administrations of a partial 5-$HT_6$ receptor agonist EMD386088 resulted in a high increase in CREB and BDNF in the PFC and a significant, but lower, increase in the Hp of the OB rats. Moreover, following the chronic administration of EMD386088, a decrease in the cFos level was observed. The obtained results show the possible role of the induction of the neurotrophin pathway signalling responsible for the antidepressant-like effect of EMD386088, observed in rat OB model.

We also can conclude that the depressive-like behavior induce by OB model [18] seems to be related just to the reduced CREB expression at level of PFC, which is reversed by acute or chronic treatment with EMD386088.

## Declaration of interest

The authors declare that they have no conflict of interest. Marcin Kołaczkowski, is an employee of Adamed Ltd.

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