**Short communication**

# Effect of repeated co-treatment with imipramine and metyrapone on the behavioral reactivity of the central serotonin, dopamine and $\alpha_1$-adrenergic systems in rats

Zofia Rogóż

Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

**Correspondence:** Zofia Rogóż, e-mail: rogoz@if-pan.krakow.pl

**Abstract:**

The aim of the present study was to examine the effect of repeated co-treatment with imipramine and metyrapone on the development of adaptive changes in the function of central serotonin 5-HT$_{1A}$ and 5-HT$_{2A}$, dopamine D$_{2/3}$ and $\alpha_1$-adrenergic receptors in rats. The obtained results showed that repeated co-treatment with imipramine (5 or 10 mg/kg) and metyrapone (50 mg/kg) (twice daily for 14 days) either induced more potent inhibition of the behavioral syndrome evoked by 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor agonists (8-OH-DPAT and (±)DOI, respectively), or did no change the action of amphetamine and quinpirole (a dopamine D$_{2/3}$ agonist) or phenylephrine (an $\alpha_1$-adrenergic agonist) compared to treatment with either drug alone. The results described in the present paper support the hypothesis that repeated co-treatment with imipramine and metyrapone may possess more effective antidepressant activity than the treatment with imipramine alone, and that, among other mechanisms, 5-HT$_{1A}$- and 5-HT$_{2A}$ (but not dopamine D$_{2/3}$- or $\alpha_1$-adrenergic) receptors may also play some role in this effect.

**Key words:**
repeated treatment, imipramine, metyrapone, behavioral test, rats

## Introduction

It has been estimated that 30–40% of patients diagnosed with depression do not respond to a conventional therapy. The problem of antidepressant-resistant depression has been the subject of extensive studies, yet with no apparent therapeutic success. Therefore, to improve therapy, a combination of antidepressant drugs (ADs) belonging to various pharmacological groups or a combination of an AD and a substance enhancing its effect, has been used in clinical practice.

Among agents that are expected to potentiate antidepressant efficacy are inhibitors of glucocorticoid synthesis. Indeed, they have shown antidepressant-like properties in some animal models of depression [5]. Also clinical studies have demonstrated an antidepressant effect of metyrapone, aminoglutethimide and ketoconazole; however, these drugs are mainly used at relatively high doses, hence, their side-effects are occasionally observed [15, 19]. It is well known that patients with endogenous depression often display an enhanced activity of the hypothalamic-pituitary-adre-

nal (HPA) axis [6, 15, 18]. Since the hyperactivity of the HPA axis may be significant for the pathogenesis of depression, and since the lack of normalization of the axis activity in the course of therapy with ADs often correlates with the absence of their therapeutic effect, it seems purposeful to study the effect of joint administration of ADs and the glucocorticoid inhibitors on drug-resistant depression. A combination of a glucocorticoid inhibitor and an AD should help decrease their doses and, in consequence, also their side-effects. Among glucocorticoid inhibitors, metyrapone (which inhibits the enzyme 11-β-hydroxylase) has the weakest effect on gonadal hormone levels [19]. It reveals antidepressant-like properties in the forced swimming test [5]; moreover, co-treatment with imipramine (IMI) and metyrapone evokes a more potent "antidepressant" effect in the forced swimming test in rats than does treatment with either drug given separately [20, 22]. In addition, our earlier preliminary studies showed that combined administration of IMI and metyrapone to drug-resistant depressed patients led to significant clinical improvement [23]. Similar result was reported by Jahn et al. [7], who also observed in a double-blind, randomized, placebo-controlled trial that the addition of metyrapone to antidepressants (nefazodone or fluvoxamine) induced a more rapid, more efficacious and sustained treatment response in patients with major depression. The above findings suggest that the observations made in animal models may also be valid in clinical practice.

In the search for mechanisms that could be a substance of that action, the aim of the present study was to examine the influence of the repeated treatment (twice daily *po*, for 14 days) with IMI (5 or 10 mg/kg) and metyrapone (50 mg/kg), given separately or jointly, on the behavioral reactivity of the central serotonin (5-HT$_{1A}$ and 5-HT$_{2A}$), dopamine D$_{2/3}$ or α$_1$-adrenergic systems in rats.

## Materials and Methods

### Animals and drug administration

The experiments were carried out on rats (male Wistar, 250–270 g). The animals had free access to food and water before the experiment and were kept on a 12–h light/dark cycle (the light on at 07:00), at a constant room temperature (22 ± 1°C). Imipramine

(IMI) at doses of 5 or 10 mg/kg and metyrapone at a dose of 50 mg/kg were dissolved in distilled water and administered repeatedly (twice daily *po*, for 14 days), separately or jointly (at a volume of 2 ml/kg). The behavioral syndrome evoked by 8-OH-DPAT, a 5-HT$_{1A}$ receptor agonist, and the head twitches induced by (±)DOI, a 5-HT$_{2A}$ receptor agonist or amphetamine, as well as quinpirole-induced hyperactivity and the exploratory behavior elicited by phenylephrine were measured 24 h after the last dose of IMI and metyrapone (or after their single administration). The animals were used only once in each experiment. Experimental protocols were approved by the Local Bioethics Commission for the Animal Experiments at the Institute of Pharmacology, Polish Academy of Sciences.

### Behavioral syndrome induced by 8-OH-DPAT

8-OH-DPAT (5 mg/kg *ip*) was given 24 h after the last dose of IMI or metyrapone (or after their single administration). Immediately after 8-OH-DPAT injection, each animal was separately placed in a cage. Observation sessions began 3 min after 8-OH-DPAT injection and were repeated every 3 min for a period of 15 min. Reciprocal forepaw treading and flat body posture were scored using a ranked intensity scale (0 point – absence; 1 point – equivocal; 2 points – present; 3 points – intense). All the scores were added up over 5 observation periods. Each group consisted of 8 rats.

### Head twitches induced by (±)DOI

(±)DOI (2.5 mg/kg *ip*) was given 24 h after the last dose of IMI or metyrapone (or after their single administration). Head twitches were recorded immediately after (±)DOI administration, and the recording was continued for 30 min. Each group consisted of 8 rats.

### D-amphetamine- and quinpirole-induced locomotor hyperactivity

Locomotor activity was measured in photoresistor actometers (two light beams, two photoresistors; L × W × H = 40 × 40 × 25 cm), starting at 24 h after the last dose of IMI and metyrapone (or after their single administration). Locomotor activity measurements started at 30 min after AMP (0.5 mg/kg *sc*) and QUI (0.3

mg/kg *sc*) administration and lasted for 1 h (AMP) or 2 h (QUI). Each experimental group consisted of 8 rats.

### Phenylephrine-induced exploratory behavior

For phenylephrine experiments, the rats were operated under pentobarbital anesthesia (30 mg/kg *ip*). They were implanted chronically and unilaterally with stainless steel guide cannula (9.00 mm long; 0.4 mm o.d.) according to the method described by Kolasiewicz and Maj [8]. After a 4-day postoperative period, the animals were given IMI (5 or 10 mg/kg *po*) and metyrapone (50 mg/kg *po*) twice daily for 14 days. Control animals were given a vehicle. Phenylephrine was injected at a dose of 25 μg/5 μl into the brain lateral ventricle at 30 min before the test using an inner injection cannula (14.6–11.6 mm long; 0.3 mm o.d.). The tip of the injection cannula was aimed at the lateral ventricle using stereotaxic coordinates (AP (–) 0.4–0.8, L 1.1–1.7). Injection of a volume of 5 μl lasted 2 min. The inner cannula was withdrawn 1 min after the termination of the injection. Control animals (operated) were treated with an appropriate volume of the solvent. Exploratory activity was assessed in the elevated open field test. A black circular elevated platform (without walls, 1 m in diameter, divided into six symmetrical sectors elevated 50 cm above the floor) was used. During the experiment, the laboratory room was dark and only the centre of the open field was illuminated with a 75 W bulb hung directly above it, at a height of 75 cm. The animals were placed in the open field and their exploratory behavior, i.e. the time of walking, number of crossings (ambulation), episodes of peeping outside the edge of the arena and rearing, was assessed for 3 min. After completion of the experiment, the rats were anesthetized with 45 mg/kg of pentobarbital, perfused through the heart with a 4% paraformaldehyde, and decapitated. The brains were cut into 50 μm sections, and the location of all the injection cannula tips was determined histologically. Only the animals with histologically confirmed injection sites were used for data analysis. Each group consisted of 8 rats.

### Drugs

D-Amphetamine sulfate (AMP) and imipramine hydrochloride (IMI; Sigma, USA); 2-methyl-1.2-di-3-pyridyl-1-propanone (metyrapone; Aldrich, USA); (±)-8-hydroxy-2(di-n-propylamino)-tetralin hydrobro-

mide (8-OH-DPAT), (±)-1-(4-iodo-2,5-dimethoxy-phenyl)-2-aminopropane hydrochloride [(±)DOI], phenylephrine hydrochloride (PHEN) and quinpirole hydrochloride (QUI; Research Biochemicals Int., USA) were used in the present study.

### Statistical analysis

The data were evaluated by a one-way analysis of variance (ANOVA), followed, when appropriate, by individual comparisons with the control using Dunnett's test.

## Results

### Behavioral syndrome induced by 8-OH-DPAT

Neither IMI (5 and 10 mg/kg) nor metyrapone (50 mg/kg), given at a single dose separately or jointly, changed the behavioral syndrome induced by the 5-HT$_{1A}$ agonist 8-OH-DPAT (5 mg/kg) (data not shown). Repeated treatment with IMI (5 or 10 mg/kg), but not metyrapone (50 mg/kg), inhibited the behavioral syndrome induced by 8-OH-DPAT in a statistically significant manner. Moreover, repeated co-administration of IMI at either dose (5 or 10 mg/kg) and metyrapone (50 mg/kg) induced a more potent (statistically significant) inhibition of 8-OH-DPAT action [a flat body posture (ANOVA; $F_{(5, 42)} = 59.68$, $p < 0.001$); Fig. 1A] and forepaw treading [(ANOVA; $F_{(5, 42)} = 84.09$, $p < 0.001$); Fig. 1B] than treatment with IMI or metyrapone alone.

### Head twitches induced by (±)DOI

When given separately or jointly at a single dose, neither IMI (5 and 10 mg/kg) nor metyrapone (50 mg/kg) affected the head twitch reaction induced by the 5-HT$_{2A}$ agonist (±)DOI (2.5 mg/kg) (data not shown). Repeated administration of IMI (5 or 10 mg/kg) or metyrapone (50 mg/kg) inhibited the behavioral effect induced by (±)DOI (by 26.5, 54.8 and 44.2%, respectively). Co-treatment with IMI (5 or 10 mg/kg) and metyrapone (50 mg/kg) induced more potent (statistically significant) inhibition of the head twitches induced by (±)DOI than did treatment with IMI or metyrapone alone [ANOVA; $F_{(5, 42)} = 15.77$, $p < 0.001$; Fig. 2].
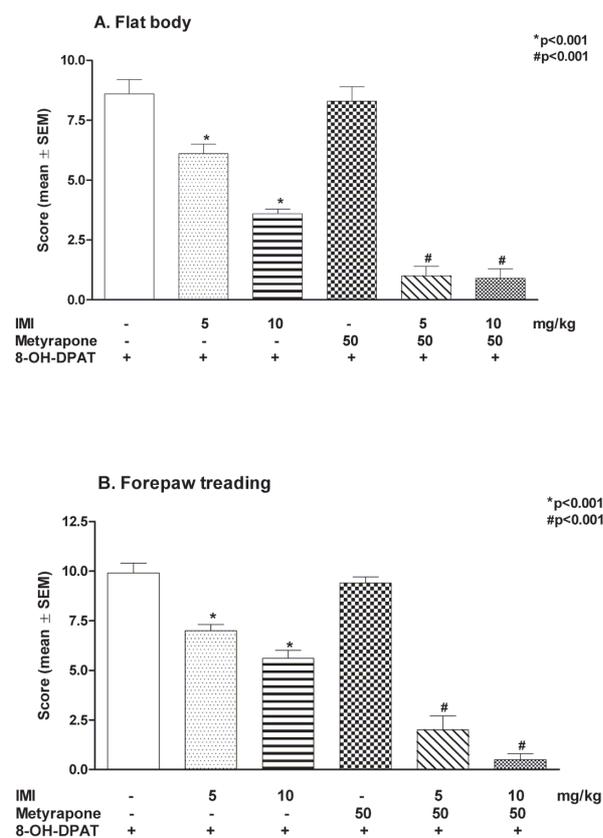
## A. Flat body

*p<0.001
#p<0.001



| IMI | - | 5 | 10 | - | 5 | 10 | mg/kg |
| Metyrapone | - | - | - | 50 | 50 | 50 | |
| 8-OH-DPAT | + | + | + | + | + | + | |

## B. Forepaw treading

*p<0.001
#p<0.001



| IMI | - | 5 | 10 | - | 5 | 10 | mg/kg |
| Metyrapone | - | - | - | 50 | 50 | 50 | |
| 8-OH-DPAT | + | + | + | + | + | + | |

**Fig. 1.** The effect of repeated co-treatment with imipramine (IMI) and metyrapone on the 8-OH-DPAT-induced behavioral syndrome [(A) flat body, (B) forepaw treading] in rats. The test was carried out 24 h after the last dose of IMI or metyrapone. 8-OH-DPAT (5 mg/kg *ip*) was injected 3 min before the test. The results are presented as the mean ± SEM of 8 animals/group. The data were statistically evaluated by ANOVA, followed by individual comparisons using Dunnett's test. * p < 0.001 *vs.* 8-OH-DPAT group, # p < 0.001 *vs.* IMI + 8-OH-DPAT or metyrapone + 8-OH-DPAT group

### D-amphetamine- and quinpirole-induced locomotor hyperactivity

Both AMP (0.5 mg/kg) and QUI (0.3 mg/kg) increased the locomotor activity of rats compared to the vehicle-treated control (by 115 and 219%, respectively). IMI (5 or 10 mg/kg) and metyrapone (50 mg/kg) given separately or jointly at a single dose affected neither the locomotor activity of naive rats nor the locomotor hyperactivity induced by AMP (0.5 mg/kg) or QUI (0.3 mg/kg) (data not shown).

Repeated treatment with IMI (10 but not 5 mg/kg) increased the locomotor hyperactivity induced by AMP or QUI (by 55 and 107%, respectively). However, repeated co-treatment with IMI (5 or 10 mg/kg)
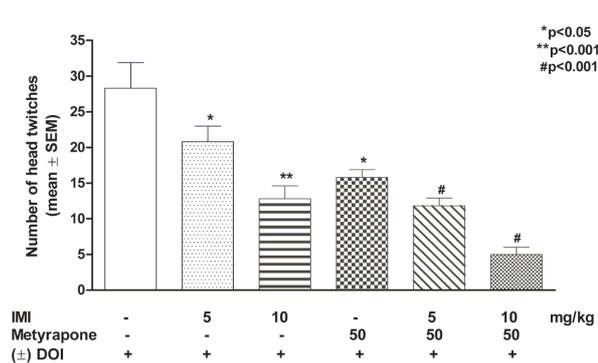


*p<0.05
**p<0.001
#p<0.001

| IMI | - | 5 | 10 | - | 5 | 10 | mg/kg |
| Metyrapone | - | - | - | 50 | 50 | 50 | |
| (±) DOI | + | + | + | + | + | + | |

**Fig. 2.** The effect of repeated co-treatment with imipramine (IMI) and metyrapone on the (±)DOI-induced head twitches in rats. The test was carried out 24 h after the last dose of IMI or metyrapone. (±)DOI (2.5 mg/kg *ip*) was injected immediately before the test. The results are presented as the mean ± SEM of 8 animals/group. The data were statistically evaluated by ANOVA, followed by individual comparisons using Dunnett's test. * p < 0.05, ** p < 0.001 *vs.* (±)DOI group, # p < 0.001 *vs.* IMI + (±)DOI or MET + (±)DOI group

and metyrapone (50 mg/kg) did not enhance the locomotor hyperactivity evoked by AMP (0.5 mg/kg) or QUI (0.3 mg/kg) given jointly with IMI (5 or 10 mg/kg) (data not shown).

### Phenylephrine-induced exploratory behavior

PHEN (25 μg/5 μl) given intraventricularly increased exploratory behavior in the open field test compared to the vehicle-treated control (time of walking rose by 81% and ambulation by 67%). IMI at a single dose (5 or 10 mg/kg) and metyrapone (50 mg/kg) given separately or jointly at a single dose, neither affected the exploratory activity of normal rats nor changed the action of PHEN (data not shown).

Repeated treatment with IMI at a dose of 10 mg/kg (but not 5 mg/kg) enhanced the effect of PHEN in the open field test: it prolonged the time of walking (by 45%) and increased the number of ambulations (by 42%). Repeated co-treatment with IMI (5 or 10 mg/kg) and metyrapone (50 mg/kg) did not change the effect evoked by PHEN (25 μg/5 μl) given jointly with AD to rats (data not shown).

## Discussion

The present study was aimed at investigating the influence of repeated treatment (twice daily for 14 days)

with IMI (5 or 10 mg/kg) and metyrapone (50 mg/kg), given separately or jointly, on the behavioral reactivity of the central serotonin (5-HT$_{1A}$ and 5-HT$_{2A}$), dopamine D$_{2/3}$ or $\alpha_1$-adrenergic systems in rats. The obtained results indicate that repeated co-treatment with IMI and metyrapone inhibits more potently 5-HT$_{1A}$ neurotransmission in behavioral tests that does IMI alone, and that neither acute nor chronic administration of metyrapone significantly alters this effect. In contrast to our current data, repeated treatment with desipramine and metyrapone was previously demonstrated to decrease the 5-HT$_{1A}$ system reactivity in behavioral experiments and to attenuate the hypothermic response to an acute challenge with 8-OH-DPAT [5]. The ability to alter the 8-OH-DPAT-induced hypothermia suggests that metyrapone and desipramine can alter 5-HT$_{1A}$ receptor sensitivity, which was previously demonstrated for other ADs [4]. While the mechanisms underlying these changes still remain unknown, the ability of metyrapone to alter the 8-OH-DPAT-induced hypothermia suggests that 5-HT$_{1A}$ receptor function is modulated by glucocorticoids. This concept is consistent with the findings that adrenalectomy can shift receptor binding characteristics and can increase 5-HT$_{1A}$ receptor mRNA expression [1], both of them being reversed by corticosterone supplementation. The above studies point to similarities between metyrapone and desipramine despite their different biochemical properties; moreover, they suggest that the mechanism underlying their antidepressant action may involve the serotonergic system. Furthermore, the present results indicate that repeated co-treatment with IMI and metyrapone also inhibits 5-HT$_{2A}$ neurotransmission in the behavioral test more potently than does IMI or metyrapone alone. The latter effect is in line with the previously demonstrated decrease in the density of 5-HT$_{2A}$ receptors, observed after repeated treatment with IMI [10] and other ADs [12, 13] in biochemical and behavioral experiments. In addition, our previous study showed that IMI given repeatedly increased brain-derived neurotrophic factor (BDNF) gene expression in either brain region studied (hippocampus and cerebral cortex) [21]. The effect of IMI in that test was similar to that produced by MAOI, tranylcypromine or ECS [16]. Moreover, metyrapone significantly increased BDNF mRNA levels in the cerebral cortex only. Also repeated co-administration of IMI and metyrapone induced a more potent increase in the level of BDNF mRNA gene expression in both brain regions examined than did

treatment with either of those drugs alone [21]. Furthermore, some other research has identified BDNF and 5-HT as two prominent signals acting in concert to regulate of neuronal plasticity in a number of brain regions. These two signals co-regulate each other in such a way that 5-HT stimulates the expression of BDNF and BDNF enhances the growth and survival of 5-HT neurons. The impaired 5-HT and BDNF signaling is pivotal to depression and anxiety disorders, and may also play an important role in the pathogenesis of several age-related disorders [14]. The results described in the present paper show that repeated co-treatment with IMI and metyrapone more potently inhibits the reactivity of the central 5-HT$_{1A}$ and 5-HT$_{2A}$ systems. These data are in line with our earlier findings which indicated that combined treatment with IMI and metyrapone produced a more potent antidepressant-like effect than did either of those drugs given alone in the forced swimming test in rats; moreover, they suggested that 5-HT receptors might contribute to the mechanism of the synergistic action of IMI and metyrapone [22]. Metyrapone is also known to reveal antidepressant properties *via* a combination of several different mechanisms, e.g. the blockade of synthesis and subsequent reduction of corticosterone (in rats) or cortisol release (in human) into the bloodstream. It has also been found that metyrapone suppresses plasma corticosterone concentration in stressed animals, but does not change the basal level of this steroid. These findings seem to be of great importance, since in the light of the corticosteroid receptor hypothesis of depression, the attenuation of elevated (but not basal) glucocorticoid levels is essential to the treatment of depression. On the other hand, ADs are known to affect not only the level of neurotransmitters and hormones, but also some immunological parameters which are disturbed in the course of depression [2]. Our previous study indicated that metyrapone decreased corticosterone level, but had no effect on the proliferation of splenocytes; moreover, combined treatment with IMI and metyrapone inhibited the stress-induced proliferative activity of splenocytes (a change beneficial to the immune system), but decreased corticosterone level to a similar extent as did metyrapone alone [20]. The lack of correlation between corticosterone level and the proliferative activity of splenocytes suggests that the synergistic action of IMI and metyrapone is probably connected with changes in the neurotransmitter level and/or their receptors. Of the possible mediators,

GABA$_A$ receptors may be involved since both tricyclic ADs and metyrapone are known to increase the levels of tetrahydroallopregnanolone, a GABA$_A$ agonists, and tetrahydroallodeoxy-corticosterone, respectively, i.e. neuroactive steroids, on the other hand, GABA$_A$ agonists potently decrease T cell proliferation [19, 24]. Moreover, it has been shown that both GABA and muscimol (a GABA$_A$ receptor agonist) stimulate BDNF expression, while pretreatment with the MAPK/ERK kinase (MEK) inhibitor U0126 attenuates the GABA-induced BDNF expression. The signaling *via* MAPK cascade and CREB transcription factor appears to play a substantial role in this process [17].

In addition, earlier studies had shown that ADs administered repeatedly (but not at a single dose) induced the up-regulation of dopamine D$_{2/3}$ receptors in the brain, evaluated in behavioral (potentiation of the hyperactivity induced by dopamine D$_{2/3}$ agonists) and biochemical experiments [3, 11, 25, 26], but also altered the responsiveness of postsynaptic $\alpha_1$-adrenergic receptors. That activity was assessed by measuring the potentiation of behavioral hyperexploration evoked by $\alpha_1$-adrenergic agonists (phenylephrine, methoxamine) in rats, as well as by estimating the increase in the binding to $\alpha_1$-adrenergic receptors in different brain regions [9, 25]. The present results show that repeated co-treatment with IMI and metyrapone does not enhance the effects evoked by amphetamine, quinpirole (a dopamine D$_{2/3}$ agonist) or phenylephrine (an $\alpha_1$-adrenergic agonist) compared to treatment with either drug alone in rats, which suggests that metyrapone does not enhance the behavioral reactivity of IMI in the central dopamine D$_{2/3}$ and $\alpha_1$-adrenergic systems in rats.

In view of all the above data, the results described in the present paper support the hypothesis that repeated joint administration of IMI and metyrapone (a corticosterone synthesis inhibitor) may possess more effective antidepressant activity than does treatment with IMI alone, and that among other mechanisms, 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors may also be involved in this action. Moreover, enhancement of the neurotrophic system and the associated augmentation of synaptic plasticity and function may constitute a basis for antidepressant efficacy and serve as a present and future focus in the search for a more rapidly acting and effective medication. These findings also seem to support greater efficacy of the combined administration of AD and metyrapone in treatment-resistant patients compared to AD alone [7, 23], and offer an alternative treatment strategy in such patients; this, however, needs to be further confirmed in clinical trials.

## References:

1. Chalmers DT, Kwak SP, Mansour A, Akil H, Watson SJ: Corticosteroids regulate brain hippocampal 5-HT$_{1A}$ receptor mRNA expression. Trends Neurosci, 1993, 13, 914–923.
2. Connor TJ, Leonard BE: Depression, stress and immunological activation: the role of cytokines in depressive disorders. Life Sci, 1998, 62, 583–606.
3. D'Aquila PS, Collu M, Gessa GL, Serra G: The role of dopamine in the mechanism of action of antidepressant drugs. Eur J Pharmacol, 2000, 405, 365–373.
4. Goodwin GM, DeSouza RJ, Green AR: Attenuation by electroconvulsive shock and antidepressant drugs of the 5-HT$_{1A}$ receptor-mediated hypothermia and serotonin syndrome produced by 8-OH-DPAT in the rat. Psychopharmacology, 1987, 91, 500–505.
5. Healy DG, Harkin A, Cryan JF, Kelly JP, Leonard BE: Metyrapone displays antidepressant-like properties in preclinical paradigms. Psychopharmacology, 1999, 145, 203–208.
6. Holsboer F: The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology, 2000, 23, 477–501.
7. Jahn H, Schick M, Kiefer F, Kellner M, Yassouridias A, Wiedemann K: Metyrapone as additive treatment in major depression: a double-blind and placebo-controlled trial. Arch Gen Psychiatry, 2004, 61, 1235–1244.
8. Kolasiewicz W, Maj J: Locomotor activity and motor disturbances induced by the competitive NMDA receptor antagonist CGP 37849. Pol J Pharmacol, 1996, 48, 467–478.
9. Maj J: Antidepressants given repeatedly: pharmacological evaluation of their action. Pol J Pharmacol Pharm, 1991, 43, 241–254.
10. Maj J, Bijak M, Dziedzicka-Wasylewska M, Rogóż Z, Skuza G, Tokarski K: The effect of paroxetine given repeatedly on the 5-HT receptor subpopulations in the rat brain. Psychopharmacology, 1996, 127, 73–82.
11. Maj J, Dziedzicka-Wasylewska M, Rogoż R, Rogóż Z: Effect of antidepressant drugs administered repeatedly on the dopamine D$_3$ receptors in the rat brain. Eur J Pharmacol, 1998, 351, 31–37.
12. Maj J, Moryl E: Effect of sertraline and citalopram given repeatedly on the responsiveness of 5-HT receptor subpopulations. J Neural Transm [Gen Sect], 1992, 88, 143–156.

13. Maj J, Moryl E: Effect of fluoxetine given chronically on the responsiveness of 5-HT receptor subpopulations to their agonists. Eur Neuropsychopharmacol, 1993, 3, 85–94.

14. Mattson MP, Maudsley S, Martin B: BDNF and 5-HT a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. Trends Neurosci, 2004, 27, 589–594.

15. Murphy BEP: Antiglucocorticoid therapies in major depression: a review. Psychoendocrinology, 1997, 22, Suppl 1, S125–S132.

16. Nibuya M, Morinobu S, Duman RS: Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci, 1995, 15, 7539–7547.

17. Obrietan K, Gao XB, Van Den Pol AN: Excitatory action of GABA increases BDNF expression *via* a MAPK-CREB-dependent mechanism – a positive feedback circuit in developing neurons. J Neurophysiol, 2002, 88, 1005–1015.

18. Pariante CM, Miller AH: Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. Biol Psychiatry, 2001, 49, 391–404.

19. Raven PW, O'Dwyer A-M, Taylor NF, Checkley SA: The relationship between the effect of metyrapone treatment on depressed mood and urinary steroid profiles. Psychoneuroendocrinology, 1996, 21, 277–286.

20. Rogóż Z, Budziszewska B, Kubera M, Basta-Kaim A, Jaworska-Feil L, Skuza G, Lasoń W: Effects of combined treatment with imipramine and metyrapone on the immobility time, the activity of hypothalamo-pituitary-adrenocortical axis and immunological parameters in the forced swimming test in the rat. J Physiol Pharmacol, 2005, 56, 49–61.

21. Rogóż Z, Legutko B: Combined treatment with imipramine and metyrapone induces hippocampal and cortical brain-derived neurotrophic factor gene expression in rats. Pharmacol Rep, 2005, 57, 840–844.

22. Rogóż Z, Skuza G, Wójcikowski J, Daniel WA: Effect of combined treatment with imipramine and metyrapone in the forced swimming test in rats. Behavioral and pharmacokinetic studies. Pol J Pharmacol, 2003, 55, 993–999.

23. Rogóż Z, Skuza G, Wójcikowski J, Daniel WA, Wróbel A, Dudek D, Zięba A: Effect of metyrapone supplementation on imipramine therapy in patients with treatment-resistant unipolar depression. Pol J Pharmacol, 2004, 56, 849–855.

24. Tian J, Chau C, Hales TG, Kaufman DL: GABA(A) receptors mediate inhibition of T cell responses. Neuroimmunology, 1999, 96, 21–28.

25. Vetulani J, Nalepa I: Antidepressants: past, present, and future. Eur J Pharmacol, 2000, 405, 351–363.

26. Willner P: The mesolimbic dopamine system as a target for rapid antidepressant action. Int Clin Psychopharmacol, 1997, 12, Suppl 3, S7–S14.