

# Antidepressant-like and anxiolytic-like effects of mild hypobaric hypoxia in mice: possible involvement of neuropeptide Y

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Previous studies have demonstrated that repeated submission of rats to mild hypobaric hypoxia reduces the persistent behavioral and hormonal depressive symptoms induced by exposure to footshock in the learned helplessness paradigm. The aim of this study was to determine whether hypoxic preconditioning of mice can also induce antidepressant- and anxiolytic-like effects that are detectable with the other commonly used behavioral tests, and to determine whether these effects are accompanied by an increase in neuropeptide Y (NPY) in the hippocampus, which may suggest the involvement of NPY in these mechanisms. The intermittent mild hypobaric hypoxia was generated by 2-h exposure of mice to 0.47 atm for 3 consecutive days. In the tail suspension test a significant decrease in the duration of immobility was observed 24 h, but not 48 h after the last hypobaric session. The elevated plus maze trials performed 48 h after preconditioning showed a significant increase in the frequency of open arm entries, a reduction in the duration of closed arm occupancy and substantially more time spent in the open arms in comparison to the control groups. The open field test demonstrated the absence of increases in general activity or unspecific exploratory behavior in hypoxia-preconditioned mice. The EIA test detected a statistically significant but relatively weak increase in the NPY content in the hippocampus 24 h after preconditioning. Together, our data demonstrate that preconditioning of mice with intermittent mild hypobaric hypoxia induces anxiolytic- and antidepressant-like effects. They are accompanied by up-regulation of NPY which may suggest its mechanistic role.

**Key words:** mice, hypoxia, preconditioning, hippocampus, elevated plus maze, tail suspension test, open field, neuropeptide Y

## INTRODUCTION

It has been established that mild hypoxia produced either by hypobaria or via brain ischemia that is sublethal to neurons, induces brain tolerance to subsequent severe hypoxia and ischemia in various animal models (Bergeron et al. 2000, Stroev et al. 2004, Rybnikova et al. 2005a, 2005b, Duszczak et al. 2009). In addition, it has been demonstrated that preconditioning with repetitive sessions of moderate hypobaric hypoxia reduces persistent behavioral and hormonal

depressive symptoms in rats exposed to unpredictable and inescapable footshock in the learned helplessness paradigm (Rybnikova et al. 2007a, 2007b, Baranova et al. 2010, 2012). These authors demonstrated that training with hypobaric hypoxia modulates the responses of the hypothalamic-pituitary-adrenal axis and the expression of NGFI-A and c-Fos in the brain when exposed to stress (Rybnikova et al. 2007a, 2007b, Baranova et al. 2010, 2012). These previous studies have been conducted exclusively on rats, and it is not clear whether this antidepressant-like behavioral effect of hypoxic preconditioning will be detected in other animal models and using different behavioral tests.

Our previous studies demonstrated that precondi-

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tioning with hypobaric hypoxia which induces tolerance to global forebrain ischemia in Mongolian gerbils, is accompanied by a significant rise in the number of neuropeptide Y (NPY)-positive neurons in the hippocampus (Duszczyk et al. 2009). NPY is a 36-amino acid peptide which is widely distributed in the brain, particularly in GABA-ergic interneurons in the hippocampus and cortex. NPY modulates excitatory neurotransmissions, which has been implicated in the mechanisms of affective disorders (Morales-Medina et al. 2010). Anxiolytic- and antidepressant-like effects of neuropeptides NPY and VIP were observed in mice and rats (Redrobe et al. 2002, Karlsson et al. 2008, Ivanova et al. 2014; for reviews see: Heilig 2004, Madaan and Wilson 2009, Morales-Medina et al. 2010). These data may suggest involvement of NPY in the mechanism of anxiolytic- and antidepressant-like effects of the hypoxic preconditioning. However, it is not known whether hypobaric hypoxia in mice results in the increase in the level of NPY in the brain.

The aim of this study was to examine whether repeated treatment with mild hypobaric hypoxia induces antidepressant- and anxiolytic-like effects in mice, and to determine if these effects coincide with increases in the NPY content in the hippocampus.

## EXPERIMENTAL METHODS

### Animals

Experiments were performed using adult male mice Balb/c57 of 20–24 g body weight, fed and supplied with water *ad libitum*. They were bred in the Animal Colony of the Mossakowski Medical Research Centre, Polish Academy of Sciences. In accordance with domestic and international regulations, all efforts were made to minimize animal suffering and the number of animals required. The Third Local Ethical Committee in Warsaw approved all the procedures. The animals were divided into control and experimental groups, of  $n=5$ –11 mice per group, and groups were assigned to particular behavioral test sessions or immunochemical studies. The experimental groups were submitted to hypoxic preconditioning in a hypobaric chamber, whilst the control groups were placed in the hypobaric chamber but were not subjected to any hypoxic treatment. Since in the tail suspension and the open field tests as well as upon determination of the level of NPY in the brain, there were no differences between the

control groups examined 24 and 48 hours after preconditioning, they were combined into one common control group.

### Hypobaric hypoxia

The procedure for hypoxic preconditioning was performed exactly as has been described previously (Rybnikova et al. 2007a, 2007b, Duszczyk et al. 2009). Briefly, three episodes of mild hypobaric hypoxia were produced in the hypobaric chamber by maintaining the pressure at 0.47 atm (equivalent to an altitude of 5000 m above sea level or 10% of the O<sub>2</sub> in normobaric air) for 2 h daily, and the hypobaric sessions were repeated on 3 consecutive days.

### Tail suspension test

The methodology of the tail suspension test was as described by Steru and others (1985) with minor modifications. A paper clip was taped to the tail of each individual mouse, approximately 2 cm from the tip of the tail. Mice were suspended vertically for 6 min at a distance of 30 cm above the surface. Data from the first 2 min of the test were not collected, then a trained observer, blinded to the animal's group, recorded how long each mouse spent immobile during the final 4 min of the test. The duration of immobility is expressed in percent of the recorded period of the test.

### Elevated plus maze

The elevated plus maze testing was performed according to methodology described by Handley and Mithani (1984), considering modifications suggested by Walf and Frye (2007). Testing was performed in a Plexiglas plus-maze elevated to a height of 35 cm. The apparatus consisted of two open arms and two closed arms both of the same dimensions (30×8 cm), with clear Plexiglas walls 20 cm high and arranged so that both the open and closed arms faced each other. The floor of the maze was constructed of black Plexiglas. Test sessions lasted 5 min. The observer, blinded to the animal's group, was situated 2 m from the maze. Evaluation of the anxiolytic-like effects was based on behavioral measures of the time spent in the open and closed arms or in the center platform (expressed as a percentage of total test time), and on

the number of open arm entries (OAE). An entry into a specific arm was scored when a mouse placed all four paws into the arm. The other parameters studied were the number of head dipping (DIP, exploratory movement of head/shoulders over the sides of the maze), and stretch-attend postures (SAP, exploratory posture in which the mouse stretches forward and retracts to original position without locomoting forward) (Ganu et al. 2011).

### Open field test

The open field arena was 70×70 cm with black walls and a white gray floor. It was lit by diffuse light and was virtually divided into 25 squares to assist with data analysis (see below). The test was initiated by placing each mouse in the middle of the arena. The behavior of the animals during 10-min sessions was continuously recorded using a videocamera, this allowed for subsequent analysis without the interference of the experimenter. The arena was cleaned after each mouse with a 70% solution of ethanol and dried. The data were analyzed using Ethovision XT 11.5 software (Noldus, Wageningen, The Netherlands). The analyzed parameters included the total distance traveled (cm); the total number of crossings of the borders of the squares dividing the arena which was calculated as the sum of entries to each of the squares; the frequency of entering the central part, the borders and corners of the arena; and the time (sec) spent by the animals immobile or in locomotion.

### NPY immunochemistry

After termination of the open field test, mice were sacrificed by i.p. injection with a lethal dose of morbital (1.5 mg/kg). Brains were removed, chilled, placed on ice-cooled plates, purified from blood and meninges and dissected to obtain the frontal cortex, hippocampus and hypothalamus as described by Glowinski and Iversen (1966). These samples were weighed, frozen on dry ice and stored at  $-70^{\circ}\text{C}$  for further processing. NPY extraction was performed using procedures described by El-Karim et al. (2003) and Baticic et al. (2011). The samples were homogenized at  $4^{\circ}\text{C}$  in the extraction solution (8 ml/g of tissue) which contained 0.5 M acetic acid and commercially available Calbiochem inhibitors of proteases and phosphatases (Merck KGaA, Darmstadt, Germany). Homogenates were heated for 10 min at

$95^{\circ}\text{C}$ , then chilled to  $4^{\circ}\text{C}$ , centrifuged for 20 min at 2200 g and then the supernatants were stored at  $-70^{\circ}\text{C}$ . Protein concentration was measured by the method of Bradford (1976) and NPY content was determined using a commercial EIA kit (Merck KGaA, Darmstadt, Germany) according to the manufacturer's instruction.

### Statistics

The results are presented as mean values  $\pm$ SEM. Statistical significance of differences between means was tested using the unpaired Student's t-test or ANOVA test (Microsoft Excel v. 2007 and R version 3.22). Significance was taken at  $P < 0.05$ .

## RESULTS

After the initial period of escape-oriented movements, all the tail suspended animals developed an immobile posture at some point during the final 4-min period of the test. In the control groups immobility lasted about 45% of the time. Immobility was significantly reduced to 30.8% in mice tested 24 h after the last hypobaric session ( $P < 0.01$ ) (Fig. 1). However, in mice tested 48 h after preconditioning, although immobility lasted for 36.9% of the time, this value was found not to be significantly different from that of the control (Fig. 1).

The elevated plus maze test (Fig. 2) was performed exclusively 48 h after the last hypobaric session. The animals from the control group preferred the closed arms where they spent about 60% of the time and the

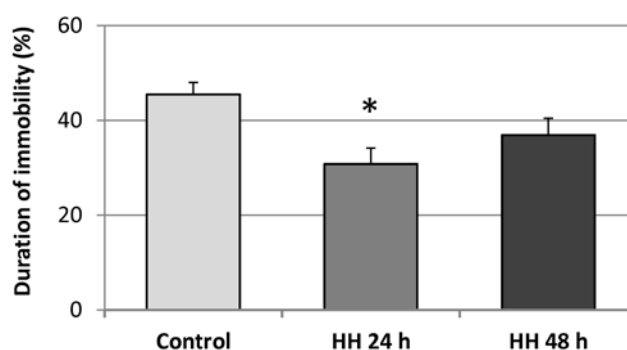


Fig. 1. The effects of preconditioning mice with mild hypobaric hypoxia on immobility in the tail suspension test performed 24 h or 48 h after the last hypobaric session. Data are expressed as percentage of total test time. Bars represent means  $\pm$ SEM ( $n=5-12$ ); \* – result significantly different from control ( $P < 0.01$ , One Way ANOVA).

center of the maze (35% of the time), whereas they spent only 5% of their time on the open arms (Fig. 2A). After the hypobaric treatment the time the mice spent in the open arms increased significantly to 36.2% ( $F=6.230$ ,  $P<0.01$ ), whereas the time they spent in the closed arms decreased to 22.6% ( $F=1.514$ ,  $P<0.002$ ). There was no significant change for time spent in the center of the maze. The number of OAE significantly increased ( $F=4.571$ ,  $P<0.0003$ ) in preconditioned mice, but there were no statistically significant changes in DIP or SAP.

To check whether the antidepressant-like effect observed in the tail suspension and elevated plus maze tests could be due to a general stimulation or increase in non-specific exploratory behavior, separate groups of mice were tested in an open field 24 and 48 h after preconditioning. The results presented in Table I demonstrate that this explanation may be rejected, since we found no significant differences in parameters characterizing mobility and exploratory behavior between control mice and those preconditioned with hypoxia ( $P>0.05$ ).

The results of immunochemical NPY assays using the EIA test (Fig. 3) showed a significant increase of 71.7% in the concentration of NPY in the hippocampus 24 h after hypoxic preconditioning ( $P<0.05$ ); however, 48 h after hypobaria the observed increase was found not to be statistically significant. No other brain regions showed any statistically significant differences in NPY content between control and preconditioned mice.

## DISCUSSION

The results of this study demonstrate that preconditioning of mice with repeated mild hypobaric hypoxia reduces immobility in the tail suspension test and increases occupancy of the open arm in the elevated plus maze, without symptoms of a general stimulation or increase in non-specific exploratory behavior in the open field test. We interpret these results as a reflection of the antidepressant- and anxiolytic-like effects of hypoxic preconditioning. These behavioral changes are accompanied by an increase in the concentration of NPY in the hippocampus 24 h after preconditioning. Although previous studies using exclusively the rat model have described similar behavioral effects of such a treatment on shock-induced depression (Rybnikova et al. 2007a, 2007b), the results of this work, using mice and distinct experimental approaches, provides evidence that antidepressant- and anxiolytic-like effects of mild hypoxia are universal and may be detected using different animals and tests. Up-regulation of NPY in the hippocampus of mice preconditioned with hypobaric hypoxia may reflect the potential defense reaction.

To make our results comparable to the data previously published by Rybnikova and others (2007a, 2007b) we used the same protocol of hypoxic preconditioning that was also previously applied in our laboratory experiments inducing ischemic brain tolerance in *Mongolian* gerbils (Duszczyk et al. 2009). The main differences between our work and studies of Rybnikova

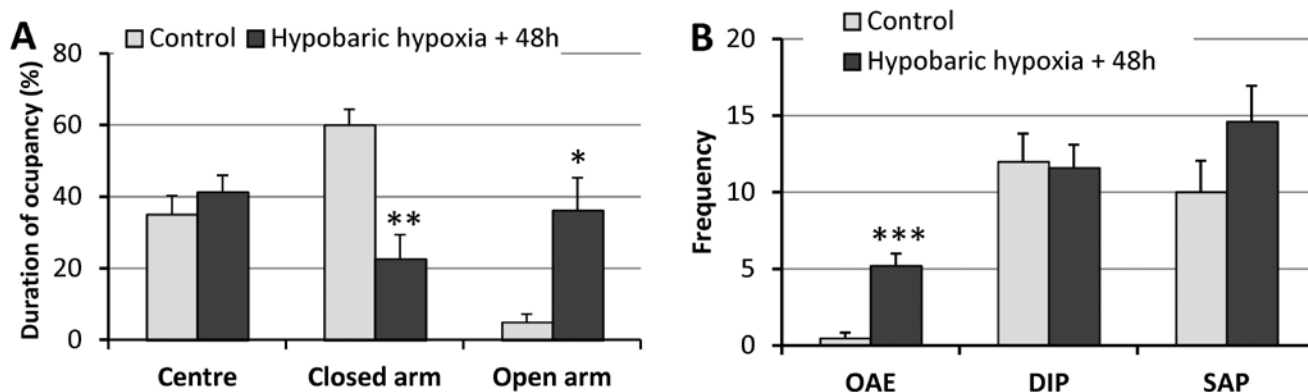


Fig. 2. The effects of preconditioning mice with mild hypobaric hypoxia on behavior in elevated maze test: (A) the occupancy of the open and closed arms and the center of the elevated plus maze, expressed as percentage of total test time, and on the number of the open arm entries (OAE). (B) the open arm head dips (DIP) and stretch-attend postures (SAP). Bars represent means  $\pm$ SEM ( $n=5-6$ ). Asterisks indicate results significantly different from control: (A) \*  $P<0.011$ , \*\*  $P<0.002$ ; (B) \*\*\*  $P<0.0003$ , unpaired Student's *t*-test.

and coworkers is the use of other species of animals and the use of different experimental protocols and behavioral tests. In the experiments of Rybnikova and others (2007a, 2007b) the rats submitted to hypobaric training were exposed to unpredictable and inescapable foot shock in the learned helplessness paradigm, and then the degree of protection against the development of stress-induced persistent depressive symptoms was tested. In contrast, the present study used behavioral tests on mice previously submitted to hypobaric preconditioning only, without any additional stresses. The tail suspension test which we utilized here has been found particularly useful for assessing the behavioral effects of antidepressant compounds and of other manipulations relevant to depression in mice (Cryan et al. 2005). Tail suspension induces a strong acute stress in the animals and the test determines how they cope with an inescapable stressful situation. The posture of immobility in this test has been interpreted as “behavioral despair” (Porsolt et al. 1978), or as a reflection of “entrapment” known from clinical depression (Lucki 2001). Numerous studies have demonstrated that pharmacological intervention with antidepressants reduces the duration of immobility in mice submitted to the tail suspension test (Cryan et al. 2005).

In contrast, the elevated plus maze is a behavioral test that has been widely used to evaluate the anxiety level (Pinheiro et al. 2007, Godlevsky et al. 2014). This test was used by Rybnikova and others (2007a, 2007b), however in our present study it has been used in different contexts and at another period of experiments. In their previous studies (Rybnikova et al. 2007a, 2007b) animals were tested 11 days after exposition of rats to inescapable foot shock, i.e. 12 days after the last hypoxia, whereas here we tested mice 2 days after the last hypobaric session and without additional stresses. In addition to the time spent in open arms, for greater accuracy in characterizing anxiety behavior we also measured the time spent in closed arms and in the center, as well as the number of OAE. Moreover, in the present study measures of the elements of exploratory behavior related to risk assessment (DIP and SAP) (Rex et al. 2002, Ganu et al. 2011) were registered. Finally, in parallel with the behavioral experiments we studied changes in NPY immunoreactivity in the hippocampus of mice after hypobaric preconditioning.

The results of our experiments demonstrated that preconditioning of mice with three consecutive sessions of mild hypobaric hypoxia induced an antidepressant-

like effect, that manifested itself as a reduction of the immobility time in the tail suspension test. However, it appears that this effect is short-lived, because although a statistically significant reduction of the immobility period was observed 24 h after preconditioning, the observed reduction was not statistically significant 48 h after hypoxia. This timing is comparable to the findings of Rybnikova and others (2007a, 2007b), who demonstrated that preconditioning rats with mild hypobaric hypoxia 24 h before inducing a depressive state provided a significant protective effect.

Data of the present study demonstrate the increased time spent in the open arms and the increased frequency of open arms entries (OAE) 48 h after preconditioning. These results reflect the fact that the significant anxiolytic-like effect of hypoxic preconditioning may persist longer than the antidepressant-like effect. However, Rybnikova and others (2007b) have shown that the anxiolytic-like effect of mild hypobaric hypoxia is limited in time and is not detectable in rats 12 days after hypoxic preconditioning. We failed to reveal any statistically significant effect of hypoxic preconditioning on the risk assessment behavior since DIP and SOP remained unchanged. The open field test demonstrated that there were no differences in the behavioral parameters characterizing mobility and exploratory behavior. This confirms that the antidepressant-like and anxiolytic-like effects that were observed in mice after mild hypobaric hypoxia cannot be attributed to increases in mobility or exploratory behavior. In addition, data from Rybnikova and others (2007b) shows that preconditioning with hypobaric hypoxia without addi-

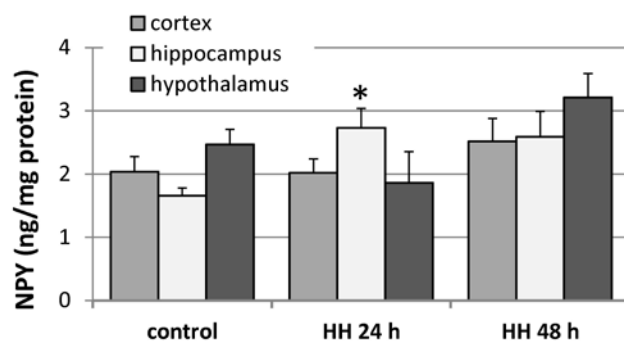


Fig. 3. The effect of preconditioning mice with mild hypobaric hypoxia on NPY concentration in frontal cortex, hippocampus and hypothalamus 24 h or 48 h after the last hypobaric session. Data are expressed as pg/mg protein. Bars represent means  $\pm$  SEM ( $n=5-11$ ); \* – result significantly different from control ( $P<0.05$ , One Way ANOVA).

tional stressors does not affect the locomotory activity of the animals.

Overall, the results of behavioral tests presented here, together with those from the literature (Rybnikova et al. 2007a, 2007b), demonstrate that the antidepressant-like and anxiolytic-like effects of preconditioning with mild hypobaric hypoxia may be induced in different species and may be visualized using various experimental approaches and behavioral tests. These data support the claims of Rybnikova and others (2008) who suggested that preconditioning with mild hypobaric hypoxia may be useful in clinical practice for the prophylaxis of stress-induced depressive episodes, and even their suggestion of its possible usefulness as complementary treatment to pharmacological therapy in other forms of depression.

The mechanism of antidepressant- and anxiolytic-like effects of preconditioning with hypobaric hypoxia is unclear. Previous studies point to alterations in the hippocampal expression of glucocorticoid receptors, in reactivity of the hypothalamic-pituitary-adrenal axis, and in intracellular signaling in preconditioned rats (Rybnikova et al. 2007a, 2007b, Baranova et al. 2010, 2012). Our experiments were aimed at a preliminary assessment of the NPY involvement in the mechanisms of this phenomenon. This supposition, which supplements and extends the explanation provided by Rybnikova and co-workers, has been based on numerous experimental data demonstrating the antidepressant and anxiolytic effects of NPY, mediated mainly by  $Y_1$  receptors (Heilig 2004, Madaan and Wilson 2009, Morales-Medina et al. 2010). Research in humans has also provided data supporting the role of NPY in regulation of mood (Garcia et al. 2012, Morales-Medina et al. 2010). Studies on the mechanisms of disturbances in appetite and blood circulation at high altitudes have shown that both prolonged and acute hypobaric hypoxia leads to increased blood NPY concentration in humans and animals (Cheng et al. 1992, Knudtzon et al. 1989, Llanos et al. 2007). We were also encouraged by our previous results demonstrating a prominent, approximately 100% increase in the number of NPY-positive neurons in the hippocampus of gerbils submitted to hypobaric hypoxia (Duszczyk et al. 2009).

The immunochemical analysis of this study showed the NPY levels in the control mouse brain cortex, hippocampus and hypothalamus in the range of 2–3 ng/mg prot. These values are one order of magni-

tude lower than 10 pmol/mg prot. (43 ng/mg prot.), found by Ruohonen and others (2008) in mice hypothalamus using radioimmunoassay. These differences may be explained by distinct methods of NPY extraction and detection. We detected increase in the NPY concentration in the hippocampus of mice preconditioned with hypobaric hypoxia, albeit this effect was rather small. Our data indicate that NPY may be considered as a potential participant in the mechanism of the antidepressant- and anxiolytic-like effects of preconditioning with mild hypobaric hypoxia. However, further research is required to establish more precisely a role of NPY in the complex mechanism(s) of brain adaptation to various stressors, including hypobaric preconditioning.

## CONCLUSIONS

Our behavioral data indicate that intermittent mild hypobaric hypoxia in mice induces antidepressant- and anxiolytic-like effects. A modest but significant increase in the content of NPY in the hippocampus was observed in mice after hypoxic preconditioning. These results may encourage further investigations as to the role of NPY in the mechanism of beneficial behavioral effects of hypobaric preconditioning.

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## REFERENCES

- Baranova KA, Rybnikova EA, Mironova VI, Samoilov MO (2010) Effects of hypoxic preconditioning on expression of transcription factor NGFI-A in the rat brain after unavoidable stress in the “learned helplessness” model. *Neurosci Behav Physiol* 40: 693–700.
- Baranova KA, Rybnikova EA, Samoilov MO (2012) Antidepressant effect of hypoxic preconditioning is associated with modification of expression of transcription

- factor c-Fos in rat brain in response to unavoidable stress. *Bull Exp Biol Med* 152: 564–567.
- Baticic L, Detel D, Kucic N, Buljevic S, Pugel EP, Varljen J (2011) Neuroimmunomodulative properties of dipeptidyl peptidase IV/CD26 in a TNBS-induced model of colitis in mice. *J Cell Biochem* 112: 3322–3333.
- Bergeron M, Gidday JM, Yu AY, Semenza GL, Ferriero DM, Sharp FR (2000) Role of hypoxia-inducible factor-1 in hypoxia-induced ischemic tolerance in neonatal rat brain. *Ann Neurol* 48: 285–296.
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248–254.
- Cheng JT, Chen CF, Shum AY, Wang JY, Chen HI (1992) Increase of plasma neuropeptide Y-like immunoreactivity following chronic hypoxia in the rat. *Neurosci Lett* 140: 211–214.
- Cryan JF, Mombereau C, Vassout A (2005) The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. *Neurosci Biobehav Rev* 29: 571–625.
- Duszczek M, Ziembowicz A, Gadamski R, Wieronska JM, Smialowska M, Lazarewicz JW (2009) Changes in the NPY immunoreactivity in gerbil hippocampus after hypoxic and ischemic preconditioning. *Neuropeptides* 43: 31–39.
- El-Karim I, Lundy FT, Linden GJ, Lamey PJ (2003) Extraction and radioimmunoassay quantitation of neuropeptide Y (NPY) and vasoactive intestinal polypeptide (VIP) from human dental pulp tissue. *Arch Oral Biol* 48: 249–254.
- Ganu G, Garud A, Agarwal V, Suralkar U, Jadhav S, Kshirsagar A (2011) Anti-anxiety activity of *Mimusops elengi* barks extract in experimental animals. *Res J Pharmacol Biol Chem Sci* 2: 405–410.
- Garcia FD, Coquerel Q, do Rego JC, Cravezic A, Bole-Feysot C, Kiive E, Déchelotte P, Harro J, Fetissov SO (2012) Anti-neuropeptide Y plasma immunoglobulins in relation to mood and appetite in depressive disorder. *Psychoneuroendocrinology* 37: 1457–1467.
- Glowinski J, Iversen LL (1966) Regional studies of catecholamines in the rat brain-I. The disposition of (<sup>3</sup>H)nor-epinephrine, (<sup>3</sup>H)dopamine and (<sup>3</sup>H)DOPA in various regions of the brain. *J Neurochem* 13: 655–669.
- Godlevsky LS, Muratova TN, Kresyun NV, van Luijtelaar G, Coenen AM (2014) Anxiolytic and antidepressive effects of electric stimulation of the paleocerebellar cortex in pentylenetetrazol kindled rats. *Acta Neurobiol Exp (Wars)* 74(4): 456–464.
- Handley SL, Mithani S (1984) Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of ‘fear’-motivated behaviour. *Naunyn Schmiedebergs Arch Pharmacol* 327: 1–5.
- Heilig M (2004) The NPY system in stress, anxiety and depression. *Neuropeptides* 38: 213–224.
- Ivanova M, Belcheva S, Belcheva I, Stoyanov Z, Tashev R (2014) Modulatory effect of VIP injected into hippocampal CA1 area on anxiety in olfactory bulbectomized rats. *Acta Neurobiol Exp (Wars)* 74(3): 317–327.
- Karlsson RM, Choe JS, Cameron HA, Thorsell A, Crawley JN, Holmes A, Heilig M (2008) The neuropeptide Y Y1 receptor subtype is necessary for the anxiolytic-like effects of neuropeptide Y, but not the antidepressant-like effects of fluoxetine, in mice. *Psychopharmacology (Berl)* 195: 547–557.
- Knudtson J, Myhre K, Rasch W, Neslein IL, Bogsnes A, Opstad PK (1989) Reduced systolic blood pressure elevations during maximum exercise at simulated altitudes. *Aviat Space Environ Med* 60: 1072–1076.
- Llanos AJ, Riquelme RA, Herrera EA, Ebensperger G, Krause B, Reyes RV, Sanhueza EM, Pulgar VM, Behn C, Cabello G, Parer JT, Giussani DA, Blanco CE, Hanson MA (2007) Evolving in thin air – lessons from the llama fetus in the altiplano. *Respir Physiol Neurobiol* 158: 298–306.
- Lucki I (2001) A prescription to resist proscriptions for murine models of depression. *Psychopharmacology (Berl)* 153: 395–398.
- Madaan V, Wilson DR (2009) Neuropeptides: relevance in treatment of depression and anxiety disorders. *Drug News Perspect* 22: 319–324.
- Morales-Medina JC, Dumont Y, Quirion R (2010) A possible role of neuropeptide Y in depression and stress. *Brain Res* 1314: 194–205.
- Pinheiro SH, Zangrossi H Jr, Del-Ben CM, Graeff FG (2007) Elevated mazes as animal models of anxiety: effects of serotonergic agents. *An Acad Bras Cienc* 79: 71–85.
- Porsolt RD, Anton G, Blavet N, Jalfre M (1978) Behavioural despair in rats: a new model sensitive to antidepressant treatments. *Eur J Pharmacol* 47: 379–391.
- Redrobe JP, Dumont Y, Fournier A, Quirion R (2002) The neuropeptide Y (NPY) Y1 receptor subtype mediates NPY-induced antidepressant-like activity in the mouse forced swimming test. *Neuropsychopharmacology* 26: 615–624.
- Rex A, Morgenstern E, Fink H (2002) Anxiolytic-like effects

- of kava-kava in the elevated plus maze test – a comparison with diazepam. *Prog Neuropsychopharmacol Biol Psychiatry* 26: 855–860.
- Ruohonen ST, Pesonen U, Moritz N, Kaipio K, R  ytt   M, Koulou M, Savontaus E (2008) Transgenic mice overexpressing neuropeptide Y in noradrenergic neurons: a novel model of increased adiposity and impaired glucose tolerance. *Diabetes* 57: 1517–1525.
- Rybnikova E, Mironova V, Pivina S, Tulkova E, Ordyan N, Nalivaeva N, Turner A, Samoilov M (2007a) Involvement of the hypothalamic-pituitary-adrenal axis in the antidepressant-like effects of mild hypoxic preconditioning in rats. *Psychoneuroendocrinology* 32: 813–823.
- Rybnikova E, Mironova V, Pivina S, Tulkova E, Ordyan N, Vataeva L, Vershinina E, Abritalin E, Kolchev A, Nalivaeva N, Turner AJ, Samoilov M (2007b) Antidepressant-like effects of mild hypoxia preconditioning in the learned helplessness model in rats. *Neurosci Lett* 417: 234–239.
- Rybnikova E, Vataeva L, Tyulkova E, Glushchenko T, Otellin V, Pelt  -Huikko M, Samoilov MO (2005a) Mild hypoxia preconditioning prevents impairment of passive avoidance learning and suppression of brain NGFI-A expression induced by severe hypoxia. *Behav Brain Res* 160: 107–114.
- Rybnikova EA, Khozhai LI, Tyul'kova EI, Glushchenko TS, Sitnik NA, Pelt  -Huikko M, Otellin VA, Samoilov MO (2005b) Expression of early gene proteins, structural changes in brain neurons in hypobaric hypoxia, and the correcting effects of preconditioning. *Neurosci Behav Physiol* 35: 383–388.
- Rybnikova EA, Samoilov MO, Mironova VI, Tyul'kova EI, Pivina SG, Vataeva LA, Ordyan NE, Abritalin EY, Kolchev AI (2008) The possible use of hypoxic preconditioning for the prophylaxis of post-stress depressive episodes. *Neurosci Behav Physiol* 38: 721–726.
- Steru L, Chermat R, Thierry B, Simon P (1985) The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology (Berl)* 85: 367–370.
- Stroev SA, Glushchenko TS, Tyulkova EI, Spyrou G, Rybnikova EA, Samoilov MO, Pelt  -Huikko M (2004) Preconditioning enhances the expression of mitochondrial antioxidant thioredoxin-2 in the forebrain of rats exposed to severe hypobaric hypoxia. *J Neurosci Res* 78: 563–569.
- Walf AA, Frye CA (2007) The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Protoc* 2: 322–328.