Original research article / Artykuł oryginalny

Farmakoterapia w Psychiatrii i Neurologii 2016, 32 (2), 79–84
© 2016 Institute of Psychiatry and Neurology. All rights reserved.
DOI: http://dx.medra.org/10.17393/fpn.2016.07.001

The long term administration of haloperidol supports the origin of DCX-expressing cells in the adult rat brain

Artur Pałasz[1], Michalina Respondek[2], Ewa Rojczyk[1], Katarzyna Bogus[1], Łukasz Filipczyk[1], Marek Krzystanek[3], Ryszard Wiaderkiewicz[1]

ABSTRACT

Aims. Continuously active neurogenic regions in the adult mammalian brain are located in the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampal dentate gyrus. The neurogenesis process is modulated by many factors e.g. growth factors, neurotransmitters and hormones. Neuropsychiatric drugs, especially antidepressants, mood stabilizers and antipsychotics may also affect the dynamics of the origin of neuronal cells. The purpose of the study was to determine the effects of long-term haloperidol treatment on adult rat neurogenesis at the level of canonical neurogenic sites.

Materials and methods. The studies were carried out on adult male Sprague-Dawley rats. Two groups of animals (5 in each) received, respectively, saline and haloperidol (2 mg/kg/day) by intraperitoneal injection for 28 days. The number of neuroblasts was evaluated using immunohistochemical detection of doublecortin (DCX) expressing cells. The total number of DCX-positive cells in the neurogenic zones was counted for each rat (which was the sum of cells from 10 slices) and the results were divided per length of the studied subependymal area (SGZ) and dentate gyri (SGZ) to obtain density of immunopositive cells per one millimeter of length.

Results. The results indicate that haloperidol has proneurogenic effects on the adult rat brain, especially in the SVZ, as the mean number of DCX-positive cells increased significantly in SVZ and there was a similar tendency in the SGZ.

Conclusions. We found that long-term treatment with haloperidol stimulated DCX-positive cell formation in the SVZ, which supports adult neurogenesis.

Received 14.02.2016
Accepted 27.06.2016

AFFILIATIONS / AFILIACJE

1 Medical University of Silesia in Katowice, School of Medicine, Department of Histology
2 Medical University of Silesia in Katowice, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Department of Pharmaceutical Chemistry
3 Medical University of Silesia in Katowice, School of Medicine in Katowice, Department and Clinic of Psychiatric Rehabilitation

KEYWORDS

- haloperidol
- adult neurogenesis
- doublecortin
- neuroblasts
- brain

CORRESPONDENCE ADDRESS / ADRES DO KORESPONDENCJI

Artur Pałasz
Śląski Uniwersytet Medyczny
ul. Medyków 18, 40-752 Katowice, Poland
phone: +48 32 20 88 377, *email*: apalasz@sum.edu.pl

Introduction

The canonical, continuously active neurogenic sites in the adult brain are located in the forebrain subventricular zone (SVZ) and subgranular zone (SGZ) of the hippocampal dentate gyrus. It has been proven that adult neurogenesis can be regulated by numerous drug treatments and diverse environmental factors. Among the pharmaceuticals that affect this process, special attention should be paid to those commonly used in psychiatric practice, mainly antidepressants, antipsychotics and mood stabilizers. The neuromodulating properties of these drugs rely on the expression of neurotrophic factors and cellular neuroprotective mechanisms slowing down the neuronal death. Many antidepressants such as monoamine oxidase blockers (MAOI) and selective serotonin/noradrenaline reuptake inhibitors (SSRI/SNRI) may distinctly stimulate the proliferation of neural stem cells (NSCs) (Duman *et al.* 2001, Hunsberger *et al.* 2009). For instance, long-term treatment with fluoxetine and reboxetine highly elevates the proliferation level in the rat hippocampus (Malberg *et al.* 2000) The mechanism of this stimulation involves brain-derived neurotrophic factor (BDNF) expression, activation of the 5HT-1A receptor (Jacobs *et al.* 1998) and the induction of the cAMP pathway through an increase in cAMP response element-binding protein (CREB) fosforylation (Duman *et al.* 2001). Previous studies have shown that mood stabilizers have also a positive impact on the course of adult neurogenesis e.g. valproate and lithium, which indirectly regulate several neuroprotective factors such as BDNF and bcl-2 expression or protein kinase C activity (Manji *et al.* 2002). Moreover, lithium inhibits the GSK-3β (glycogene synthase kinase 3β) pathway and raises the β-catenin level (Fiorentini *et al.* 2010). The β-catenins facilitate the synthesis of key growth factors including IGFs, BDNF and VEGF, which directly stimulate the proliferation and survival of newborn neurons (Wada *et al.* 2009). Some reports indicate that brain-specific angiogenesis inhibitor 2 (BAI2) may also influence hippocampal neurogenesis (Okajima *et al.* 2011).

In turn, numerous drugs negatively influence adult neurogenesis, e.g. opiates and anxiolytics. For instance, long-term morphine or heroin use decreases the rate of proliferation and viability of the new neurons in the adult rat hippocampus (Eisch *et al.* 2000). Phenobarbital and clonazepam may also inhibit neurogenesis in the SVZ; however their effects were observed in young immature rats only, but negative changes were still present in adults, even after the discontinuation of treatment (Chen *et al.* 2009). It is worth noting that cyclooxygenase-2 inhibitors (COX-2) – nimesulide and meloxicam – are also strong inhibitors of neurogenesis. They can completely depress neurogenesis in the SVZ after 5-days of administration, probably by reducing the activity of microglia (Goncalves *et al.* 2010).

Commonly used antipsychotics like olanzapine, haloperidol and risperidone also influence the formation of new cells in the adult mammalian brain (Dawirs *et al.* 1998, Keilhoff *et al.* 2010, Wakade *et al.* 2002, Wang *et al.* 2004). Their effect on adult neurogenesis is manifested by an increased number and survival of newly formed cells. Olanzapine and risperidone enhance cell proliferation in the SVZ, and in the prefrontal cortex, but in the case of haloperidol the number of BrdU labeled cells was unchanged (Wakade *et al.* 2002, Wang *et al.* 2004). Dawirs *et al.* 1998 observed neurogenic activity with haloperidol but only if the dose was very high – 15 mg daily in three separated doses. They showed that the number of newly formed cells in the SVZ region increased significantly. Both haloperidol and risperidone increased the survival rate of new neurons, mainly through an increase in the level of anti-apoptotic proteins (Bcl-2) and also via VEGF and matrix metalloproteinases (MMPs) activation (Keilhoff *et al.* 2010). The aim of the study was to determine the effect that the long-term administration of typical neuroleptic haloperidol would have on the origin of new neural cells in the rat brain by counting doublecortin (DCX)-positive cells in the canonical neurogenic sites.

Materials and methods

The studies were carried out on adult (2 months old, 180–220 g) male Sprague-Dawley rats from the Medical University of Silesia Experimental Centre housed at 22°C with a regular 12/12 light-darkness cycle with access to standard Murigan chow and water *ad libitum*. All procedures were approved by the Local Ethical Committee for Experiments on Animals at the Medical University of Silesia (decision no. 36/2012) and were conducted in a manner consistent with NIH Guidelines for the Care and Use of Laboratory Animals.

Two groups of animals (5 in each) received, respectively, saline and haloperidol (2 mg/kg/day, dissolved in isotonic sodium chloride solution) by intraperitoneal injection for 4 weeks. Haloperidol is a butyrophenone type potent antagonist of the dopamine D_2 receptors which also affects adrenergic and serotonergic signalling. 24 hours after the last drug administration the rats were quickly anaesthetized with isoflurane and then immediately sacrificed via decapitation. After that, the brains were excised, fixed with 4% paraformaldehyde PBS (pH 7.2–7.4), dehydrated, embedded in paraffin and finally sectioned on the microtome (Leica Microsystems, Germany) in the coronal planes for the SVZ and the SGZ (0.36 to -0.50 and -2.00 to -2.80 mm from bregma, respectively) at 7 µm thick slices.

After blockage with 5% goat serum sections were incubated overnight with the goat antibody against rat doublecortin (DCX) (1:1000, Santa Cruz

Pharmaceuticals). Primary antibodies were followed by biotynylated goat anti-rabbit secondary antibodies, and then an avidin-biotin-horseradish peroxidase complex (Vectastain ABC kit, Vector Labs). Finally, 3,3'-diaminobenzidine (DAB) was used to complete the reaction. All sections were mounted on glass slides, dehydrated and coverslipped. Additionally, after overnight incubation with primary antibodies for DCX, several brain sections were kept in darkness with secondary antibodies labeled with FITC (1:200, Abcam) and then mounted on slides with a DAPI-containing medium. For the calculation of DCX-positive cells, 10 slices (every tenth one) per rat for each brain region were used. All images (8 per slice) were captured with Nikon Coolpix optic systems and processed using Image ProPlus software (Media Cybernetics, USA). Anatomically comparable sections were analyzed and immunopositive cells were counted using ImageJ 1.43u software. We counted the total number of DCX-positive cells in the neurogenic zones for each rat (which was the sum of cells from 10 slices) and then divided the results per length of the analyzed subependymal regions (SGZ) and dentate gyri (SGZ) to obtain density of immunopositive cells per one millimeter of length. Data are presented as mean ± standard error of the mean (SEM). The relatively small number of animals examined may be a limitation of our study.

Statistical analyses were performed using Statistica (Systat Software). Mean differences between experimental groups were analyzed using the non-parametric Kruskall-Wallis test. Differences were considered statistically significant at $p \leq 0.05$.

Results

The mean density of DCX-immunopositive cells in the SVZ of animals exposed to haloperidol was 474.19 ± 32.27, whereas in control slices it was 374. 5 ± 36.32 ($p = 0.043$, $z = 2.021$; Fig. 2). Thus, the increase in neuroblast formation for the SVZ was 21.11%. All relatively densely packed DCX-immunoreactive neuroblasts were located beneath the wall of the lateral ventricles (Fig. 1 C,D). The mean density of DCX-immunopositive cells in the SGZ was 107.6 ± 46.69 for animals treated with haloperidol and 71.62 ± 16.67 for the control group ($p = 0.286$, $z = 0.866$; Fig. 2). Consequently, the increase in neuroblast formation in the SGZ was 33.5%. However, this result turned out to be statistically insignificant. In analyzed slices containing the SGZ, neuroblasts were dispersed in the subgranular zone of the dentate gyrus and (contrarily to the SVZ) did not form any clustered forms (Fig. 1 E,F).

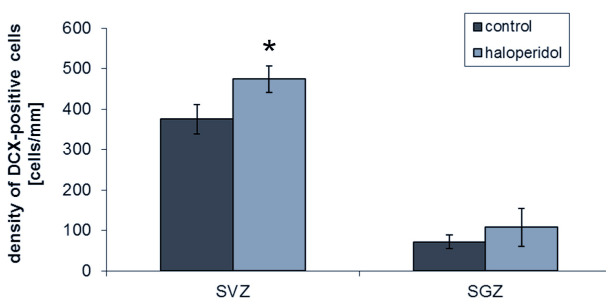

Figure 2 Number of doublecortin (DCX)-positive cells in the SVZ and SGZ after haloperidol administration. Values are expressed as means ± SEM. Differences between experimental groups were analyzed using non-parametric Kruskall-Wallis test. $p < 0.05$ is considered as statistically significant.

Discussion

Two groups of neuroleptics have so far been used in experimental studies: a group of atypical antipsychotics (olanzapine, clozapine, risperidone) and a group of typical neuroleptics – mainly haloperidol (Dawirs *et al.* 1998, Keilhoff *et al.* 2010, Wakade *et al.* 2002, Wang *et al.* 2004, Halim *et al.* 2004). The previous studies have provided unclear data about the positive effects of haloperidol on neurogenesis. In the current experiment, with the use of immunohistochemical stainings we have been able to show the differences in the number of neuroblasts in the classical neurogenic areas in the experimental vs. control group. The first studies on the effects of haloperidol on cell proliferation were performed on young rats (11-days old) by Backhouse *et al.* 1989; they reported that 24 hours after drug administration (a single dose of 20 mg/kg) the number of newly formed cells decreased by about 50%. Our data shows the stimulatory impact of haloperidol on neurogenesis in adult rat brain. The pharmaceuticals were administered intraperitoneally for 28 days in a daily dose of 2 mg/kg. This resulted in significantly increased neuroblast formation in the SVZ region, suggesting that haloperidol positively modulates neurogenesis in this brain region. Besides the number of cells we have also determined their locality. Qualitative analysis of brain slices has revealed that a large concentration of new neuroblasts is present in the region just under the ependymal lining of the lateral ventricles. The highest density of new cells was observed in the anterior part of the ventricles, where the rostral migratory stream (RMS) begins. Comparing brain slices from experimental and control groups there were no significant differences in the locality of neuroblasts. The data on the neurogenic effect of haloperidol published so far are ambiguous. Keilhoff *et al.* showed a potent increase of cell proliferation in the SGZ after a 10 day-long administration of a low (0.078 mg/kg) dose of haloperidol. That result indicates a higher proliferation potential than we found in our study. Similar results were obtained

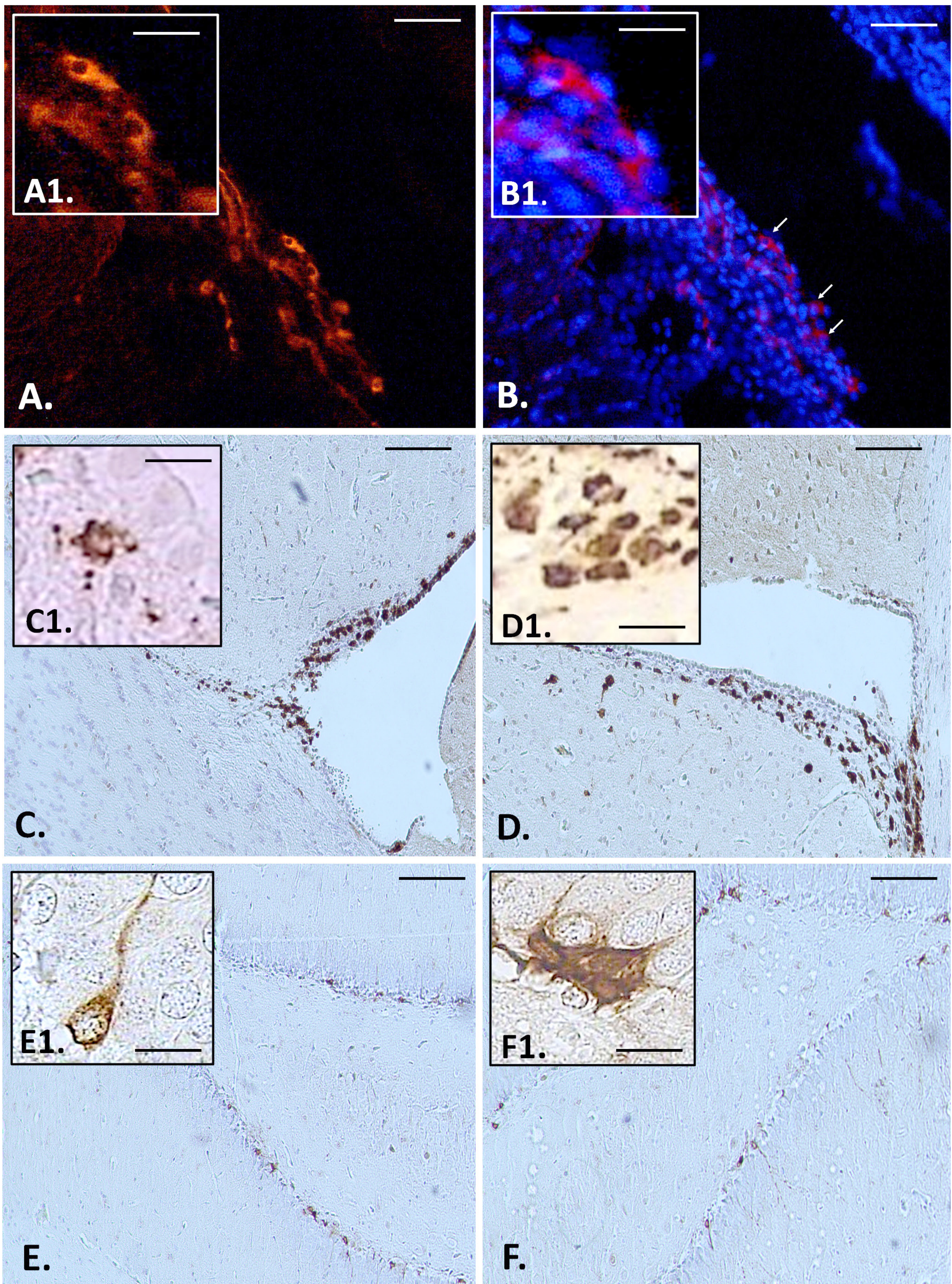

Figure 1 Doublecortin (DCX) immunopositive cells in the SVZ and SGZ. Fluorescence; DCX-expressing cells (red) in the SVZ after treatment with haloperidol (A), merged with DAPI (B, arrows). Classical DAB-immunostaining in the SVZ; controls and experimental animals (C and D) in the SGZ (E and F) respectively. Insets show the cell morphology under higher magnifications. Scale bars: 50 μm (A,B), 100 μm (C-F) 20 μm (all insets).

by Dawirs *et al.* 1998, who showed a high increase of the number of newly formed neural cells in the rat SGZ after a one-day long drug administration. However, in his experiment the drug was delivered 3 times a day at a 5 mg/kg dose. Thus, the daily dose used was 25 times higher than in Keilhoff's experiment and 7.5 times higher than in our experiment. The positive effect of haloperidol was also observed in the rat cellular model, where the haloperidol was delivered for 14 and 30 days with subcutaneous osmotic pumps at a daily dose of 0.05, 0.25 and 2.00 mg/kg and the number of primary neurospheres derived from forebrain lateral ventricle subependyma was determined to index the quantity of in vivo neural stem cell proliferation (Kippin *et al.* 2005). Some other studies also confirm the positive effect of haloperidol, and additionally show that the most BrdU positive cells express such markers as neuronal nuclear antigen (NeuN), doublecortin (DCX), nestin and glial fibrillary acid protein (GFAP) (Keilhoff *et al.* 2010). A number of the published papers provide inconsistent results with regard to the proneurogenic effect of haloperidol. Wang *et al.* did not show any proneurogenic effect of haloperidol, although they administered l a haloperidol dose identical to that in our experiment (Malberg *et al.* 2000, Wang *et al.* 2004). However, it should be underlined that they administered the drug for a shorter period (3 weeks). Wakade *et al.* also did not obtain a positive effect of haloperidol, but they used smaller dose of the drug (0.4 mg/kg), administered for 21 days. The protocols of drug administration differ from study to study, so it is difficult to formulate objective conclusions. However, we suggest that the highest impact on the cell proliferation is made by the long-term administration of drug at a dose of 2 mg/kg. The proneurogenic effects of haloperidol can be compared with other antipsychotics which have an influence on neural proliferation in the adult brain. For example, both risperidone and olanzapine are able to increase proliferation potential in the SVZ by 2–3 times (Wakade *et al.* 2002). In another study the neurogenic effect of olanzapine was very weak (a few percent) and limited to the striatum (Wang *et al.* 2004). After clozapine administration, a great increase in cell proliferation was observed in the dentate gyrus (Halim *et al.* 2004). Comparing the proneurogenic effects of haloperidol with other antipsychotic medications, it has to be stated that risperidone, clozapine and olanzapine have a higher neurogenic potential than haloperidol (Wang *et al.* 2004). The proneurogenic effect of haloperidol is caused by the inhibition of dopamine D_2 receptors. NSCs in the adult rat brain have the D_2 receptors in the cytoplasmic membrane, and their stimulation suppresses cell proliferation. Haloperidol, acting as a D_2 receptor antagonist, prevents the negative effect of dopamine on NSCs mitotic potential (Kippin *et al.* 2005). Haloperidol can also increase the level of prolactin, which has proneurogenic properties and increases neurogenesis in pregnant mice (Shingo *et al.* 2003). Prolactin may act directly through its receptors located on NSCs, and indirectly by increasing the expression of the anti-apoptotic Bcl-2 protein (Torner *et al.* 2009). Moreover, haloperidol increases expression of the MMPs and nerve growth factor (NGF), which influences the proliferation and survival of NSCs in the hippocampus (Keilhoff *et al.* 2010). On the other hand haloperidol reduces the level of BDNF, choline acetyltrasferase and tyrosine hydroxylase (TOH) in the cortex and ventral pallidum (Parikh *et al.* 2004, Meredith *et al.* 2004). However, it should be emphasized that a decreased level of neurotrophins does not necessarily suppress adult neurogenesis (Nandra and Agius 2012). Conversely, some evidence suggests that haloperidol may increase the level of the proapoptotic proteins Bax and p53 as well as reactive oxygen species (ROS) to stimulate neuronal apoptosis in the hippocampus (Lezoualc'h *et al.* 1996, Sagara 1998, Post *et al.* 2002). It should also not be excluded that our results reflect, rather, a development of tolerance to haloperidol-induced catalepsy/sedation and alterations in neurogenesis secondary to the behavioural effects of the drug. Further studies are needed to reveal whether the observed effect of haloperidol on DCX-positive cell formation is due to increased proliferation, reduced apoptosis or both processes.

Conclusions

The results cautiously but clearly indicate that the long term administration of haloperidol can support adult neurogenesis in the rat SVZ. In the future, it would also be of interest to study in detail the features and fate of newly formed neurons in both the SVZ and the SGZ after treatment with classical and atypical neuroleptic medications. ■

Acknowledgements

The authors thank Magdalena Bandyszewska, MSc and Barbara Rojczyk, MSc Eng., MA for their technical assistance.

This work was supported by the Medical University of Silesia grant KNW-1-128/N/5/0.

Conflict of interest: The authors declare no conflict of interest.

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans, EU Directive 2010/63/EU for animal experiments, and Uniform Requirements for manuscripts submitted to biomedical journals.

Authors' contributions: AP, MR – the idea and performance of the experiment as well as the preparation of the text of the manuscript; ER, ŁF – morphometric determination; KB – morphometric statistics; MK, RW – text review, content supervision.

References

1. Backhouse B, Barochovsky O, Malik *et al.*. Effects of haloperidol on cell proliferation in the early postnatal rat brain. Neuropathol Appl Neurobiol 1982; 8: 109–116.
2. Chen J, Cai F, Cao J *et al.* Long-term antiepileptic drug administration during early life inhibits hippocampal neurogenesis in the developing brain. J Neurosci Res 2009; 87: 2898–2907.
3. Dawirs RR, Hildebrandt K, Teuchert-Noodt G. Adult treatment with haloperidol increases dentate granule cell proliferation in the gerbil hippocampus. J Neural Transm 1998; 105: 317–327.
4. Duman RS, Malberg J, Nakagawa S. Regulation of adult neurogenesis by psychotropic drugs and stress. J Pharmacol Exp Ther 2001; 299: 401–407.
5. Eisch AJ, Barrot M, Schad CA *et al.* Opiates inhibit neurogenesis in the adult rat hippocampus. Proc Natl Acad Sci U S A 2000; 97: 7579–7584.
6. Fiorentini A, Rosi MC, Grossi C *et al.* Lithium improves hippocampal neurogenesis, neuropathology and cognitive functions in APP mutant mice. PLoS One 2010; 5: e14382.
7. Goncalves MB, Williams EJ, Yip P *et al.* The COX-2 inhibitors, meloxicam and nimesulide suppress neurogenesis in the adult mouse brain. Br J Pharmacol 2010; 159: 1118–1125.
8. Halim ND, Weickert CS, McClintock BW *et al.* Effects of chronic haloperidol and clozapine treatment on neurogenesis in the adult rat hippocampus. Neuropsychopharmacology 2004; 29: 1063–1069.
9. Hunsberger J, Austin DR, Henter ID *et al.* The neurotrophic and neuroprotective effects of psychotropic agents. Dialogues Clin Neurosci 2009; 11: 333–348.
10. Jacobs B, Tanapat P, Reeves A *et al.* Serotonin stimulates the production of new hippocampal granule neurons via the 5HT1A receptor in the adult rat. Soc Neurosci Abstr 1998; 23: 1992.
11. Keilhoff G, Grecksch G, Bernstein HG *et al.* Risperidone and haloperidol promote survival of stem cells in the rat hippocampus. Eur Arch Psychiatry Clin Neurosci 2010; 260: 151–162.
12. Kippin TE, Kapur S, van der Kooy D. Dopamine specifically inhibits forebrain neural stem cell proliferation, suggesting a novel effect of antipsychotic drugs. J Neurosci 2005; 25: 5815–5823.
13. Lezoualc'h F, Rupprecht R, Holsboer F *et al.* Bcl-2 prevents hippocampal cell death induced by the neuroleptic drug haloperidol. Brain Res 1996; 738: 176–179.
14. Malberg JE, Eisch AJ, Nestler EJ *et al.* Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 2000; 20: 9104–9110.
15. Manji HK, Chen G. PKC, MAP kinases and the bcl-2 family of proteins as long-term targets for mood stabilizers. Mol Psychiatry 2002; 7 Suppl 1: S46–56.
16. Meredith GE, Switzer RC 3rd, Napier TC. Short-term D2 receptor blockade induces synaptic degeneration, reduces levels of tyrosine hydroxylase and brain-derived neurotrophic factor and enhances D2-mediated firing in the ventral pallidum. Brain Res 2004; 995: 14–22.
17. Nandra KS, Agius M. The differences between typical and atypical antipsychotics: the effects on neurogenesis. Psychiatr Danub 2012; 24: 95–99.
18. Okajima D, Kudo G, Yokota H. Antidepressant-like behavior in brain-specific angiogenesis inhibitor 2-deficient mice. J Physiol Sci 2011; 61: 47–54.
19. Parikh V, Khan MM, Terry A *et al.* Differential effects of typical and atypical antipsychotics on nerve growth factor and choline acetyltransferase expression in the cortex and nucleus basalis of rats. J Psychiatr Res 2004; 38: 521–529.
20. Post A, Rücker M, Ohl F *et al.* Mechanisms underlying the protective potential of alpha-tocopherol (vitamin E) against haloperidol-associated neurotoxicity. Neuropsychopharmacology 2002; 26: 397–407.
21. Sagara Y. Induction of reactive oxygen species in neurons by haloperidol. J Neurochem 1998; 71: 1002–1012.
22. Shingo T, Gregg C, Enwere E *et al.* Pregnancy-stimulated neurogenesis in the adult female forebrain mediated by prolactin. Science 2003; 299: 117–120.
23. Torner L, Karg S, Blume A *et al.* Prolactin prevents chronic stress-induced decrease of adult hippocampal neurogenesis and promotes neuronal fate. J Neurosci 2009; 29: 1826–1833.
24. Wada A. Lithium and neuropsychiatric therapeutics: neuroplasticity via glycogen synthase kinase-3beta, beta-catenin, and neurotrophin cascades. J Pharmacol Sci 2009; 110: 14–28.
25. Wakade CG, Mahadik SP, Waller JL *et al.* Atypical neuroleptics stimulate neurogenesis in adult rat brain. J Neurosci Res 2002; 69: 72–79.
26. Wang HD, Dunnavant FD, Jarman T *et al.* Effects of antipsychotic drugs on neurogenesis in the forebrain of the adult rat. Neuropsychopharmacology 2004; 29: 1230–1238.