

**Extended neuroleptic administration affects the NMDAR subunits
immunoexpression in the rat diencephalon and neocortex**

Marek Krzystanek ^a, Katarzyna Bogus ^{b*}, Artur Pałasz ^{b*}

Anna Wiaderkiewicz ^b, Łukasz Filipczyk ^b, Ewa Rojczyk ^b, John Worthington ^c, Ryszard
Wiaderkiewicz ^b

^a Department and Clinic of Psychiatric Rehabilitation,
School of Medicine in Katowice, Medical University of Silesia
ul. Ziolowa 45/47 Katowice 40-635, Poland

^b Department of Histology
School of Medicine in Katowice, Medical University of Silesia
ul. Medyków 18, Katowice 40-752, Poland

^c Manchester Immunology Group,
Faculty of Life Sciences, University of Manchester
Greater Manchester, M13 9PT, UK
and Biomedical and Life Sciences,
Faculty of Health and Medicine, Lancaster University
Lancaster, LA1 4YQ, UK

* both authors contributed equally to this work

Correspondence:

Marek Krzystanek, M.D., Ph.D., Assistant Professor, Department of Psychiatry and Psychotherapy, Medical University of Silesia ul. Ziolowa 45/47 Katowice 40-635, Poland, e-mail: krzystanekmarek@gmail.com

Extended neuroleptic administration modulates NMDA-R subunit immunoexpression in the rat neocortex and diencephalon.

Abstract

Background: This study aimed to evaluate the effect of extended olanzapine, clozapine and haloperidol administration on NMDA-R subunit immunoexpression in the rat neocortex and diencephalon.

Methods: To explore NR1, NR2A and NR2B subunit protein expression, densitometric analysis of immunohistochemically stained brain slices was performed.

Results: Interestingly, all neuroleptics caused a downregulation of NMDA-R subunit expression in the thalamus but increased the level of NR1 in the hypothalamus. Olanzapine upregulated hypothalamic NR2A expression, while clozapine and haloperidol decreased hypothalamic levels. We observed no significant changes in NR2B immunoreactivity. None of the studied medications had significant influence on NMDA-R subunit expression in the neocortex.

Conclusions: Neuroleptic-induced reduction in the expression of thalamic NMDA-R subunits may play an important role in the regulation of glutamatergic transmission

disorders in cortico–striato–thalamo–cortical loop in schizophrenia. A decrease in NMDA signaling in this region after long-term neuroleptic administration may also cautiously explain the incomplete effectiveness of these drugs in the therapy of schizophrenia-related cognitive disturbances.

Key words: NMDA receptor, neuroleptics, glutamate, neocortex, diencephalon

Introduction

It has been established that during schizophrenia there are changes in NMDA receptor (NMDA-R) subunit composition in various regions of the brain [1-4, Paoletti and Neyton 2007]. However, current results are often inconsistent and our understanding of the influence of neuroleptics on NMDA-R expression remains incomplete. Nevertheless, on the basis of this data we can still try to create a hypothetical model of NMDA-R subunit expression changes in patients suffering from schizophrenia. In this model, NR1 subunit expression decreases in majority of brain structures of schizophrenia patients. Accordingly, these dysfunctions result from an NMDA-R dependent deficit in glutamatergic transmission in certain forebrain areas, such as the prefrontal cortex (PFC) and hippocampus [5-8]. The NMDA receptors indirectly regulate dopamine release in the brain cortex, suggesting that the dopaminergic hypofunction, typical in schizophrenia, is secondary to glutamatergic action [9]. Alternatively, dopaminergic receptors participate in the regulation of NMDA-R expression [10], and therefore, a final consensus on the sequence of signaling events underlying schizophrenia remains open. The ionotropic NMDA-R is

a heterotetramer, composed of one mandatory subunit NR1, and three different subunits: NR2, NR3 and NR4 [11,12]. At present, eight splice variants of subunit NR1, and at least four classes of the subunit NR2: A, B, C and D have been identified. In patients suffering from schizophrenia, NR1 and NR2A subunit expression decreases in dorso-lateral PFC [13, 14]. Transport of NR2B subunits is also disturbed, a lack of which can in turn impair NMDA-R function [15]. In frontal and occipital cortex there is an increase of NR2A subunit expression [16]. On the other hand, in the temporal cortex an increase of the number of NR2B subunits has been observed [17]. NR1 subunit expression is probably diminished in the thalamus [16] with simultaneous increase of the number of NR2B subunits in the dorso-medial area [18]. However, some studies have revealed no abnormalities in the thalamus [17]. In the hippocampus NR1 subunit expression decreases, while formation of NR2B subunits probably increases [19]. Moreover, the number of NMDA-Rs increases in putamen [20]. As the NR1 subunit is present in all NMDA-R, the results concerning putamen can be indirectly interpreted as an overall decrease in the number of NR1 subunits. Similarly, expression of NR1 subunit in substantia nigra is probably decreased in schizophrenia [16]. Described changes of NMDA-R subunit composition can result from disease pathogenesis or from the influence of neuroleptics. Indeed, antipsychotics can change NMDA-R subunit gene expression and lead to modifications of receptor function [21]. We previously examined an effect of chronic treatment with haloperidol, clozapine and olanzapine on NMDA-R subunits expression in the rat hippocampus [22]. In the present article we aim to report and discuss the effect of these neuroleptics on NMDA receptor composition in the rat hypothalamus, thalamus and neocortex. It is especially worth mentioning that haloperidol is a classical neuroleptic with a wide spectrum of unfavorable dyskinetic and cardiac side effects (Leucht et al. 2013, Stracina et al. 2015), while olanzapine and clozapine are new generation atypical medications which are more suitable in drug resistant schizophrenia (Wahlbeck et al. 2000, Duggan et al 2003., Essali et al. 2009). In the context of this, comparing potential effects of these drugs on NMDA-R immunoexpression in various brain regions seems to be relatively novel and valid.

Materials and methods

Adult (5 month old, 210-240 grams weight) male Sprague-Dawley rats from Medical University of Silesia Experimental Centre were housed at 22⁰C with regular 12/12 light-dark cycle and access to standard Murigran chow and water *ad libitum*.

Four groups of animals (n=6) received either control vehicle, haloperidol (1 mg /kg/day), clozapine (20 mg/kg/day) or olanzapine (10mg/kg/day) by intraperitoneal injection for 4 weeks. Three hours after the last drug administration, rats were anaesthetized with isoflurane and their brains removed and fixed in 4% paraformaldehyde PBS (pH 7.2-7.4). 7 µm thick sections were cut on a microtome (Leica Microsystems, Germany) in the coronal plane (-3.30 to -3.80 from bregma, according to Paxinos & Watson's The Rat Brain in Stereotaxic Coordinates).

Sections were blocked with 5% goat serum and incubated with the following rabbit polyclonal primary antibodies against the NMDA receptor subunits; anti-NR1 (1:1500; AB17345), anti-NR2A (1:500; AB14596) and anti-NR2B (1:400; AB65875) were purchased from Abcam Company, Cambridge, UK.

Primary antibodies were followed by biotinylated goat anti-rabbit secondary antibody, and then visualized via avidin-biotin-horseradish peroxidase complex (Vectastain ABC kit, Vector Labs), and 3,3'-diaminobenzidine. Sections were mounted on gelatin-coated glass slides, dehydrated and coverslipped.

The same level of cortical, thalamic and hypothalamic sections were chosen from each slide, and three standardized areas were studied (Fig 1.). The relatively poorly investigated parietal cortex was examined. The hypothalamic frame contained ventro- and dorsomedial (VMH, DMH) as well as paraventricular nuclei (PVN) and lateral area (LH). In the thalamus we focused on the main laterodorsal and ventrobasal nuclei involved in the crucial sensory processing. The immunoreactivity of NR1, NR2A and NR2B, containing cells in the randomly selected regions, were measured as the integrated optical density (IOD) and a mean ± SEM was calculated. The data were collected and analyzed using Nikon optic systems and software Image ProPlus (Media Cybernetics, USA). Statistical analyses were performed using Kruskal-Wallis and HSD test. Differences were considered statistically significant at $p < 0.05$.

Results

Following neuroleptic treatment, changes in rat NMDA-R subunit expression were greatly dependent on the area of rat brain analyzed. In the thalamus each neuroleptic treatment caused a decrease in expression of all three NMDA subunits examined, most notably the general subunit - NR1. Interestingly olanzapine and clozapine caused equivalent reductions in each NMDA subunit (Fig. 2B, Fig 3.). In the hypothalamus all examined neuroleptics increased NR1 expression and also, in the case of olanzapine administration, we observed increased NR2A expression (Fig. 2A and B). However, treatment with clozapine and haloperidol caused reduced NR2A subunit expression in the hypothalamus. No significant changes in NR2B expression were observed in this region (Fig. 2A and B.) In the cortex, drug treatment caused an increased trend in NR1 expression, but the result was not statistically significant. Moreover, none of the studied antipsychotics had significant influence on NR2A and NR2B expressions in the cortex.

Discussion

Collectively, these results suggest that the described neuroleptics inhibit the expression of NMDA receptors in the thalamus, which in turn can decrease the activity of the thalamic glutamatergic system. Alternatively, the NMDA-R subunit expression changes in the hypothalamus suggest an increase of glutamatergic circuit activity in this structure. In our analysis, we focused on the expression of the receptor subunits via protein quantification. Although, this may be a distinct limitation, we

decided not to analyze expression at the mRNA level, given previous studies [23] and since changes at the mRNA level are rather difficult to interpret without specific information related to translation of receptor molecules [14]. For this reason, in the present experiment, we have emphasized a necessity of immunohistochemical assessment of the expression of NMDA receptor protein subunits. Neuroleptic-induced reduction of the expression of thalamic NMDA-R subunits has so far not been described in the literature. This effect may play an important role in the regulation of glutamatergic transmission disorders in cortico–striato–thalamo–cortical loop in the schizophrenia. Decreased expression of the NMDA-R subunits in the thalamus after neuroleptic administration may correspond with our previous results reporting that all studied drugs decreased the number of NMDA receptor molecules in the CA1 and CA2 hippocampal areas [22]. Interestingly, treatment with a potent NMDA-R antagonist phencyclidine (PCP) markedly increased c-fos expression in the rat centromedial and mediodorsal thalamic nuclei as well as in the amygdala (Celada et al. 2013). Clozapine administration completely reversed the elevation in firing rate produced by PCP in thalamic and PFC glutamatergic neurons (Kargieman et al., 2007; Santana et al., 2011). Furthermore, some behavioural alterations after NMDA-R blockers administration in rats are completely abolished by neuroleptics (Carlsson and Carlsson, 1989; Geyer et al., 2001). However, the neuronal pathways involved in these effects remains so far poorly investigated. Our results suggest cautiously, that the effect of neuroleptics might be based on the decrease of glutamatergic activity in the thalamic neurons. This downregulation may be particularly important for understanding the influence of antipsychotics on the cognitive symptoms of schizophrenia. For instance, clozapine has been shown to improve spatial memory in some animal models. The effects of this neuroleptic on delayed spatial alternation deficits in rats with NMDA-induced excitotoxic hippocampal damage was tested. The results obtained suggest that long-term, but not acute, drug administration enables animals with hippocampal dysfunction to develop new spatial learning (Bardgett et al. 2006). It may have potential implications for the treatment of cognitive deficits in schizophrenic patients.

According to the glutamatergic model of schizophrenia, dysfunction of NMDA receptors causes a dysfunctional hyperactivity of glutamatergic pyramidal neurons of the cerebral cortex and an increased glutamatergic transmission [24, 25].

Neuroleptics could potentially limit this hyperactivity of the glutamatergic system by reducing the number of receptors. The increase of NRI expression in the hypothalamus may be related to the blockade of D2 receptors by neuroleptics. Dopaminergic D2 and NMDA receptors remain in a functional antagonistic relationship [26]. Chronic inhibition of D2 receptors by neuroleptics and their upregulation leads to the decreased expression of the NMDA-R [27]. Moreover, a decrease of NR2A subunit expression causes an increase in the proportion of NR2B regulatory subunits, which can also lead to increased activity of the NMDA receptor. [15]. In summary, we found that all of the examined neuroleptics increased glutamatergic activity in the rat hypothalamus. Results from this brain region are in line with results obtained by Riva *et al.* [28], who have demonstrated that a 3 week-long haloperidol and clozapine administration decreased the expression of NR2A subunits in the hypothalamus. It should not be excluded that the effect observed may play an alternative role in the central mechanism of neuroleptic-dependent weight-gain.

The observed trend for an increase in NR1 subunit expression in the sensory cortex is consistent with the findings of Ulas *et al.* [29] who have seen an increase in NR1 expression in the rat parietal cortex after 3 weeks of haloperidol administration. The results of various independent studies have revealed that changes in the composition of NMDA subunits can be specific for particular cortex areas. In some experiments it was observed, that after haloperidol treatment there was a slight increase of NR1 expression in the rat temporal cortex. In the studies performed by Hanaoka *et al.* [30] 2 week-long administration of haloperidol and clozapine leads to a decrease of NR1 and NR2B subunit protein level in the frontal cortex. On the other hand, clozapine administered for 6 months decreases the number of NR1 subunits in the dorsolateral part of the PFC. These above-mentioned neuroleptics cause a decrease of NR2A subunits expression in the rat PFC [31]. Presented changes in NMDA-R subunit expression in different areas of the sensory cortex suggest the regional specificity of the influence of neuroleptics on the NMDA receptor composition. Special attention should be given to the comparison of obtained results with changes of NMDA receptor subunit expression in patients suffering from schizophrenia. Human studies have revealed an increased expression of NR2B subunits in the hippocampus, thalamus and in some parts of the cerebral cortex. This effect is opposite to the one

obtained in animal studies, where an inhibition of NR2B subunit expression was observed after exposure to neuroleptics. The data from experiments on humans suggests that an increase of NR2B subunits expression is related to the pathogenesis of schizophrenia, rather than with exact effects of neuroleptics used in the therapy. It may indicate the importance of the modulating influence of neuroleptics on the NMDA receptor composition and thereby on their activity. An inhibitory effect of neuroleptics on NMDA-R expression as well as the drug-induced reduction of thalamic glutamatergic activity could be important elements of the mechanism of neuroleptic anti-schizophrenic activities. A decreased NR1 phosphorylation was also found in some other brain regions in schizophrenia patients [32]. Mice with mutation in the NR1 molecule exhibit an impairment in social behaviour [16]. However, it seems that the specific activity of the NMDA-R has mostly been determined via the analysis of the subunit NR2 [6,33]. Varied expression of the alternative subunit classes could determine a level of receptor sensitivity to different antipsychotic actions. Haloperidol, clozapine and olanzapine, are dopaminergic receptor antagonists and despite their lack of affinity to the NMDA-R, present a significant increase in glutamatergic transmission [14]. Some studies suggested that the blockage of the dopamine receptors D_2 and D_4 by haloperidol, cause an increase in phosphorylation of the NR1 subunit via protein kinase₁, leading to sensitization of the glutamate receptor [28]. However, alternative studies, demonstrate no influence of haloperidol on NMDA-R activity in the rat PFC [30]. Conversely, it has also been reported that atypical antipsychotic drugs enhance glutamatergic activity in the PFC [31]. In turn, clozapine, may also indirectly influence the activity of the glutamatergic system, by inhibiting glutamate reuptake, through a decreased expression of the glycine transporters, EAAT 3 and EAAT 2, in neurons and astrocytes. Perhaps, the observed dynamic changes in composition of NMDA-R subunits could explain differences in the spectrum of cognitive process modification in schizophrenic patients, treated with classical and atypical antipsychotic drugs [26]. A dysfunction of PFC has been associated with the augmented response to stress observed in schizophrenic patients. Interestingly, a recent report suggests that the hypofunction of prefrontal NMDA-Rs does not affect the sensitivity to acute stress of dopamine and noradrenaline projections to amygdala but impairs the acquisition of aversive memory (Del Arco et al. 2015). On the other hand, it is known that schizophrenic patients often show abnormal affective multisensory integration and an emotion

recognition impairment (Van den Stock et al. 2011). We can not exclude that these disturbances may be associated with some deficits in glutamatergic signaling in the limbic structures.

It has been also proposed that NMDA-Rs deficits in the prefronto-thalamo-cerebellar circuit may play a role in the pathogenesis of schizophrenia (Andreasen et al. 1999, Yeganeh-Doost et al. 2011). A study by Schmitt et al. (2010) reported that clozapine may be superior to haloperidol in restoring a deficit in NMDA-R subunit NR2C expression in the cerebellum of rats chronically treated with these neuroleptics. Noteworthy, there was no significant difference in the gene expression of NR1, NR2A, NR2B, NR2C and NR2D subunits or NMDA receptor binding between control animals and groups receiving drug treatment. In the current study we have investigated whether chronic administration of haloperidol, clozapine and olanzapine affects the immunoexpression of the NR1, NR2A and NR2B subunits in the rat hypothalamus, thalamus and PFC. We postulate that the possible changes in NMDA-R expression among the examined neuroleptics may contribute to their different pharmacological effects, especially in the context of cognitive dysfunction therapy.

Conclusions

Our results support a hypothesis that the neuroleptic-dependent changes in expression of NMDA receptor subunits in the rat thalamus, may theoretically and cautiously explain the incomplete effectiveness of these medications in the therapy of cognitive dysfunctions in schizophrenia. On the other hand, the increase of hypothalamic glutamatergic activity may be related to common neuroleptic side effects such as body mass increase.

Conflict of interest

Authors declare no conflict of interest

- [1] Belforte JE, Zsiros V, Sklar ER, Jiang Z, Gu Y, Li Y et al. Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes. *Nat Neurosci* 2010;13:76-83.
- [2] Bridges TM, Williams R, Lindsley CW. Design of potent GlyT1 inhibitors; in vitro and in vivo profiles. *Curr Opin Mol Ther* 2008;10:591-601.
- [3] Halene TB, Ehrlichman RS, Liang Y, Christian EP, Jonak GJ, Gur TL et al. Assessment of NMDA receptor NR1 subunit hypofunction in mice as a model for schizophrenia. *Genes Brain Behav* 2009;8:661-75.
- [4] Kristansen LV, Patel SA, Haroutunian V, Meador-Woodruff JH. Expression of the NR2B-NMDA receptor subunit and its Tbr-1/CINAP regulatory proteins in post mortem brain suggest altered receptor processing in schizophrenia. *Synapse* 2010;64:495-502.
- [5] Labrie V, Roder JC. The involvement of NMDA receptor D-serine/glycine site in pathophysiology and treatment of schizophrenia. *Neurosci Biobehav Rev* 2010;34:351-72.
- [6] Thomsen MS, Christensen DZ, Hansen HH, Redrobe JP, Mikkelsen JD. Alpha (7) nicotinic acetylcholine receptor activation prevents behavioral and molecular changes induced by repeated phencyclidine treatment. *Neuropharmacology* 2009;56:1001-09.
- [7] Javitt DC. Phenomenology, aetiology and treatment of schizophrenia. *Novartis Found Symp* 2008;289:4-16.
- [8] Gao C, Wolf ME. Dopamine receptors regulate NMDA receptor surface expression in prefrontal cortex neurons. *J Neurochem* 2008;106: 2489-2501.
- [9] Millan MJ. NMDA receptor-coupled GlycineB receptors in the pathogenesis and treatment of schizophrenia: a critical review. *Curr Drug Targets* 2002;1: 191-213.

- [10] Wędzony K, Czyrak K. The distribution of the NMDA R1 subunit in the rat hippocampus – an immunohistochemical study. *Brain Res* 1997;768:333-37.
- [11] Vrajova M, Stastny F, Horacek J, Lachman J, Sevy O, Pekova S et al. Expression of the hippocampal NMDA receptor GluN1 subunit and its splicing isoforms in schizophrenia: postmortem study. *Neurochem Res* 2010;35:994-1002.
- [12] Li B, Devidze N, Berengolts D, Prostack N, Sphicas E, Apicella AJ et al. NMDA receptor phosphorylation at a site affected in schizophrenia controls synaptic and behavioral plasticity. *J Neurosci* 2009;29:11965-72.
- [13] Dracheva S, Marras SA, Elhakem SL, Kramer FR, Davis KL, Haroutunian V. N-methyl-D-aspartic acid receptor expression in the dorsolateral prefrontal cortex of elderly patients with schizophrenia. *Am J Psychiatry* 2001;158:1400-10.
- [14] Fumagalli F, Frasca A, Racagni G, Riva MA. Dynamic regulation of glutamatergic postsynaptic activity in rat prefrontal cortex by repeated administration of antipsychotic drugs. *Mol Pharmacol* 2008;73:1484-90.
- [15] Kristiansen LV, Bakir B, Haroutunian V, Meador-Woodruff JH. Expression of the NR2B-NMDA receptor trafficking complex in prefrontal cortex from a group of elderly patients with schizophrenia. *Schizophr Res* 2010;119:198-209.
- [16] Millan MJ. N-Methyl-D-Aspartate receptors as a target for improved antipsychotic agents: novel insights and clinical perspectives. *Psychopharmacology (Berl)* 2005;179:30-53.
- [17] Grimwood S, Slater P, Deakin JF, Hutson PH. NR2B-containing NMDA receptors are up-regulated in temporal cortex in schizophrenia. *Neuroreport* 1999;10(3):461-5.
- [18] Clinton SM, Haroutunian V, Meador-Woodruff JH. Up-regulation of NMDA receptor subunit and post-synaptic density protein expression in the thalamus of elderly patients with schizophrenia. *J Neurochem* 2006;98(4):1114-25.
- [19] Gao XM, Sakai K, Roberts RC, Conley RR, Dean BT. Ionotropic glutamate receptors and expression of N-methyl-D-aspartate receptor subunits in subregions of human hippocampus: effects of schizophrenia. *Am J Psychiatry* 2000;157:1141-49.

- [20] Aparicio-Legarza MI, Davis B, Hutson PH, Reynolds GP. Increased density of glutamate/N-methyl-D-aspartate receptors in putamen from schizophrenic patients. *Neurosci Lett* 1998;241:143-46.
- [21] Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* 1994;12(3):529-40.
- [22] Krzystanek M, Bogus K, Pałasz A, Krzystanek E, Worthington JJ, Wiaderkiewicz R. Effects of long-term treatment with the neuroleptics haloperidol, clozapine and olanzapine on immunoexpression of NMDA receptor subunits NR1, NR2A and NR2B in the rat hippocampus. *Pharmacol Rep* 2015 Oct; 67(5):965-9.
- [23] Ossowska K, Pietraszek M, Wardas J, Dziedzicka-Wasylewska M, Nowicka D, Wolfarth S. Chronic treatments with haloperidol and clozapine alter the level of NMDA-R1 mRNA in the rat brain: an in situ hybridization study. *Pol J Pharmacol* 2002;54:1-9.
- [24] Bubeníková-Valesová V, Horáček J, Vrajová M, Höschl C. Models of schizophrenia in humans and animals based on inhibition of NMDA receptors. *Neurosci Biobehav Rev* 2008;32:1014-23.
- [25] Homayoun H, Moghaddam B. NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. *J Neurosci* 2007;27:11496-500.
- [26] Tarazi FI, Baldessarini RJ, Kula NS, Zhang K. Long-term effects of olanzapine, risperidone, and quetiapine on ionotropic glutamate receptor types: implications for antipsychotic drug treatment. *J Pharmacol Exp Ther* 2003;306:1145-51.
- [27] Tarazi FI, Zhang K, Baldessarini RJ. Long-term effects of olanzapine, risperidone, and quetiapine on dopamine receptor types in regions of rat brain: implications for antipsychotic drug treatment. *J Pharmacol Exp Ther* 2001 May;297(2):711-7
- [28] Riva MA, Tascetta F, Lovati E, Racagni G. Regulation of NMDA receptor subunit messenger RNA levels in the rat brain following acute and chronic exposure to antipsychotic drugs. *Brain Res Mol Brain Res* 1997;50:136-42.

- [29] Ulas J, Nguyen L, Cotman CW. Chronic haloperidol treatment enhances binding to NMDA receptors in rat cortex. *Neuroreport* 1993 Aug;4(8):1049-51.
- [30] Hanaoka T, Toyoda H, Mizuno T, Kikuyama H, Morimoto K, Takahata R et al. Alterations in NMDA receptor subunit levels in the brain regions of rats chronically administered typical or atypical antipsychotic drugs. *Neurochem Res* 2003;28:919-24.
- [31] Schmitt A, Zink M, Müller B, May B, Herb A, Jatzko A et al. Effects of long-term antipsychotic treatment on NMDA receptor binding and gene expression of subunits. *Neurochem Res* 2003;28:235-41.
- [32] Kruusmägi M, Kumar S, Zelenin S, Brismar H, Aperia A, Scott L. Functional differences between D(1) and D(5) revealed by high resolution imaging on live neurons. *Neuroscience* 2009;164:463-69.
- [33] Del'Guidice T, Beaulieu JM. Messing up with traffic: different effects of antipsychotic agents on glutamate receptor complexes in vivo. *Mol Pharmacol* 2008;73:1339-42.

Duggan L, Fenton M, Dardennes RM, El-Dosoky A, Indran S. Olanzapine for schizophrenia. *Cochrane Database Syst Rev*. 2003;(1):CD001359.

Wahlbeck K, Cheine M, Essali MA. Clozapine versus typical neuroleptic medication for schizophrenia. *Cochrane Database Syst Rev*. 2000;(2):CD000059.

Essali A, Al-Haj Haasan N, Li C, Rathbone J. Clozapine versus typical neuroleptic medication for schizophrenia. *Cochrane Database Syst Rev*. 2009 Jan 21;(1):CD000059.

Leucht S, Cipriani A, Spineli L, Mavridis D, Orey D, Richter F et al. Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. *Lancet*. 2013 Sep 14;382(9896):951-62.

Stracina T, Slaninova I, Polanska H, Axmanova M, Olejnickova V, Konecny P et al. Long-Term Haloperidol Treatment Prolongs QT Interval and Increases Expression of Sigma 1 and IP3 Receptors in Guinea Pig Hearts. *Tohoku J Exp Med.* 2015;236(3):199-207.

Paoletti P, Neyton J. NMDA receptor subunits: function and pharmacology. *Curr Opin Pharmacol.* 2007 Feb;7(1):39-47.

Van den Stock J, de Jong SJ, Hodiament PP, de Gelder B. Perceiving emotions from bodily expressions and multisensory integration of emotion cues in schizophrenia. *Soc Neurosci.* 2011;6(5-6):537-47

Del Arco A, Ronzoni G, Mora F. Hypofunction of prefrontal cortex NMDA receptors does not change stress-induced release of dopamine and noradrenaline in amygdala but disrupts aversive memory. *Psychopharmacology (Berl).* 2015 Jul;232(14):2577-86.

Celada P, Lladó-Pelfort L, Santana N, Kargieman L, Troyano-Rodriguez E, Riga MS, Artigas F. Disruption of thalamocortical activity in schizophrenia models: relevance to antipsychotic drug action. *Int J Neuropsychopharmacol.* 2013 Nov;16(10):2145-63.

Bardgett ME, Griffith MS, Foltz RF, Hopkins JA, Massie CM, O'Connell SM. The effects of clozapine on delayed spatial alternation deficits in rats with hippocampal damage. *Neurobiol Learn Mem.* 2006 Jan;85(1):86-94.

Schmitt A, Koschel J, Zink M, Bauer M, Sommer C, Frank J, Treutlein J, Schulze T, Schneider-Axmann T, Parlapani E, Rietschel M, Falkai P, Henn FA. Gene

expression of NMDA receptor subunits in the cerebellum of elderly patients with schizophrenia. *Eur Arch Psychiatry Clin Neurosci.* 2010 Mar;260(2):101-11.

Kargieman L, Santana N, Mengod G, Celada P, Artigas F. Antipsychotic drugs reverse the disruption in prefrontal cortex function produced by NMDA receptor blockade with phencyclidine. *Proc Natl Acad Sci USA* (2007) 104:14843–14848.

Santana N, Troyano-Rodriguez E, Mengod G, Celada P, Artigas F. Activation of thalamocortical networks by the N-methyl-D-aspartate receptor antagonist phencyclidine: reversal by clozapine. *Biol Psychiatry*(2011) 69:918–927.

Carlsson M, Carlsson A Dramatic synergism between MK-801 and clonidine with respect to locomotor stimulatory effect in monoamine-depleted mice. *J Neural Transm* (1989) 77:65–71.

Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology* (2001)156:117–154.

Yeganeh-Doost P, Gruber O, Falkai P, Schmitt A. The role of the cerebellum in schizophrenia: from cognition to molecular pathways. *Clinics (Sao Paulo)*. 2011;66 Suppl 1:71-7.

Andreasen NC, Nopoulos P, O'Leary DS, Miller DD, Wassink T, Flaum M. Defining the phenotype of schizophrenia: cognitive dysmetria and its neural mechanisms. *Biol Psychiatry.* 1999;46:908–20

Fig 1. Standardized frames used for the integrated optical density (IOD) measurement in the examined brain regions after immunohistochemical procedure. Nissl staining. Scale bar: 500 μm .

Fig 2. Representative expression of NR1, NR2A and NR2B in the hypothalamus of control and indicated neuroleptic treated animals. The immunopositive perikarya and dendrites of multipolar neurons are visible (A). Scale bars: 100 μm . Changes in the expression of NR1, NR2A and NR2B subunits in rat hypothalamus and thalamus (B.). Results are based on the mean values of integrated optical density (IOD), and expressed as percentages of control (100%) for n=6 rats. OLZ – olanzapine, KLO – clozapine, HAL – haloperidol. * $p < 0,05$, ** $p < 0,01$.

Fig 3. The NMDA-Rs expressing cells in the thalamus. Immunopositive perikarya and processes of multipolar neurons are visible. Scale bars: 100 μm