SciMedCentral

Annals of Psychiatry and Mental Health

Short Communication

# Effect of Neuroleptic Administration on Neuronal Changes and Nitric Oxide in a Rat Model of Schizophrenia

Gonzalo Flores[1]* and Julio Cesar Morales-Medina[2]

[1]Laboratory of Neuropsychiatry, Benemérita Universidad Autónoma de Puebla, Puebla, Mexico
[2]Center Animal Reproduction Research, Cinvestav Autonomous University of Tlaxcala, Mexico

*Corresponding author
Gonzalo Flores, Laboratory of Neuropsychiatry, Instituto de Fisiología, Benemérita Universidad Autónoma de Puebla, 14 Sur 6301, CP72570, Mexico, Tel: 522-2295500; Email: gonzaloflores56@gmail.com, gonzalo.flores@correo.buap.mx

Submitted: 07 March 2016

Accepted: 05 April 2016

Published: 06 April 2016



ISSN: 2374-0124

OPEN ACCESS

Keywords
- Nitric oxide
- Animal model
- Schizophrenia
- Dendritic morphology
- Neuroleptics

**Abstract**

Schizophrenia is a debilitating disorder that affects a considerable number of people worldwide with positive, negative and cognitive domains. The etiology of this disorder is not yet fully established. Frequently used medications to treat symptoms of schizophrenia include haloperidol and clozapine. While haloperidol is a typical neuroleptic commonly used for acutely ill patients, clozapine is an atypical neuroleptic and its prescription is restricted to refractory patients. Also, cerebrolysin has recently been used to treat cognitive deficits in schizophrenics. The neonatal ventral hippocampal lesion (nVHL) has emerged as a key model of schizophrenia related behavior producing numerous behavioral deficits, neuronal hypotrophy, reducing the number of neurons in the basolateral amygdala (BLA) and increasing nitric oxide (NO) levels. Our group has shown that clozapine and cerebrolysin reshape neurons in the prefrontal cortex (PFC), (BLA) and striatum. Cerebrolysin treatment also increases the spine density and the number of cells in the (PFC) in the (nVHL) rat. Moreover, clozapine and haloperidol normalize the abnormal high levels of (NO) in the (PFC). Clozapine and haloperidol target dopamine and serotonin neurotransmitter systems respectively, and (NO) modulates both of these systems. Thus the (nVHL) is a key model to understand schizophrenia and (NO) seems to be an ultimate effector.

## INTRODUCTION

Schizophrenia is one of the diseases described in ancient medical writings. In every stage of the human history, attempts were made to explain this complex disorder with the available scientific elements. A new stage in understanding of schizophrenia occurred with the development of neuroleptics and antipsychotics in the 1950s. The first generation of neuroleptics chlorpromazine, haloperidol, trifluperacine etc., suggested that the dopamine (DA) neurotransmitter dysfunction is implicated in the etiology of this disorder [1-3]. Further progress in the study of neuromorphology and neurochemistry of the limbic system supports new hypothesis in the etiology of schizophrenia. In the last two decades, animal models of schizophrenia-related behavior have emerged and have tried to explain biochemical and signaling systems or synaptic communication changes in relation to the development and the appearance of symptoms after puberty, which are comparable to symptoms present in human. The main symptoms of schizophrenia are classified as: positive, negative and cognitive deficits (Figure 1). While positive symptoms comprise auditory and visual hallucinations, delusions and thought disorders, negative symptoms include deficits in social interaction and emotional expression with poor quality of speech and inability to initiate and persist in goal-directed activities. Finally, schizophrenic patients exhibit cognitive deficits such as attention, visual and verbal learning and memory, working memory and executive functioning such as time to respond [3,4]. A new generation of neuroleptics such as clozapine, olanzapine, risperidone, aripiprazole, etc. appeared in 1990s (Figure 1). While the first generation of neuroleptics is known as typical (Figure 1), the second generation is better known as atypical neuroleptics. The typical or atypical term implicates the therapeutic action. While typical neuroleptics modulate positive symptoms, atypical neuroleptics have an effect on positive and negative symptoms [5,6]. Interestingly, both types of neuroleptics have limited and poor effect on the cognitive impairment [6]. While haloperidol blocks (DA) receptors, clozapine is the first atypical antipsychotic with known effects on (DA), serotonin, glutamate and gamma Aminobutyric acid (GABA) receptors [7]. Haloperidol is used

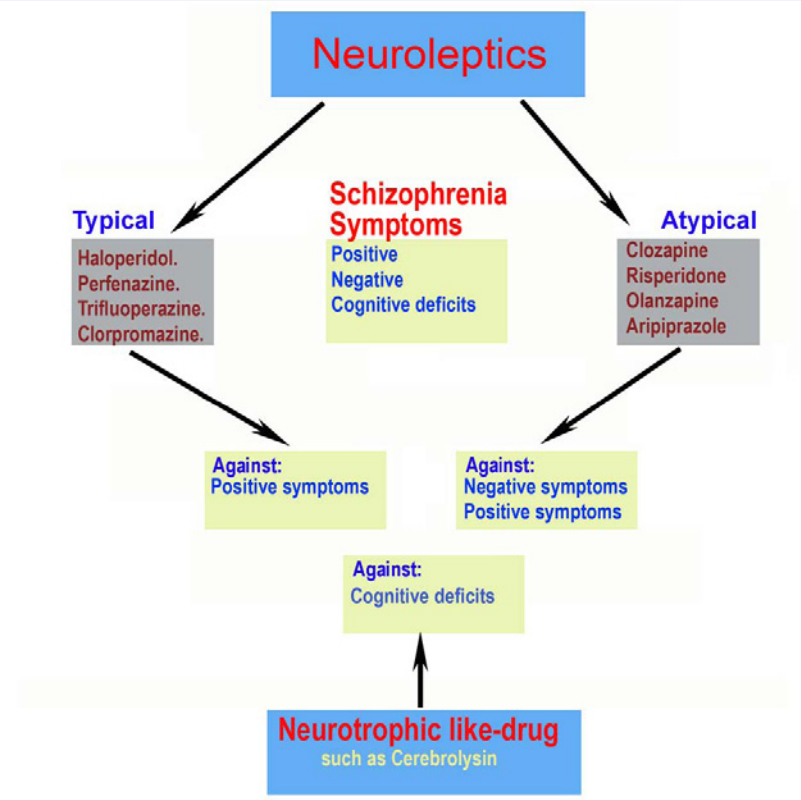


**Figure 1** This diagram shows the relationship between schizophrenia symptoms and various types of neuroleptics.

in acutely-ill patients and in contrast clozapine prescription is restricted to refractory patients [7]. Recently, cerebrolysin is a preparation of peptides derived from porcine brain with effects in neuronal survival, adulth neurogenesis and neuroplasticity [8,9]. Moreover, cerebrolysin mimics the action of endogenous neurotrophic factors [8], has shown cognitive improvement in schizophrenic patients [10]. The present manuscript review recent literature of the behavioral and neuronal effects of clozapine, haloperidol and cerebrolysin in the neonatal lesion of the ventral hippocampus (nLVH) model of schizophrenia-related behavior

## Hippocampus-prefrontal cortex pathway

Communication between the hippocampus and prefrontal cortex (PFC) has been known for several decades. Optimal connectivity is critical in the development of various behaviors, such as spatial memory. To better understand the function of a given structure, it is essential to describe how these structures are connected to different brain regions. At the end of the first month of gestation in humans, the neural tube is closed embryonic day 25. The six layers of the (PFC) and three layers of the hippocampus are fully established at gestational week (GW) 22 [11] as well as the connection between thalamus and (PFC) starts at the end of the five months of gestation and finishes 4 weeks later (GW)22-27 [11,12]. Interestingly, the connection between hippocampus, (PFC) and amygdala is established at the end of the seventh month of gestation [13,14]. Accordingly, there is an order of communication among various brain regions. When this order is altered, wrong communications may develop. Finally, between the eighth and ninth month of gestation, the communication between these structures receives and sends input to other regions as well. For example, both (PFC) and hippocampus send glutamatergic projections to nucleus accumbens NAcc [3]. The main population of neurons in the (PFC) and hippocampus is glutamatergic pyramidal neurons (Figure 2). Hippocampus may regulate the activity of (GABA)ergic neurons of the NAcc, and medium spine neurons, directly and indirectly via (PFC) [3]. The medium spine neurons of the NAcc send (GABA)ergic projections to the ventral pallidum (Figure 2). The main neurons of the ventral pallidum send (GABA)ergic projections to the dorso-medial (DM) nucleus of the thalamus. Finally, the (DM) nucleus of the thalamus sends glutamatergic projections back to the (PFC) (Figure 3).

## Neonatal ventral hippocampus lesion in rats

Pharmacological models of schizophrenia were predominant until the advent of the (nLVH) model. Developmental aspects of disrupting the prefrontal-hippocampus connectivity were addressed in this lesion [1,3,14-17]. Key research in schizophrenia in this model has been based on three aspects: multi-faceted behavioral effects, alterations of circuits and neurotransmitters and the periadolescent onset of abnormal neuronal and behavioral consequences, all of these symptoms are similar to what is seen in the schizophrenic patients [14]. All together numerous authors have suggested (nLVH) as a heuristic neurodevelopment model of schizophrenia. Indeed, (nLVH) rats present a delayed, post pubertal onset of behavioral changes such as locomotor hyper-responsiveness to novel environment

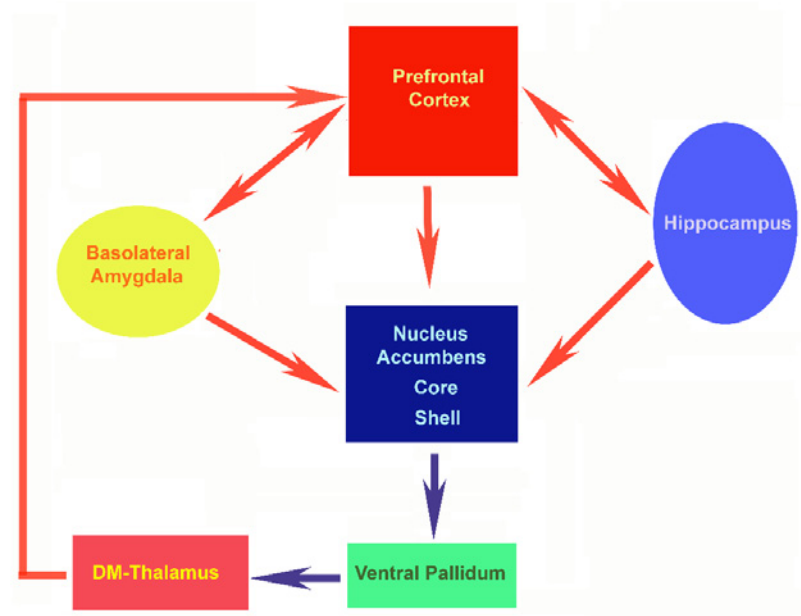


**Figure 2** This diagram illustrates the connections among the prefrontal cortex, the hippocampus and the basolateral amygdala, as well as the nucleus accumbens and dorso-medial (DM) thalamus. Blue lines indicate (GABA)ergic projections and red lines show glutamatergic projections.

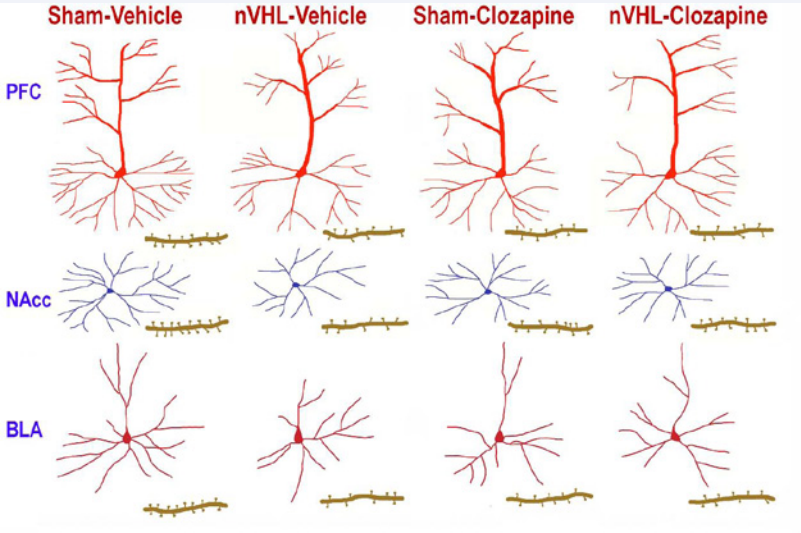


**Figure 3** This figure displays alterations in dendritic arborization and spine density in the pyramidal neurons of the prefrontal cortex (PFC), nucleus accumbens (NAcc) and basolateral amygdala (BLA) in adult rats with neonatal ventral hippocampus lesion (nVHL), an animal model of schizophrenia. Also, the schematic diagrams show how clozapine, an atypical neuroleptic leads to changes in the number of dendritic spines and dendritic arborization in this animal model of schizophrenia.

and stress, deficits in social interaction, sensor motor gating and learning and memory [3]. These behavioral changes after puberty are associated with decreased (DA) D3 receptors in the NAcc [16], increased nitric oxide (NO) levels in the (PFC) [18,19] and dendritic arbor atrophy and reduced spinogenesis in the (PFC) [17,18,20,21]. It is important to note that the behavioral and neurochemical effects of these lesions are different in several aspects if the lesions were performed in adult animals [22,23]. Therefore, the effects of this lesion may not be explained only in terms of the loss of ventral hippocampal neurons as the developing brain is an important additional factor.

## Neuronal changes in the neonatal ventral hippocampus lesion before and after Neuroleptic administration

The shape of dendritic arbor of a neuron determines the number and distribution of receptive synaptic contacts [24]. Moreover, dendritic spines are the main site of input and therefore alterations in spine density results in either gain or loss of connectivity [25]. Modifications in dendritic arbor and spine

density disrupt neuronal stability. Neuronal rearrangement and alterations in spine density are observed in postmortem brains of the patients with schizophrenia [26] and animal models of schizophrenia-related behavior [3]. The (nLVH) induced hypotrophy and decreased dendritic spine density in the (PFC), basolateral amygdala (BLA) and NAcc [18,20, 21,27]. In addition, the number of neurons is decreased in the (BLA) and (PFC) in (nVHL) rats [27]. Treatment with clozapine and cerebrolysin results in specific neuronal modifications in the (nVHL) rat. Clozapine increased the arborization in NAcc, (BLA) and (PFC) neurons with no modification in spine density [18] (Figure 3). Cerebrolysin increased the neuronal arborization without modifying the spine density in the (BLA). Moreover, cerebrolysin increased the number of cells in the (BLA) [27]. In the (nVHL) rat, cerebrolysin normalized the dendritic arborization and spine density in the (PFC) and arborization in NAcc neurons [21,27]. Finally, cerebrolysin treatment increased the number of cells in the (BLA) and (PFC) in the (nVHL) rat [21,27]. But the possible effects of haloperidol in dendritic arborization and spine density are yet to be described.

### Nitric oxide levels in the neonatal ventral hippocampus lesion before and after neuroleptic administration

(NO) is a soluble, short-lived and freely diffusible gas considered as a key inter- and intra-cellular messenger [28]. (NO) is involved in numerous physiological processes including synaptic and neuronal plasticity that may produce functional modifications in brain circuits [29]. Indeed, processes known to be involved in the pathogenesis of schizophrenia [3] and accumulated evidence show involvement of (NO) in schizophrenia [30-32]. For example, schizophrenic subjects present increased level of plasma (NO) [33] and polymorphism of the neural nitric oxide synthase increases the genetic risk of schizophrenia [34]. Moreover, postnatal blockade of (NO) resulted in amphetamine- and novel-induced hyper locomotion and neuronal hypotrophy in the (PFC) and hippocampus [35,36]. In line with preclinical and clinical data, the (nVHL) rat presents increased levels of (NO) in the (PFC), occipital cortex (OC) and striatum [18,19,37]. Moreover, adult rats with (nVHL) also showed an increased (NO) S immunostaining in the (PFC) [38]. Clozapine administration decreased (NO) levels in the striatum with reduced locomotion in the (nVHL) rat [18]. This pharmaceutical also increased (NO) levels in the (PFC) and OC in sham animals with any behavioral modification [18]. Haloperidol reduced (NO) levels in the (PFC) and striatum as well as normalized locomotion in the (nVHL) rat [19]. But the effect of cerebrolysin on (NO) levels in the (nVHL) rat still needs to be investigated. However, recent reports show that the cerebrolysin treatment was able to reduce elevated hippocampal (NO) levels in an animal model of streptozotocin-induced diabetes mellitus [39]. In addition, several reports have demonstrated that cerebrolysin increases neurotrophins such as nerve growth factor (NGF) and brain-derived growth factor (BDNF) [40-42]. Both of these neurotrophins are implicated in synaptic plasticity [43].

Pioneer work from our group suggests that haloperidol and clozapine reduce behavioral deficits and neuronal hypotrophy in diverse key brain regions by modifying (NO) levels and display different receptor targets. However, it is well established that (NO) interacts with glutamate, serotonin and (DA) neurotransmitters [32]. In particular (NO) is a second messenger of N-methyl-D-aspartate (NMDA) receptors, a subtype of glutamate receptors. Our working hypothesis suggests that upon activation of (NMDA) receptors, haloperidol and clozapine interact with dopaminergic and serotoninergic pathways [44]. Therefore both haloperidol and clozapine have been able to achieve similar behavioral and neuronal effects.

## CONCLUSION

Schizophrenia is a devastating disorder with numerous symptoms negative, positive, and cognitive domains. Clinical and preclinical results suggest that this disorder present neuronal remodeling in several brain regions [3]. In particular, the (nVHL) model produced neuronal reshaping in the (PFC), (BLA) and NAcc [18,21,27]. The long term administration of clozapine as well as cerebrolysin reversed the behavioral deficits and normalized the dendritic arborization in (nVHL) rats. Moreover haloperidol and clozapine reduced the abnormal high levels of (NO) observed in (nVHL) rats. Furthermore, cerebrolysin also increased the number of cells in the (PFC) and (BLA). The beneficial effects of an increase in the number of cells may be based on antioxidant properties of cerebrolysin. Further studies are warranted to test the effects of other antipsychotics on (NO) levels and neuronal reshaping in the brain.

## ACKNOWLEDGEMENTS

GF and JCMM acknowledge the membership of National Research System of Mexico. Authors would like to thank Mira Thakur for editing the English language text. This study was supported by grants from ProDES (CA-BUAP-120) and CONACYT grant (No. 129303) to G. Flores. None of the funding institutions had any further role in the study design, collection of data, analyses and interpretation of data, writing of the report or in the decision to submit the paper for publication.

## REFERENCES

1. Flores and Atzori M. The Potential of Cerebrolysin in the Treatment of Schizophrenia. Pharmacology & Pharmacy. 2014; 5: 691-704.
2. Wu HE, Okusaga OO. Antipsychotic medication-induced dysphoria: its meaning, association with typical vs. atypical medications and impact on adherence. Psychiatr Q. 2015; 86: 199-205.
3. Flores G, Morales-Medina J, Diaz A. Neuronal and brain morphological changes in animal models of schizophrenia. Behav Brain Res. 2016; 301: 190-203.
4. Feifel D, Shilling PD, MacDonald K. A Review of Oxytocin's Effects on the Positive, Negative, and Cognitive Domains of Schizophrenia. Biol Psychiatry. 2016; 79: 222-233.
5. Maher AR, Maglione M, Bagley S, Suttorp M, Hu JH, Ewing B, et al. Efficacy and comparative effectiveness of atypical antipsychotic medications for off-label uses in adults: a systematic review and meta-analysis. JAMA. 2011; 306: 1359-1369.
6. Karson C, Duffy RA, Eramo A, Nylander AG, Offord SJ. Long-term outcomes of antipsychotic treatment in patients with first-episode schizophrenia: a systematic review. Neuropsychiatr Dis Treat. 2016; 12: 57-67.
7. Leucht S, Heres S, Kissling W, Davis JM. Evidence-based pharmacotherapy of schizophrenia. Int J Neuropsychopharmacol. 2011; 14: 269-284.

8. Masliah E, Díez-Tejedor E. The pharmacology of neurotrophic treatment with Cerebrolysin: brain protection and repair to counteract pathologies of acute and chronic neurological disorders. Drugs Today (Barc). 2012; 48: 3-24.

9. Plosker GL, Gauthier S. Cerebrolysin: a review of its use in dementia. Drugs Aging. 2009; 26: 893-915.

10. Xiao S, Xue H, Li G, Yuan C, Li X, Chen C, et al. Therapeutic effects of cerebrolysin added to risperidone in patients with schizophrenia dominated by negative symptoms. Aust N Z J Psychiatry. 2012; 46: 153-160.

11. Stiles J, Jernigan TL. The basics of brain development. Neuropsychol Rev. 2010; 20: 327-348.

12. Cooper JA. A mechanism for inside-out lamination in the neocortex. Trends Neurosci. 2008; 31: 113-119.

13. Weinberger DR, Lipska BK. Cortical maldevelopment, anti-psychotic drugs, and schizophrenia: a search for common ground. Schizophr Res. 1995; 16: 87-110.

14. Tseng KY, Chambers RA, Lipska BK. The neonatal ventral hippocampal lesion as a heuristic neurodevelopmental model of schizophrenia. Behav Brain Res. 2009; 204: 295-305.

15. Lipska BK, Jaskiw GE, Weinberger DR. Postpubertal emergence of hyperresponsiveness to stress and to amphetamine after neonatal excitotoxic hippocampal damage: a potential animal model of schizophrenia. Neuropsychopharmacology. 1993; 9: 67-75.

16. Flores G, Barbeau D, Quirion R, Srivastava LK. Decreased binding of dopamine D3 receptors in limbic subregions after neonatal bilateral lesion of rat hippocampus. J Neurosci. 1996; 16: 2020-2026.

17. Flores G, Alquicer G, Silva-Gómez AB, Zaldivar G, Stewart J, Quirion R, et al. Alterations in dendritic morphology of prefrontal cortical and nucleus accumbens neurons in post-pubertal rats after neonatal excitotoxic lesions of the ventral hippocampus. Neuroscience. 2005; 133: 463-470.

18. Bringas ME, Morales-Medina JC, Flores-Vivaldo Y, Negrete-Diaz JV, Aguilar-Alonso P, León-Chávez BA, et al. Clozapine administration reverses behavioral, neuronal, and nitric oxide disturbances in the neonatal ventral hippocampus rat. Neuropharmacology. 2012; 62: 1848-1857.

19. Negrete-Díaz JV, Baltazar-Gaytán E, Bringas ME, Vazquez-Roque RA, Newton S, Aguilar-Alonso P, et al. Neonatal ventral hippocampus lesion induces increase in nitric oxide [(NO)] levels which is attenuated by subchronic haloperidol treatment. Synapse. 2010; 64: 941-947.

20. Alquicer G, Morales-Medina JC, Quirion R, Flores G. Postweaning social isolation enhances morphological changes in the neonatal ventral hippocampal lesion rat model of psychosis. J Chem Neuroanat. 2008; 35: 179-187.

21. Vázquez-Roque RA, Ramos B, Tecuatl C, Juárez I, Adame A, de la Cruz F, et al. administration of the neurotrophic agent cerebrolysin ameliorates the behavioral and morphological changes induced by neonatal ventral hippocampus lesion in a rat model of schizophrenia. J Neurosci Res. 2012; 90: 288-306.

22. Lipska BK, Jaskiw GE, Chrapusta S, Karoum F, Weinberger DR. Ibotenic acid lesion of the ventral hippocampus differentially affects dopamine and its metabolites in the nucleus accumbens and prefrontal cortex in the rat. Brain Res. 1992; 585: 1-6.

23. Wood GK, Lipska BK, Weinberger DR. Behavioral changes in rats with early ventral hippocampal damage vary with age at damage. Brain Res Dev Brain Res. 1997; 101: 17-25.

24. Koleske AJ. Molecular mechanisms of dendrite stability. Nat Rev Neurosci. 2013; 14: 536-550.

25. Fiala JC, Spacek J, Harris KM. Dendritic spine pathology: cause or consequence of neurological disorders? Brain Res Brain Res Rev. 2002; 39: 29-54.

26. Glausier JR, Lewis (DA). Dendritic spine pathology in schizophrenia. Neuroscience. 2013; 251: 90-107.

27. Vázquez-Roque RA, Ubhi K, Masliah E, Flores G. Chronic cerebrolysin administration attenuates neuronal abnormalities in the basolateral amygdala induced by neonatal ventral hippocampus lesion in the rat. Synapse. 2014; 68: 31-38.

28. Garthwaite J, Charles SL, Chess-Williams R. Endothelium-derived relaxing factor release on activation of (NMDA) receptors suggests role as intercellular messenger in the brain. Nature. 1988; 336: 385-388.

29. Prast H, Philippu A. Nitric oxide as modulator of neuronal function. Prog Neurobiol. 2001; 64: 51-68.

30. Bernstein HG, Bogerts B, Keilhoff G. The many faces of nitric oxide in schizophrenia. A review. Schizophr Res. 2005; 78: 69-86.

31. Bernstein HG, Becker A, Keilhoff G, Grecksch G, Bogerts B. Schizophrenia and the nitric oxide controversy: do all things fall into place now? Synapse. 2011; 65: 545-546.

32. Bernstein HG, Keilhoff G, Steiner J, Dobrowolny H, Bogerts B. Nitric oxide and schizophrenia: present knowledge and emerging concepts of therapy. CNS Neurol Disord Drug Targets. 2011; 10: 792-807.

33. Yanik M, Vural H, Kocyigit A, Tutkun H, Zoroglu SS, Herken H, et al. Is the arginine-nitric oxide pathway involved in the pathogenesis of schizophrenia? Neuropsychobiology. 2003; 47: 61-65.

34. Reif A, Herterich S, Strobel A, Ehlis AC, Saur D, Jacob CP, et al. A neuronal nitric oxide synthase (NO)S-I) haplotype associated with schizophrenia modifies prefrontal cortex function. Molecular Psychiatry. 2006; 11: 286-300.

35. Morales-Medina JC, Mejorada A, Romero-Curiel A, Flores G. Alterations in dendritic morphology of hippocampal neurons in adult rats after neonatal administration of N-omega-nitro-L-arginine. Synapse. 2007; 61: 785-789.

36. Morales-Medina JC, Mejorada A, Romero-Curiel A, Aguilar-Alonso P, León-Chávez BA, Gamboa C, et al. Neonatal administration of N-omega-nitro-L-arginine induces permanent decrease in (NO) levels and hyperresponsiveness to locomotor activity by D-amphetamine in postpubertal rats. Neuropharmacology. 2008; 55: 1313-1320.

37. Flores G, Negrete-Díaz JV, Baltazar-Gaytán E, Bringas ME, Vazquez-Roque RA, Newton S, et al. Nitric oxide in neonatal ventral hippocampus lesion rats. Synapse. 2011; 65: 547.

38. Bernstein HG, Grecksch G, Becker A, Höllt V, Bogerts B. Cellular changes in rat brain areas associated with neonatal hippocampal damage. Neuroreport. 1999; 10: 2307-2311.

39. Georgy GS, Nassar NN, Mansour HA, Abdallah (DM). Cerebrolysin Ameloriates Cognitive Deficits in Type III Diabetic Rats. PLoS One. 2013; 8: 64847.

40. Chen H, Tung YC, Li B, Iqbal K, Grundke-Iqbal I. Trophic factors counteract elevated FGF-2-induced inhibition of adult neurogenesis. Neurobiol Aging. 2007; 28: 1148-1162.

41. Menon PK, Muresanu DF, Sharma A, Mössler H, Sharma HS. Cerebrolysin, a mixture of neurotrophic factors induces marked neuroprotection in spinal cord injury following intoxication of engineered nanoparticles from metals. CNS Neurol Disord Drug Targets. 2012; 11: 40-49.

42. Ubhi K, Rockenstein E, Vazquez-Roque R, Mante M, Inglis C, Patrick C, et al. Cerebrolysin modulates pronerve growth factor/nerve growth factor ratio and ameliorates the cholinergic deficit in a transgenic model of Alzheimer's disease. J Neurosci Res. 2013; 91: 167-177.

43. Mariga A, Mitre M, Chao MV. Consequences of Brain-Derived Neurotrophic Factor withdrawal in CNS neurons and implications in disease. Neurobiol Dis. 2016.

44. Brenman JE, Bredt DS. Synaptic signaling by nitric oxide. Curr Opin Neurobiol. 1997; 7: 374-378.

**Cite this article**

*Flores G, Morales-Medina JC (2016) Effect of Neuroleptic Administration on Neuronal Changes and Nitric Oxide in a Rat Model of Schizophrenia. Ann Psychiatry Ment Health 4(3): 1065.*