

Effects of Prefrontal Cortical Dopamine Depletion on Parvalbumin-Containing GABAergic Interneurons

Aziza M. Hart, Peter Vollbrecht, Sheila V. Kusnoor, Bonnie Garcia, and Ariel Y. Deutch

KEYWORDS. Dopamine, parvalbumin, interneurons, schizophrenia, gamma-aminobutyric acid

BRIEF. The purpose of this work is to see the effects of reducing the dopamine input to the prefrontal cortex on parvalbumin-containing GABAergic interneurons.

ABSTRACT. Schizophrenia is a psychological disorder characterized by disturbances in thought, cognitive deficits and social withdrawal. Cognitive deficits of schizophrenia are thought to reflect physiological changes in the prefrontal cortex, a brain region known for focusing and planning. Post-mortem studies of schizophrenic patients show a decreased number of a specific subset of parvalbumin containing GABAergic interneurons has been reported. Parvalbumin is a calcium-binding protein, and GABA (gamma-aminobutyric acid) is a neurotransmitter that these interneurons use. Knowing that PFC PV interneurons receive a dopamine input, we hypothesize that a decreased dopamine input to the PFC would result in a decreased amount of PV interneurons. Immunohistochemistry was used to reveal PV interneurons. The number of PV interneurons in the PFC was determined using stereological methods. Immunoblotting was used to determine PV levels in the PFC of rats. A trend towards a decrease in PV interneurons in response to dopamine depletion was found. Finding a definite decrease in the number of PV interneurons in response to dopamine depletion brings promise to the advent of improved antipsychotic medications.

INTRODUCTION.

Schizophrenia is a disorder characterized by disturbances in thought, cognitive deficits, and social withdrawal [1]. The symptoms of schizophrenia can be divided into three categories: positive, negative and cognitive [2]. Positive symptomatology includes hallucinations, delusions, and thought disorders such as disorganized thinking or thought blocking [2]. Negative symptoms include reduced emotional expression and interaction with others and motivational problems [2]. Cognitive symptoms include impaired ability to plan, trouble with focusing or paying attention, and problems with working memory [2]. Current treatments include typical and atypical antipsychotic medications (APDs) and psychotherapy. The main type of treatment is the use of APDs. APDs do not effectively treat the negative and cognitive symptoms of schizophrenia [3]. Without medications that alleviate these symptoms, schizophrenic patients have greater difficulty of living independent and productive lives. Due to over 2 million people in the United States alone being diagnosed with schizophrenia and it still being the seventh most costly medical illness, investing time in better understanding the neuronal pathology of schizophrenia is of national importance.

Dopamine is a catecholamine neurotransmitter that participates in the regulation of motor functions and of cognitive processes such as learning and memory [4]. Dopaminergic neurons (neurons whose primary neurotransmitter is dopamine) are produced in several areas of the brain including the substantia nigra pars compacta and the ventral tegmental area (VTA) of the midbrain. Various studies have shown that dopamine has a major role in regulating the excitability of prefrontal cortical neurons upon which working memory depends [5]. In fact, 6-hydroxydopamine lesions of the VTA were performed in an experiment to denervate the prefrontal cortex [6].

Evidence suggests that the prefrontal cortex (PFC) is responsible for complex cognitive behaviors due to theta and gamma oscillations being induced in the prefrontal cortex during cognitive tasks [7]. Postmortem studies of schizophrenic patients have found that the PFC is thinner but with no decrease in

overall cell number [5]. However, the loss of particular subsets of neurons has been reported. In particular, there appears to be a decrease in a subset of inhibitory local circuit neurons (interneurons) in the PFC that use gamma-aminobutyric acid (GABA) as a neurotransmitter [1]. PFC interneurons can be defined by expression of one of three different calcium-binding proteins: calretinin, calbindin, and parvalbumin (PV). The PV interneurons receive a dopamine input, in contrast to other interneurons. We hypothesized that dopamine denervation of the PFC would result in a decrease in the number of PV-containing interneurons. The results could help contribute to our current understanding of schizophrenia.

MATERIALS AND METHODS.

Animals/Tissue preparation.

12 Sprague-Dawley adult male rats (Harlan, Indianapolis, IN) were given food and water ad libitum. 25 mg/kg of desipramine, a norepinephrine reuptake inhibitor, was administered prior to 6-OHDA (neurotoxin that targets dopaminergic neurons injection. Six rats underwent 6-hydroxydopamine (6-OHDA) lesion destruction of the dopamine neurons in the VTA. The rate of 6-OHDA injection into the VTA was 0.15 μ l/min and the overall volume of the neurotoxin injected was 0.75 μ l. Perfusion using 0.1 M phosphate buffered-saline followed by 4% paraformaldehyde of prefrontal and midbrain segments was performed. Frozen sections through the PFC and midbrain were cut at 42 μ m. 6-OHDA and perfusion was performed by project advisors.

Immunohistochemistry.

Midbrain sections of the six lesioned animals and the prefrontal sections of both control and lesioned animals were stained immunohistochemically to identify tyrosine hydroxylase (TH) in the VTA using an immunoperoxidase method and to identify parvalbumin interneurons within the PFC, respectively. One subject had to be eliminated from analysis due to a unilateral lesion.

Stereological analysis.

Stereological analysis, an unbiased method of determining the number of cells in an area, was performed using Stereo Investigator (MBF Bioscience, Williston, VT).

Immunoblotting.

Tissue for immunoblotting was used from a different set of animals that were deeply anesthetized with isoflurane, and then the PFCs (and other areas) were dissected from the brain. Prefrontal cortical samples were stored at -80°C until subsequent analysis. The immunoblot was made using a method previously stated [8], with the exception of different antibodies. Multiple film x-ray exposures were obtained. Films were digitally scanned and the optical density of the bands was determined by ImageJ.

RESULTS.

6-hydroxydopamine lesioning of the VTA.

6-hydroxydopamine (6-OHDA) is a neurotoxin that kills dopaminergic neurons. 6-OHDA lesions of the VTA resulted in a marked decrease in the number of dopamine neurons in the VTA. Separate groups of animals exhibited an overall 70% decrease in the dopamine concentration in the PFC [6]. An example of the physical result of 6-OHDA lesioning can be seen in Figure 1.

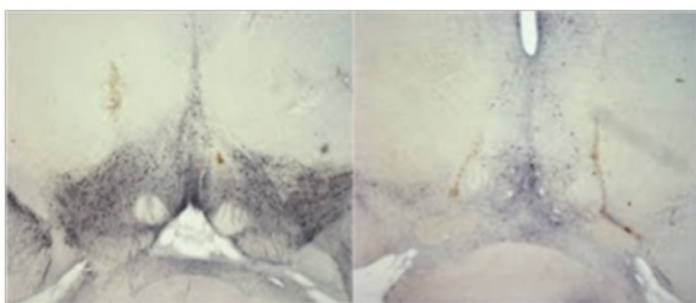


Figure 1. Tyrosine hydroxylase-stained dopamine neurons in the VTA in the control condition are displayed in the left panel with marked loss of dopamine neurons in the lesioned rat in the right panel.

Stereological Analysis.

Although the lesioned animals did not show a significant decrease in the number of parvalbumin (PV) interneurons, a trend towards a decrease in PFC PV interneurons was observed ($p=0.2584$) (Figure 2).

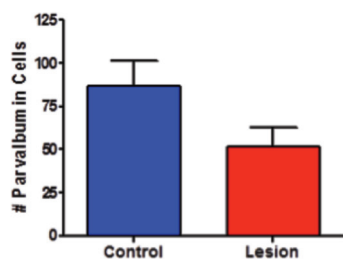


Figure 2. Stereology, an unbiased counting method, was used to count the number of PV interneurons within the prelimbic cortex (outlined in orange in Figure 4 of the supplemental material), a homologous structure to the prefrontal cortex.

Immunoblotting.

Parvalbumin protein levels in the PFC did not significantly differ between the control and lesioned groups (see Figure 3).

DISCUSSION.

We did not observe a statistically significant decrease in the number of PV-containing interneurons in response to PFC dopamine depletion. However, we did see a trend toward a decrease in PV interneurons in the PFC. A reason for these results may lie in the unknown compensatory mechanisms for PV due to dopamine depletion. While a decrease in PV-containing interneurons may occur due to dopamine depletion, the amount of PV per cell in the decreased population of PV interneurons may balance to that of the control group (no marked decrease in PV interneurons) and therefore not show a marked decrease in the level of PV on the immunoblot. By verifying this trend of a decrease in PV cells, this could possibly elaborate the model of schizophrenia. This also implies that the loss of PV cells contribute to the cognitive deficits of schizophrenia. PV appears to be the next focus of antipsychotic medications.

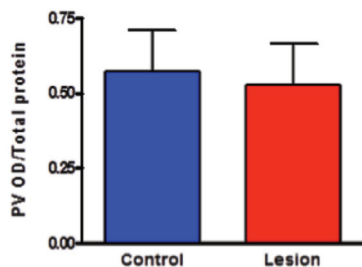


Figure 3. Figure 3 shows a graph of the immunoblotting results. The optical density of the x-ray exposed was divided by the optical density of the ponceau-stained membrane to create a relative level of protein for the controlled and lesioned group (Figure 5 of the supplemental material section shows ponceau stained membrane below the x-ray exposed film of parvalbumin protein).

Methylazoxymethanol acetate (MAM) is a chemical known to cause a decrease in parvalbumin-containing interneurons. Rats that are treated with MAM display a loss of parvalbumin interneurons along with decreased oscillatory activity [7]. Similar results have occurred when phencyclidine (PCP) is a NMDA

receptor antagonist known to create behavioral deficits in adulthood that produce similar behavioral impairments observed in schizophrenic patients.) was administered to adult rats [1]. While these papers gave valuable insight into PV's role in cognition and potential ways to compensate for the marked decreases in PV, the present study is more applicable to as better understanding how to make/enhance current antipsychotic medication more efficient due to the majority of them targeting D2 dopamine receptors.

There has been debate within the field of whether glutamate or dopamine has the heaviest influence on the symptomatology of schizophrenia [3]. This study strengthens the dopamine hypothesis of schizophrenia.

A larger animal set is needed to show a definitive decrease in PV interneurons within the PFC as a result of dopamine depletion. Only the prelimbic (PrL) area was analyzed in this study, and to get a more elaborate view of the PFC the infralimbic area would need to be looked at as well. Morphology of PV interneurons is also vital to understanding PV's role in neuronal pathology of schizophrenia. Soma (cell body) size, axonal length, and deep/superficial layer analysis could elucidate the health of the cell, its capability to transmit signals and the distribution of PV in the PFC, respectively.

Having definitive proof that dopamine depletion influences parvalbumin interneuron reduction in the prefrontal cortex could be the catalyst towards improved antipsychotic medications. Also PV's role in cognition still needs to be found. Indeed, there is evidence of parvalbumin being found within and having influence on motor systems [9] but investigating PV's specific molecular pathway in relation to cognition under the umbrella of dopaminergic influence would help the field of neuroscience to better understand the pathophysiology of schizophrenia.

ACKNOWLEDGMENTS. I would like to thank Peter Vollbrecht, Sheila Kusnoor, Bonnie Garcia and Ariel Deutch for being my advisors, instructors and most importantly my teachers that have given me a detailed glimpse into the field of neuroscience. I would like to thank the School for Science and Math at Vanderbilt for not only giving me the opportunity to participate in a high caliber research laboratory, but also to be a part of a school that encourages me to be creative in becoming the critical thinker necessary for what I want to pursue in life. Lastly, I would like to thank God for his impeccable timing of this entire research experience; my "orchestrating skills" are incomparable to his.

SUPPORTING INFORMATION.

Figure S1. Stereological image of prefrontal cortex section with prelimbic area highlighted

Figure S2. Total protein on ponceau-stained membrane prior to administration of antibodies

REFERENCES.

1. Wang CZ, Yang SE, Xia Y, Johnson KM, *Neuropsych*. **33**, 2442 (2008)
2. Freedman R. *The New Eng Journ of Med* **349** 1738, (2003).
3. Coyle JT, *Cell and Mole Neurobio* **26** 365, (2006)
4. Cadet JL, Jayanthi S, McCoy MT, Beauvais G, Cai NS, *CNS Neurol Disord Drug Target* **9**, 526(2010).
5. Goldman-Rakic PS, Selemon LD, *Schizo Bull*. **23** 437, (1997).
6. Wang H, Deutch AY. *Neuropsych*. **33**, 1276 (2008)
7. Lodge DJ, Behrens MM, Grace AA, *The Journal of Neuroscience*
8. Kusnoor SV, et al., *The Journ of Comp Neurol*. **518** 2525, (2010). **29**, 2344 (2009)
9. Lannrgren J, Elzinga G, Stienen GJ, *Journ of Phys-London* **463**, 123 (1993).



Aziza Hart is a student at Glencliff High School and enrolled in the School for Science and Math at Vanderbilt.