Possible Alterations in Parvalbumin Immunopositive Interneurons in and 5 in Area 9 of the Prefrontal Cortex in Schizophrenia

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Abstract

Schizophrenia is a devastating disorder which affects approximately 1% of the population in the Unites States it affects roughly 2.2 million people. Research suggests that it is a neurodevelopmental disorder emerging in late adolescences to early adulthood. Previous studies have shown that the prefrontal cortex, more specifically areas 9 and 32 are affected in individuals with schizophrenia. The prefrontal cortex is important as it controls executive functioning. Research from our lab has shown a variety of alterations with the pyramidal cells, projection neurons in these areas. Research by Lewis et. a;l., (2013) suggests that schizophrenia maybe caused by an imbalance of signaling between the pyramidal cells and the interneurons in the region. Therefore we have begun to examine two types of interneurons basket cells and chandelier cells. We hypothesize that theses two populations based on their connections to the pyramidal cells will have an altered cell density in both areas 9 and 32 of the prefrontal cortex.

Introduction

Schizophrenia is known for having a large magnitude of deficits in several cognitive domains. The cognitive deficits of schizophrenia include impairments in current IQ, sustained attention, category fluency, verbal memory, response inhibition. (Lewis 1) The area of the brain that are associated with this issue is the prefrontal cortex. (specifically, the dorsal lateral prefrontal cortex or DLPC). Lower gamma oscillations that are detected from this area are some of the physical characteristics of schizophrenia. Gamma oscillations are often between the range of 32 to 80 Hz. In order for normal gamma oscillations to occur there needs to be stable interactions between several pyramidal cells and parvalbumin cells. There also needs to be a satisfactory amount of GABA and GAD to regulate feedback within the cells. During several studies with animal subjects, those with lower GABA had a lower frequency within regions of the brain and lower cognitive function. Higher amounts of GABA and GAD lead to increased gamma oscillations and stronger cognitive control. To determine the effects of lower GAD and GABA on the DLPC we must observe the presence of cells that require or synchronize with these chemicals. Postmortem observations of several brains help us gain a hypothesis.

Methods

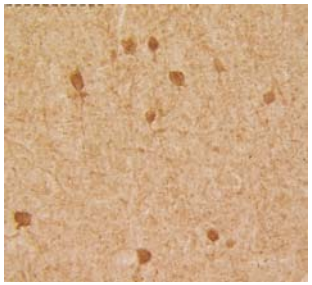
Subjects and Tissue:

This study was conducted on postmortem brain tissue received from the Harvard Brain Tissue Resource Center (HBTRC) at McLean Hospital. Tissue was obtained from eight subjects diagnosed with schizophrenia and eight subjects matched for age, sex and postmortem interval who served as controls. Tissue was collected at the time of the autopsy in accordance with a protocol approved by an Institutional Review Board. Diagnoses conformed to DSM-IV criteria and were established retrospectively by reviewing medical records and interviewing knowledgeable informants. Tissue was not included in the study if there was evidence of a neuropathology (e.g. Alzheimer's disease) or substance dependence as determined by the HBTRC. Tissue was coded by the brain bank and researchers performing quantitative procedures were blind to diagnoses. All specimens were formalin-fixed in a consistent manner and 1-cm thick sections from each cortical region of interest were dissected in the coronal plane by qualified neuropathologists at the HBTRC. The accuracy of the dissections was confirmed by examining thionin-stained sections prepared from each block. Specimens from area 9 were taken from the dorsolateral extent, which is founded on the middle third of the superior frontal gyrus (Rajkowska and Goldman-Rakic, 1995a). Compared with the medial region, the lateral region of area 9 is more differentiated. In addition to having a distinguishable layer IV, it has a layer II with a distinct upper margin and the supragranular cell density is conspicuously higher than the infragranular cell density (Barbas and Pandya, 1989). Compared with area 46, area 9 is less differentiated, layers III and V are more sublimated, and the neurons are less differentiated (Rajkowska and Goldman-Rakic, 1995b).

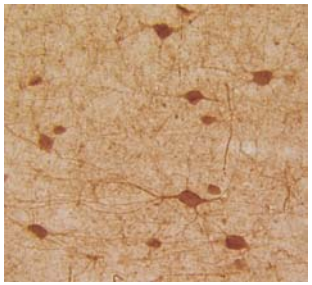
Histological Procedures:

Coronal sections were cut at 50 micrometers on a freezing sledge sliding microtome. Sections were collected into sequentially numbered wells and stored in cryoprotectant at -70º C. Odd numbered sections were stained with thionin and every tenth section processed for Parvalbumin immunocytochemistry. Pairs matched for age, sex and postmortem interval were processed together to control for variability. To maximize impregnation and antibody exposure, a free-floating method of immunocytochemistry was used. Sections intended for Parvalbumin immunocytochemistry were removed from the cryoprotectant, washed in phosphate buffered saline (PBS) and pretreated with 0.3% H_2O_2 for 5 minutes to remove endogenous peroxidases. The sections were washed in PBS and then incubated in a 0.4% instant milk PBS solution (BLOTTO) with 0.2% Triton X-100 for 1 hour. Sections were then incubated in anti-parvalbumin antibody (Chemicon) diluted 1:300 in BLOTTO for 48 hours at room temperature on an orbital shaker. A positive control was not necessary since tissues were processed as matched pairs (control vs. schizophrenic). Tissue was removed from primary antibody solution, washed three times for 10 minutes in PBS and incubated for 1 hour in a biotinylated anti-mouse secondary (Vector Laboratories) at a 1:100 dilution. Finally, sections were incubated in HRP-avidin/biotin complex according manufacturer's protocol followed by a standard diaminobenzadine reaction to view the immunocytochemical staining. All staining was done in 12-well plates. Sections were then mounted onto glass slides and cover-slipped.

Control 3878



Schizophrenic 3742



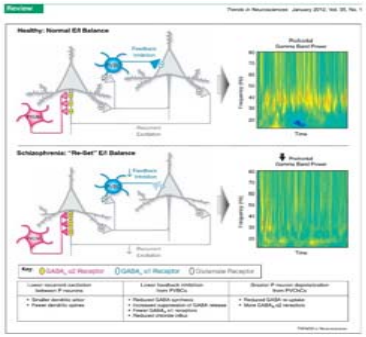
Control	7504.05	414.89 +/-
Schizophrenia	6629.49	148.05 +/-

Two-tailed paired t-test p= 0.084
t= 2.015

Brain #	Distributive Dx	Age	Sex	PMI	Layers	Area	Storage time (Months)
3673	schizophrenia	43	M	20.8	III & V	32	10
3748	Control	44	M	23	III & V	32	8
3833	schizophrenia	67	M	21.3	III & V	32	13
3878	Control	65	M	13	III & V	32	11
3742	schizophrenia	71	M	19	III & V	32	7
3875	Control	68	F	14	III & V	32	12
3915	schizophrenia	34	M	17.4	III & V	32	10
3932	Control	38	M	21.9	III & V	32	10
3546	schizophrenia	69	M	17.83	III & V	32	13
3619	Control	74	F	19.8	III & V	32	11
3557	schizophrenia	66	F	16.75	III & V	32	13
3625	Control	70	F	18.25	III & V	32	11
3634	schizophrenia	71	M	18	III & V	32	15
3626	Control	65	F	16.6	III & V	32	11

Discussion

The preliminary data from the study supports the current hypothesis that schizophrenia is a result of alterations in the excitatory and inhibitory balance in the cortex. Previous data from the lab shows dramatic decrease in spine density on the pyramidal cells as well as loss of basilar dendrites, and alterations in MAP2, neurogranin, and calmodulin immunostaining. Our data suggests an increase in parvalbumin immunopositive cells in area 9 of the prefrontal cortex in schizophrenia. Preliminary research to examine cahiges in immunostainign which would suggest a change in expression of the protein show an increase in immunostaining in layer II/III suggest an increase in production of parvalbumin possibly do to an increase in inhibitory neuron activity or increase in immunpositive cells. Parvalbumin positive cells are most abundant in layers II/III and synapse on pyramidal cells in layer III (7). There is additional evidence that layer III inhibitory cells make long distance connections, which may explain the increase in parvalbumin immunostaining in the subcortical white mater (7). Preliminary data suggests a decrease in immunostaining in layer V would suggest a possible decrease in parvalbumin expression and possible activity. The data taken together with previous research in the lab suggests a possible increase in inhibition of layer III pyramidal cells with a decrease in excitation on the same cells as seen by the loss of spines and dendrites on those cells. The decrease in immunostainging in layer V along with a decrease in spines and dendrites on the pyramidal cells suggest compensation for the loss excitatory synaptic surface area by decreasing parvalbumin expression. Much research suggests that this may be the case in the dorsolateral prefrontal cortex in schizophrenia. Research from the lab on an animal model for prefrontal cortical development suggest loss of thalamic input during development results in loss of spines and basilar dendrites in the dorsolateral prefrontal cortex. The loss of spines is more pronounced in layers II/III than in layer V. The data taken together is suggests that schizipohrenia has its roots in development and may be related to a loss of excitatory input that leads to alterations in the excitation inhibition balance as seen in alterations in parvalbumin immunostaining, loss of spines and loss of dendrites. More research needs to be done to confirm the preliminary result.



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