Hippocampal Dysfunction in Schizophrenia: The Role of Parvalbumin-Positive Inhibitory Interneurons

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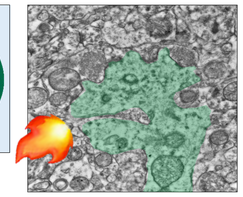


INTRODUCTION

•Schizophrenia (SZ) is a neuropsychiatric disorder affecting roughly 1% of the world's population.
•Prominent features of this disorder include psychotic episodes, or reality distortions, such as hallucinations or delusions. Other symptoms consist of cognitive impairments, such as difficulty concentrating or making decisions, and negative symptoms.
•Replicated studies have implicated a link between hyperactivity in the hippocampus and symptoms of psychosis and cognitive deficits.
•Balanced excitation-inhibition play an important role in higher cognitive and neural function.
•The majority of cells within the hippocampus are glutamatergic cells communicate with each other via the trisynaptic pathway.
•Theories propose this is due to a disinhibition of glutamatergic pyramidal cells and a decreased number of interneurons.
•A subtype of interneuron implicated in schizophrenia are parvalbumin-positive (PV) inhibitory interneurons.
• To test for abnormalities in hippocampal PV interneurons, human postmortem SZ tissue was immunostained and analyzed for expression of PV protein.
•This study was focused on the PV interneurons in the CA1 region of the trisynaptic pathway.

METHODS

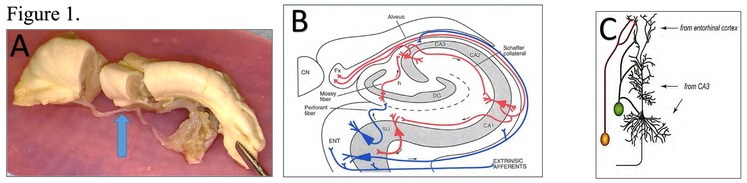
Brain tissue and processing: Human postmortem brain tissue was obtained from the Alabama Brain Collection and Maryland Brain Collection. All cases (CTRL=6, SZ=5) were chosen based on best matches of age, race, sex, postmortem interval (PMI), pH, and storage time (Table 1). The hippocampus body was sub-dissected and fixed in 4% paraformaldehyde and 1% glutaraldehyde in 0.1M phosphate buffer.
Immunohistochemistry: Hippocampal tissue was prepared and vibrotomed at 40-μm for immunohistochemical localization of PV inhibitory interneurons. The sections of tissue were incubated in 10% normal house serum (NHS) pre-incubation buffer in 0.01M PBS for 60 minutes while being agitated. The sections were then incubated for 48 hours at 4°C in PV monoclonal mouse primary antibody in a solution of 3% NGS in 0.01 M PBS at a dilution of 1:1000 to detect presence of antigen. Sections were then incubated in horse anti-mouse secondary antibody at a dilution of 1:800 and 3% NGS in 0.01 M PBS for one hour, followed by an avidin biotin complex for 45 minutes. The samples were then developed in DAB for 15 minutes in order to be visualized. The immunostained tissue sections were prepared for light microscopy by standard techniques.
Light microscopy and Image Analysis: For each case, representative images of labeled cells from the CA1 region were taken at 20x under the light microscope and analyzed using ImageJ software. Sets of images where significant labeling was observable were taken for each case. For each image from every case, measurements were collected and placed into categories of: interneurons, neuropil, and background. Three analyses were performed: Optical density of PV+ interneurons, optical density of PV+ neuropil and the density of PV+ neurons per μm^2. Optical density of background regions were subtracted from that of interneurons and neuropil. Neuronal density was calculated by dividing total number of neurons per total area of the CA1 region.
Statistics: We did not test for outliers due to our small sample size. The data was tested for Normality, and all data was normally distributed, so we used an unpaired t-test for group comparisons of data and a chi-square test for quantitative demographics (race, sex and hemisphere). SPSS was used for all statistical analysis with the exception of Vasser Stats that we used for a two-correlation coefficient analysis.

TABLE 1	SZ (n = 5)	NC (n = 6)	Statistics
Age in years	58.8 ± 12.3	43.7 ± 21.0	P<0.190
Race	W = 3; B = 2	W = 4; B = 2	P<0.819
Sex	M = 2; F = 3	M = 3; F = 3	P<0.740
PMI in hours	5.2 ± 1.3	6.2 ± 1.5	P<0.284
pH	6.9 ± 0.3	6.9 ± 0.4	P<0.898
Hemisphere	R = 2; L = 3	R = 2; L = 4	P<0.358

Table 1. Demographics
SZ: Schizophrenia; NC: Normal Control; W: White; B: Black; M: Male; F: Female; PMI: Postmortem Interval in hours. Data = mean± standard deviation.

ANATOMY of the HIPPOCAMPUS

Figure 1.



A. Fixed and sub-dissected human hippocampus. B. Trisynaptic pathway: perforant pathway (entorhinal cortex to granule cells of dentate gyrus); mossy fibers (dentate gyrus granule cells to CA3 pyramidal cells); and Schaffer collaterals (CA3 pyramidal cells to CA1 Pyramidal cells) C. Interneuron connections onto an excitatory pyramidal neuron: Parvalbumin (green) and somatostatin (red) interneuron control neural firing by their inhibitory biology.

RESULTS

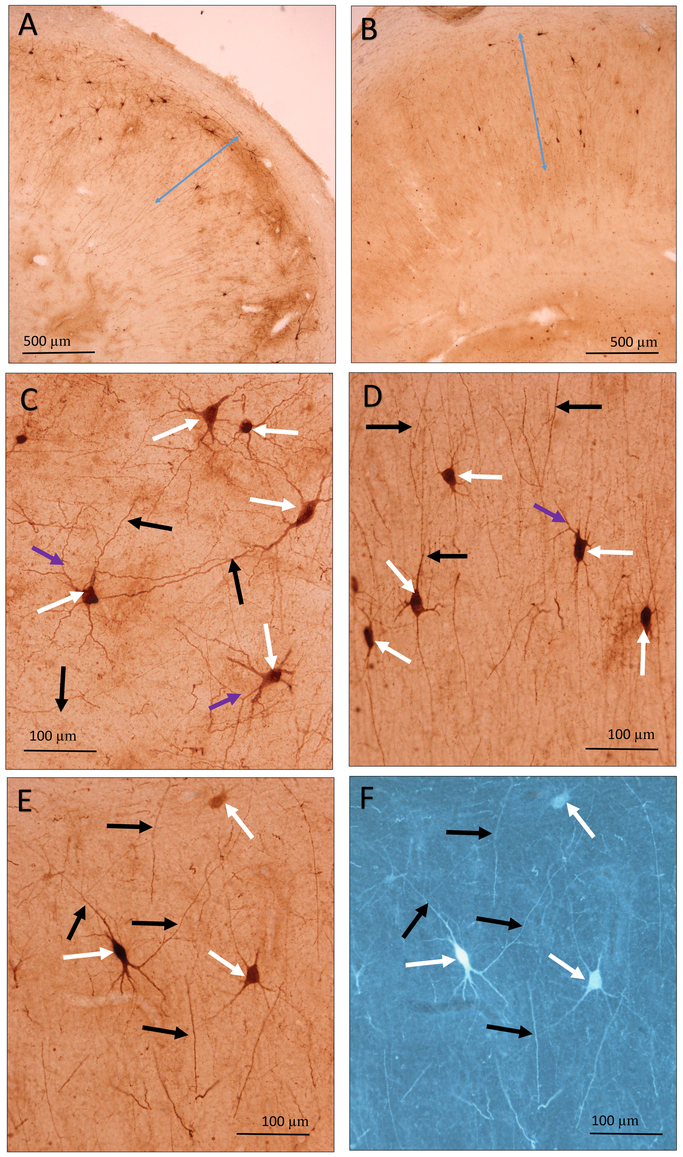


Figure 2. Light microscopy images of controls (A,C) and patients with schizophrenia (B, D-F). Neuronal density was calculated using 4x images (A, B). Stratum pyramidale layers are indicated with arrows. (C,D) Labeled processes (black arrows), cell bodies of PV+ interneurons (white arrows) and branched dendrites (purple arrows). E) Image of neurons and neuropil and (F) inverted 20x image from ImageJ that is used to measure and outline (as shown) neurons and neuropil.

RESULTS CONT.

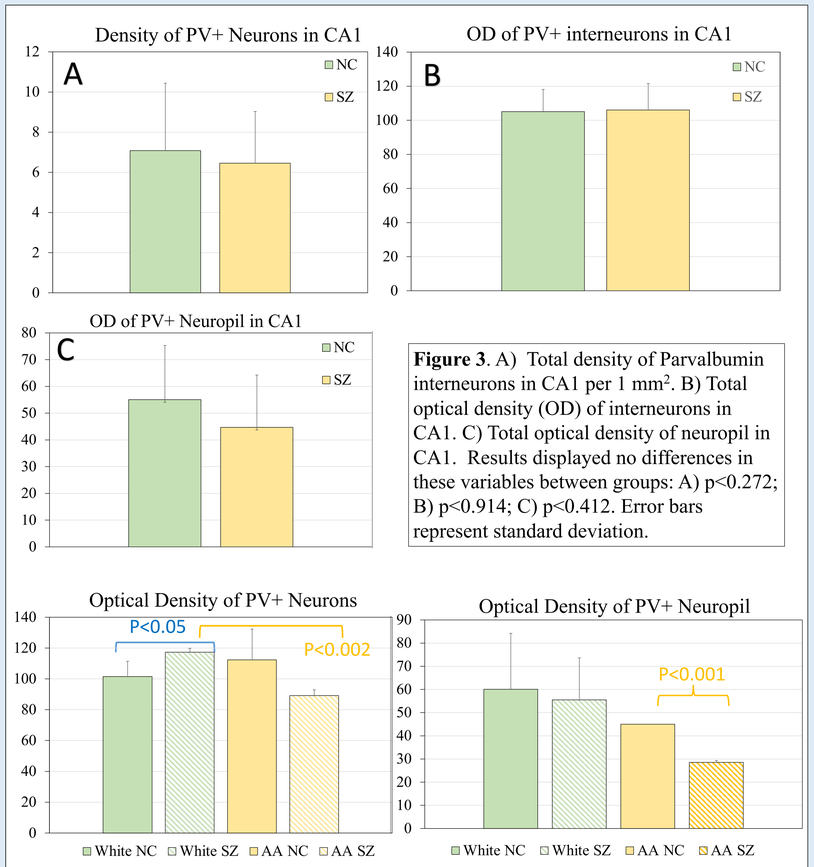


Figure 3. A) Total density of Parvalbumin interneurons in CA1 per 1 mm^2. B) Total optical density (OD) of interneurons in CA1. C) Total optical density of neuropil in CA1. Results displayed no differences in these variables between groups: A) p<0.272; B) p<0.914; C) p<0.412. Error bars represent standard deviation.

Figure 4. Correlations between optical density of parvalbumin neurons and race (A). Correlation between optical density of neuropil and race (B). When we separated and analyzed the variables independently, we reached significance between schizophrenia group and race. Correlation between race and OD of neurons is 0.426 (p=NS) in NCs and -0.986 in SZ (p<0. 002); 2 correlation coefficient shows significant differences p<0.0013.

CONCLUSIONS and FUTURE DIRECTIONS

• When analyzing group comparisons between controls and patients schizophrenia, no significant differences were found.
• Say something about Konradi
• As a preliminary study we found that AA SZ had lower PV neuropil levels than AA NCs. AA SZ had fewer PV+ neurons than white SZs. Thus racial differences need to accounted for.
• Limitations of our study include a small cohort, thus future investigations with a larger sample size are necessary.
• Future directions include analyzing PV tissue at the electron microscopic level will extend and clarify our findings. For example the number of mitochondria per neuron and terminal could be eval electron microscopy; **Fig. 5** displays PV terminals in human cortex, it represents our future aims at this level.

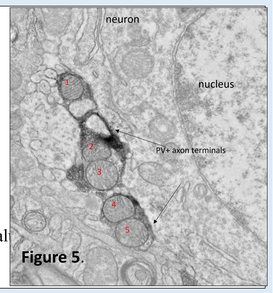


Figure 5.

ACKNOWLEDGEMENTS

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