PRESENTER:

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Postmortem hippocampal blood-brain barrier leakage is increased in schizophrenia compared to controls as well as peripheral endothelial vascular dysfunctions and perfusion.

Hippocampal blood-brain barrier damage and peripheral vascular compromise in schizophrenia share endothelial dysfunction: preliminary evidence from postmortem and in vivo testing

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Introduction

Schizophrenia spectrum disorder (SSD) is one of the most severe forms of mental illness, yet mechanisms remain unclear. A widely established brain finding in SSD is hippocampal atrophy, and a coherent explanation similarly is lacking. Epidemiological evidence suggests increased cerebrovascular and cardiovascular complications in SSD independent of lifestyle and medication, pointing to disease-specific pathology. Endothelial cell contributions to blood-brain barrier (BBB) compromise may influence neurovascular unit and peripheral vascular function, and we hypothesize that downstream functional and structural abnormalities may be explained by endothelial deficits.

Methods

Postmortem human hippocampus sections (n=27 controls, n=25 SSD) were obtained from the NIH NeuroBioBank and Maryland Brain Collection. Leakage phenomena was observed using a secondary IgG-only (Vector Laboratories, 1:250) staining technique, to demonstrate endogenous IgG extravasation. IgG leak was quantified using unbiased stereology (MBF Bioscience, VT) to measure the areas of IgG immunoreactivity under blinded analysis. A ratio was then calculated of fraction of leak in the tissue compared to overall tissue area.

Post-occlusive reactive hyperemia was used with simultaneously administered brachial artery reactivity testing (Philips iE33 Ultrasound, Germany) and laser speckle contrast imaging (Perimed Inc., Sweden) of the macrovascular- and microvascular endothelial cell response, respectively, in n=26 community controls and n=34 SSD participants. Flow-mediated dilation was captured in the brachial artery by calculating the percent dilation at one-minute post-occlusion compared to baseline, while perfusion measures captured using the Laser Contrast Imager with area under the curve (AUC) and time relative to baseline perfusion measures. The primary measure of BBB leak between diagnostic groups was compared using raw means as well as covarying for age, sex, and PMI with reported estimated marginal means. Multiple comparisons were corrected using false discovery rate with q-value set at <0.05.

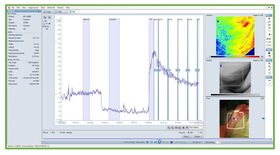
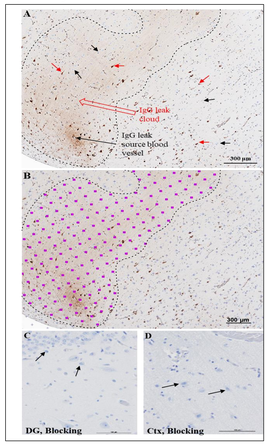
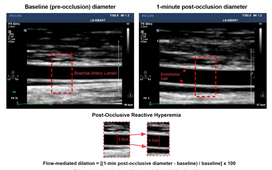


Fig 1. Semi-quantitative approach to quantify BBB breach extent





Clinical Demographics and Tissue Characteristics

		Control (n=27)	Schizophrenia (n=25)	Test statistic	p-value
Demographics	Age [years] (SD)	51.8 (13.4)	56.9 (14.6)	t=-1.3	0.2
	Sex (% male)	58%	56%	χ^2=0.2	0.9
	Race % (C/AA)	20 / 7	17 / 8	χ^2=0.2	0.6
Section Characteristics	PMI [hours] (SEM)	14.5 (1.0)	11.9 (1.3)	t_{50}=1.6	0.12
	Average section surface area (μm^2) (SEM)	$1.8 \cdot 10^8$ ($1 \cdot 10^7$)	$1.6 \cdot 10^8$ ($1 \cdot 10^7$)	t_{50}=1.4	0.17
	sIgG Leaked Area Fraction (μm^2) (SEM)	$8.9 \cdot 10^6$ ($2 \cdot 10^6$)	$1.5 \cdot 10^7$ ($3 \cdot 10^6$)	t_{50}=-1.9	0.06
	Fraction of sIgG Leaked Area	0.05 (.01)	0.11 (.02)	t_{50}=-2.3	0.02
	Fraction (SEM)	0.047 (.02) [a]	0.12 (0.2) [a]	$F_{1,48}$=7.3[a]	0.009[a]
	SD – Standard deviation; C – Caucasian; AA – African American; PMI-Postmortem Interval; SEM-Standard Error of Mean				

Participant Clinical and Demographic Information

	Control (n=39)	Schizophrenia (n=37)	Test statistic	p-value
Age [years] (SD)	41.7 (15.3)	39.0 (12.1)	t=-0.82	0.4
Sex (% male)	47.8	81.3	χ^2=7.5	0.006
BMI (SD)	27.6 (7.0)	30.0 (5.1)	t=-1.5	0.2
Current smoking status (%)	27.2	25.8	χ^2=0.14	0.9
Skin tone# (SEM)				
L*	51.4 (2.23)	50.5 (1.90)	t=0.30	0.8
a*	9.7 (0.60)	9.8 (0.5)	t=-0.14	0.9
b*	12.4 (0.87)	12.3 (0.86)	t=0.03	1.0
Heart rate, bpm (SEM)	69.5 (2.2)	75.7 (1.9)	t=-2.1	0.04
Respiratory rate, rpm (SEM)	15.3 (0.5)	18.1 (0.7)	t=-2.9	0.005
Systolic BP (mmHg) (SEM)	113.5 (2.1)	121.0 (2.5)	t=-2.2	0.04
Diastolic BP (mmHg) (SEM)	72.5 (1.6)	76.2 (1.8)	t=-1.5	0.14
Mean Arterial Pressure (SEM)	86.2 (1.6)	91.2 (1.9)	t=-1.9	0.06
Pulse Pressure (SEM)	41.0 (1.4)	44.8 (1.8)	t=-1.6	0.12
SpO2 (SEM)	98.6 (0.3)	97.2 (0.3)	t=3.3	0.002
Forearm Temperature (SEM)	89.6 (0.5)	89.4 (0.6)	t=0.30	0.8
Oral Temperature (SEM)	97.6 (0.1)	97.8 (0.6)	t=-1.34	0.19

L* values determine the lightness/darkness of a color used to relate skin color. a* values measure cutaneous erythema, proportional to the melanin composition and cutaneous blood flow. b* values display constitutional pigmentation.

Results

Fig 2. Temporal cortex near hippocampus of SZ patients demonstrates increased BBB permeability and selective interaction between sIgGs and pyramidal neurons.

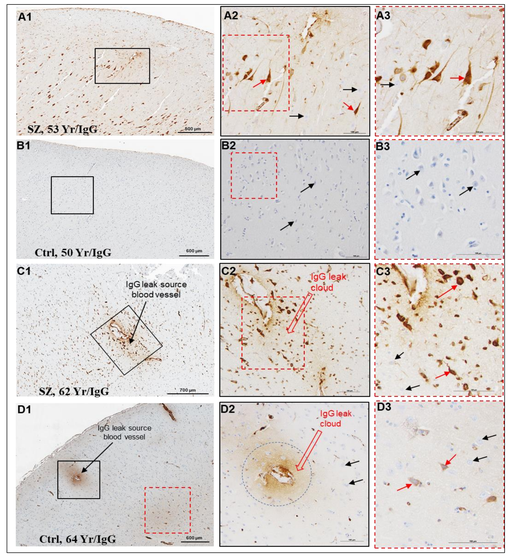
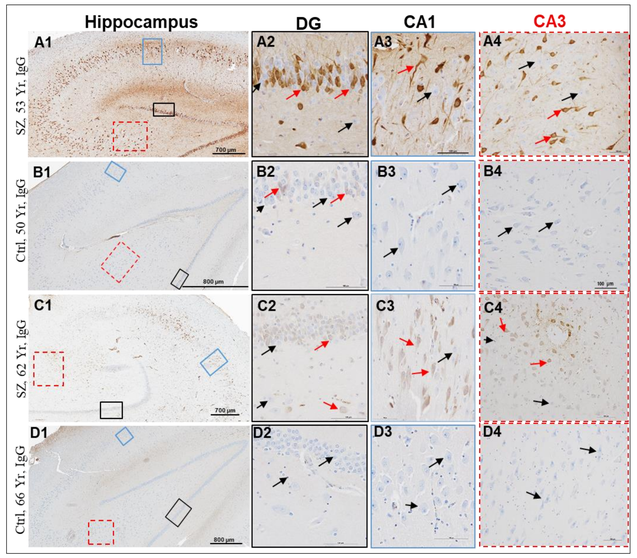


Fig 3. Hippocampus proper of SZ patients demonstrates increased BBB permeability and selective interaction between sIgGs and neurons.



Postmortem samples demonstrated 11% BBB leakage in SSD compared to 5% in controls (t_{50}=-2.3, p=0.02). Linear regression was performed to determine age, sex, and PMI in addition to diagnosis as predictors of BBB leak, and the model remained significant ($F_{4,47}$=3.0, p=0.03) with a significant diagnosis (t=3.3, p=0.002) but no significant age (p=0.5), sex (p=0.2), or PMI (p=0.5) effects. We further explored the impact of age on BBB permeability by splitting our sample population in two age-groups, 30-50 or 50-70 years-old, based on mean and medians. BBB leak fraction was also significantly increased in older aged schizophrenics vs controls (t_{39}=2.5, p=0.02). We found no significant sex difference in BBB permeability in the combined sample (p=0.99), however, there was a significant increase in BBB permeability only in male schizophrenia subjects compared to male control subjects (t_{51}=2.7, p=0.009) on post-hoc analysis, while females did not (p=0.9), with a diagnosis x sex interaction (p=0.03).

Fig 4. Comparison of the extent of BBB breakdown in the hippocampus and surrounding temporal cortex of the SZ and Ctrl subjects with respect to diagnosis, age, and sex.

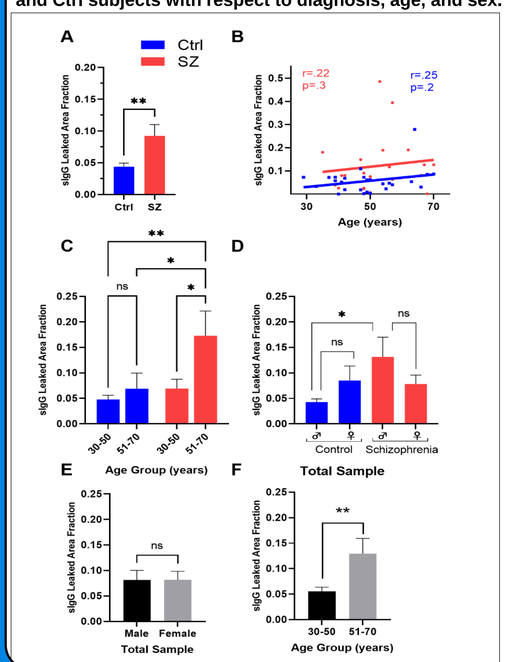
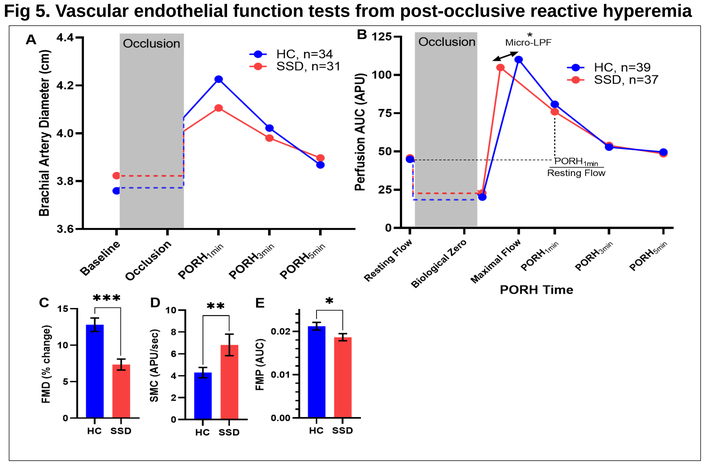


Fig 5. Vascular endothelial function tests from post-occlusive reactive hyperemia



Microvascular perfusion flux indices and derived kinetic measures

	Ctrl (n=39)	SSD (n=37)	Test statistic	p-value
Maximal flow (APU) (SE)	98.1 (4.7)	103.5 (6.6)	t=-.63	.53
Time to maximal flow (TMF), sec (SE)	24.8 (2.9)	15.6 (1.5)	t=3.0	.005
AUC of time to maximal flow (SE)	71.4 (3.9)	65.0 (4.4)	t=1.1	.29
Amplitude difference from resting to maximal flow (AMP_{RF})	54.4 (3.9)	61.4 (4.3)	t=-1.17	.25
Amplitude difference from biological zero to maximal flow (AMP_{BZ})	77.4 (4.5)	82.4 (5.7)	t=-.67	.51
Percentage increase from resting to maximal flow (PI_{RF})	126.5 (9.9)	164.5 (15.4)	t=-2.0	.055
Percentage increase from biological zero to maximal flow (PI_{BZ})	417.7 (41.7)	545.1 (90.2)	t=-1.2	.24
AUC_{RF} (AU·s)	1.7 (.09)	1.7 (.08)	t=.08	.94
AUC_{BZ} (AU·s)	3.9 (.3)	4.1 (.5)	t=-.20	.84
ORC_{MF} (AU·s^{-1})	4.2 (.7)	6.9 (1.2)	t=-1.9	.068

APU = Arbitrary Perfusion Units; AMP = Amplitude of the response expressed as a difference between maximal flow (i.e. peak perfusion) and resting flow (i.e. baseline) or biological zero ("BZ") (i.e. occlusion); PI = Percentage increase represented as the ratio between maximal flow and resting flow or biological zero; AUC = area under the 1-minute hyperemic curve with respect to resting flow or zero perfusion; ORC = Overshoot rate of change as a function of maximal flow. All values were adjusted for mean arterial pressure (MAP).

	Brachial artery reactivity testing (cm)				Microvascular perfusion measurement (APU)			
	Ctrl	SSD	Test statistic	p-value	Ctrl	SSD	Test statistic	p-value
Baseline (SE)	3.5 (.19)	3.8 (.13)	t=-1.48	.15	43.7 (1.7)	42.2 (3.7)	t=.36	.7
Occlusion	N/A				20.7 (1.6)	21.1 (2.2)	t=-.14	.9
1-minute post-occlusion	3.9 (.2)	4.1 (.14)	t=-.97	.34	73.5 (4.1)	69.4 (5.6)	t=-.58	.6
3-minute post-occlusion	3.7 (.2)	4.0 (.15)	t=-1.18	.25	51.0 (2.8)	49.0 (5.0)	t=-.34	.7
5-minute post-occlusion	3.6 (.19)	3.9 (.14)	t=-1.36	.18	48.7 (3.3)	45.6 (4.4)	t=.55	.6

Post-occlusive reactive hyperemia experiments were performed in the peripheral vascular compartments to translate the findings of central endothelial cell dysfunction in the context of BBB permeability. Group differences were significantly present amongst the peripheral endothelial vascular measures shown using flow-mediated dilation of the brachial artery, a gold-standard cardiovascular measurement. Flow-mediated dilation was significantly reduced in the SSD group compared to controls, indicating endothelial damage (12% vs 8%, p=0.02), after covarying age, sex, and body-mass index. Microvascular endothelial perfusion of the time to reach maximal flow was significantly shorter in SSD compared to controls (16 seconds vs 24 seconds, p=0.006) as well.

Conclusions

The contributions that vascular abnormalities may have, even after accounting for psychopharmacologic and lifestyle regimens, may shed light onto mechanistic underpinnings that can be harnessed in both detection and treatment approaches for schizophrenia. Psychoneuropathology within SSD may be mediated by endothelial function within the various vascular compartments, including that of the brain. These results provide evidence for robust explorations of how endothelial dysfunction underserves impairments in neural correlates of SSD, with supporting preliminary data.

Support & Acknowledgements

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