Exploring Relationships Between Brain Structure, Peripheral Cytokines and Neurofilament Light Polypeptide in a Sample of Heavy Drinkers

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Background

- Chronic alcohol consumption is associated with structural brain changes and increased inflammatory signaling throughout the brain and body.
- Heavy drinkers show increased pro-inflammatory cytokines measured in blood (1).
- Preclinical evidence suggests that alcohol causes inflammation in the brain (2)
- Neuroinflammation is associated with structural brain damage (3).
- Neurofilament light polypeptide (NfL) is released into circulation following neuronal damage (4).
- Given that NfL is produced following axonal damage and can be measured in blood, it has been proposed as a biomarker for several neurodegenerative diseases.
- NfL has not yet been explored in connection with alcohol use disorder (AUD).

Hypotheses

- We tested associations between alcohol consumption, the pro-inflammatory cytokine IL-6, NfL and brain structure (white and gray matter) in heavy drinkers.
- We hypothesized that alcohol consumption will be associated with increased IL-6, that IL-6 and alcohol consumption will be correlated with decreased frontal gray matter (GM) volume and compromised white matter (WM) integrity, and that WM and GM damage will be associated with higher levels of circulating NfL .

Methods

Study Procedures. 182 individuals participated in a study examining the effects of Mindfulness-Based Relapse Prevention (MBRP) compared to Relapse Prevention (RP) on drinking outcomes, and a subset of participants underwent MRI scanning. The present analyses focus on measurements (self-reported alcohol consumption via Timeline Followback, blood biomarkers, and structural MRI) taken at baseline, prior to the intervention. 74 participants provided blood samples and had useable MRI data.

Inclusion/Exclusion Criteria. Inclusion: between 21-60 years of age, interested in reducing drinking, reported consuming an average of 14+ drinks/week in the past 3 months. Exclusion: undergoing alcohol withdrawal, using medications to treat bipolar or psychotic disorders, endorsed current suicidality, met criteria for psychotic or bipolar disorder or current major depressive episode, reported using illicit drugs in the 30 days prior to beginning the study, or were pregnant.

Neuroimaging. Scans were conducted at the University of Colorado on a 2016 Siemens Magnetom 3T PrismaFit scanner with a 32-channel coil.

Structural Scans. For optimal contrast between gray matter (GM), white matter (WM) and cerebral spinal fluid (CSF) at 3T, we used a multi-echo MPRAGE (MEMPR) sequence with the following parameters: TR/TE/TI = 2300/2.74/900 ms, flip angle = 8°, FOV = 256x256 mm, Slab thickness = 176 mm, Matrix = 256x256x176, Voxel size =1x1x1 mm, Number of echos = 4, Pixel bandwidth =650 Hz.

Diffusion Scans. An echo planar image spin echo acquisition was used to obtain diffusion weighted images with the following parameters: TR/TE = 4000/77.00 ms, flip angle = 84°, FOV = 248x248 mm, Slice thickness = 3 mm, Matrix = 248x248x168 mm, b-value = 2400 s/mm^2, Voxel size = 2x2x2 mm, Multiband acceleration factor = 3.

Blood Samples. 10 ml whole blood was drawn to measure protein levels of the proinflammatory cytokine IL-6, as well as protein levels of circulating NfL. IL-6 was assessed in plasma using a multiplex assay (Quanterix Corp, Billerica MA). Neurofilament-Light (NfL) protein levels were assessed using the UmanDiagnostics NF-Light assay (Quanterix Corp, Billerica MA).

Analysis Plan

- Gray matter thickness was extracted from bilateral rostral and caudal middle frontal gyrus regions using Freesurfer.
- An average of left and right rostral and caudal middle frontal regions (MFG) was created for use in GM analyses.
- Axial, radial and mean diffusivity values were extracted using Tract Based Spatial Statistics (TBSS)
- A WM composite score was created consisting of the body of corpus callosum, fornix, external capsule, superior longitudinal fasciculus, and cingulate gyrus. An average of the axial, radial and mean diffusivity composites was taken to form the overall WM score used in all WM analyses.
- For each relationship of interest, we ran Ordinary Least Squares (OLS) regression models in SPSS (Version 27, IBM), in which each outcome variable of interest was regressed on the predictor of interest and gender (Table 1)
- For models in which GM was the criterion variable, total estimated intracranial volume (total ICV) was also included as a covariate
- For associations involving WM or GM metrics, we followed up on significant results using whole brain Monte Carlo simulations in Freesurfer (for GM) and TBSS (for WM).

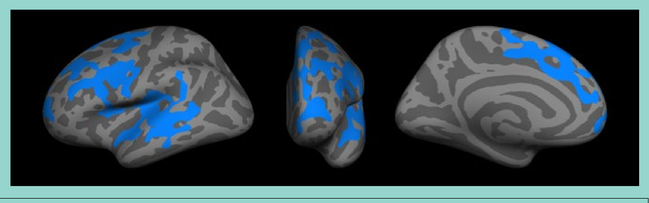

Figure 1. Effect of % heavy drinking days on cortical thickness (threshold $p < 0.05$)

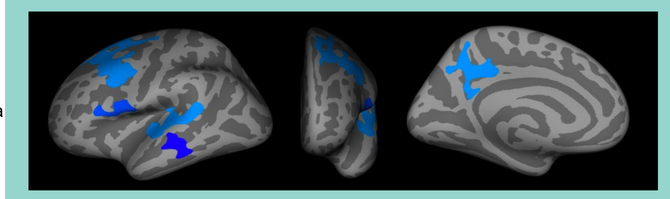

Figure 2. Effect of NfL on cortical thickness (threshold at $p < 0.05$)

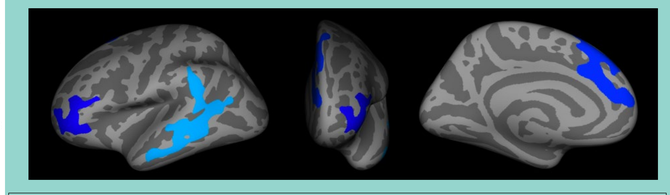

Figure 3. Effect of IL-6 on cortical thickness (threshold $p < 0.05$)

Results

- Regression models showed significant negative relationships between GM and heavy drinking days, IL-6 and GM, and NfL and GM (Table 1).
- Significant positive relationships emerged between IL-6 and total drinking days, WM and heavy drinking days and NfL and WM (note: higher WM diffusivity suggests greater damage; Table 1).
- No associations emerged between IL-6 and WM.
- Follow-up analyses in Freesurfer using whole brain Monte Carlo simulations (threshold p<.05) showed that:
  - % heavy drinking days was negatively associated with cortical thickness in the middle and superior middle temporal, insula, supramarginal, pars opercularis, rostral and caudal middle frontal, precentral, medial orbitofrontal and superior frontal regions (Fig. 1).
  - NfL was negatively associated with cortical thickness in the middle temporal, superior temporal, pars opercularis, rostral middle frontal, caudal middle frontal, precentral, postcentral, superior frontal and precuneus regions (Fig. 2)
  - IL-6 was negatively associated with cortical thickness in the middle and superior temporal, rostral middle frontal, superior frontal and cingulate regions (Fig. 3)
- Follow-up TBSS analyses demonstrated that
  - NfL was associated with radial, mean and axial diffusivity within numerous white matter tracts including the body of the corpus callosum, internal capsule, corona radiata and fornix (Fig. 4; p<.05).
  - heavy drinking days was associated with radial, mean and axial diffusivity within numerous white matter tracts including the body of of the corpus callosum, external capsule, corona radiata, fornix and cingulate gyrus (Fig. 5; no threshold).

Figure 4. Effect of NfL on radial (top), mean (middle) and axial (bottom) diffusivity. Threshold p<.05

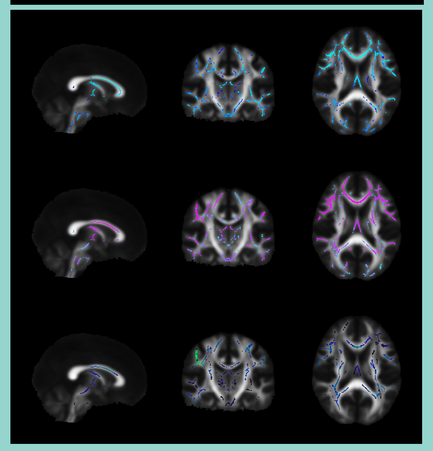

Figure 5. Effect of % heavy drinking days on radial (top), mean (middle) and axial (bottom) diffusivity. No threshold.

Table 1. Regression model results

Model	B	Std Error	beta	t	p	F	df	p	R^2
Criterion: IL-6									
Overall model						4.81	2, 112	.01	.08
Gender	-.28	.13	-.19	-2.07	**.04**				
Total drinking days (TLFB)	.02	.01	.23	2.47	**.02**				
Criterion: NfL									
Overall model						6.91	2, 66	.002	.17
Gender	-1.47	.75	-.22	-1.95	**.06**				
WM:AD/RD/MD Composite	1.15	.40	.32	2.87	**.006**				
Criterion: GM (MFG)									
Overall model						1.69	3, 72	.18	.07
Gender	-.02	.03	-.08	-.54	.59				
Total ICV	<.001	<.001	.01	.054	.96				
% heavy drinking days	-.08	.04	-.25	-2.19	**.03**				
Criterion: WM AD/RD/MD Composite									
Overall Model						3.48	2, 71	.04	.09
Gender	-.42	.22	-.22	-1.91	**.06**				
% heavy drinking days	.65	.32	.23	2.04	**.045**				
Criterion: GM (MFG)									
Overall Model						1.61	3, 70	.20	.06
Gender	.002	.03	.01	.06	.95				
Total ICV	<.001	<.001	.003	.02	.99				
IL-6	-.05	.02	-.25	-2.19	**.03**				
Criterion: NfL									
Overall Model						5.68	3, 67	.002	.20
Gender	1.30	.94	.19	1.38	.17				
Total ICV	<.001	<.001	-.01	-.05	.96				
GM (MFG)	-12.56	3.50	-.39	-3.59	**.001**				

Conclusion

- Data suggest that drinking is associated with inflammation, which is linked with structural brain damage and increased circulating NfL.
- This is the first study to demonstrate an association between brain structure and NfL in heavy drinkers.
- Results suggest that NfL may be a useful AUD biomarker with potential research and treatment implications.
- IL-6 alone may not be an adequate marker of peripheral inflammation; further research is needed to identify inflammatory biomarkers that may correlate with structural brain changes and NfL
- Results should be replicated in diagnosed AUD populations

References and Acknowledgements

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