Supplementary Information for

White matter alterations in chronic MDMA users: Evidence from diffusion tensor imaging and neurofilament light chain blood levels

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Supplementary Methods

White-matter tract segmentation

We applied TractSeg, a deep-learning based automatic white-matter bundle segmentation tool, to reconstruct anatomically well-known white matter tracts (Wasserthal et al., 2018). In total, 48 white matter tracts were reconstructed per participant:

- Arcuate fascicle (bilateral)
- Anterior Thalamic Radiation (bilateral)
- Corpus callosum (divided into seven subdivisions)
    - Rostrum
    - Genu
    - Rostral body (premotor)
    - Anterior midbody (primary motor)
    - Posterior midbody (primary somatosensory)
    - Isthmus
    - Splenium
- Cingulum (bilateral)
- Corticospinal tract (bilateral)
- Fronto-pontine tract (bilateral)
- Inferior occipito-frontal fascicle (bilateral)
- Inferior longitudinal fascicle (bilateral)
- Middle cerebellar peduncle
- Optic radiation (bilateral)
- Parieto-occipital pontine tract (bilateral)
- Superior cerebellar peduncle (bilateral)
- Superior longitudinal fascicle I, II and III (bilateral)
- Superior Thalamic Radiation (bilateral)
- Uncinate fascicle (bilateral)
- Thalamo-premotor tract (bilateral)
- Thalamo-parietal tract (bilateral)
- Thalamo-occipital tract (bilateral)
- Striato-fronto-orbital tract (bilateral)
- Striato-premotor tract (bilateral)

Supplementary Results

Tractometry

Each reconstructed white matter tract was subdivided into 100 segments and mean fractional anisotropy (FA) values were computed per segment and participant. Two sample t-tests were applied after regressing out confounding variables to compare FA values per segment between MDMA users and MDMA-naïve control participants. Resulting p-values were corrected for multiple comparison using resampling methods. In total, 11 white-matter tracts showed areas with significantly increased FA values in MDMA users. All tract containing significant areas are listed below and mean FA per segment and group are displayed in Figure S1.

- Corpus callosum
  - Genu
  - Rostral body (premotor)
  - Posterior midbody (primary somatosensory)
  - Isthmus
- Corticospinal tract (bilateral)
- Inferior occipito-frontal fascicle (left)
- Superior longitudinal fascicle I (left)
- Uncinate fascicle (right)
- Thalamo-parietal (right)
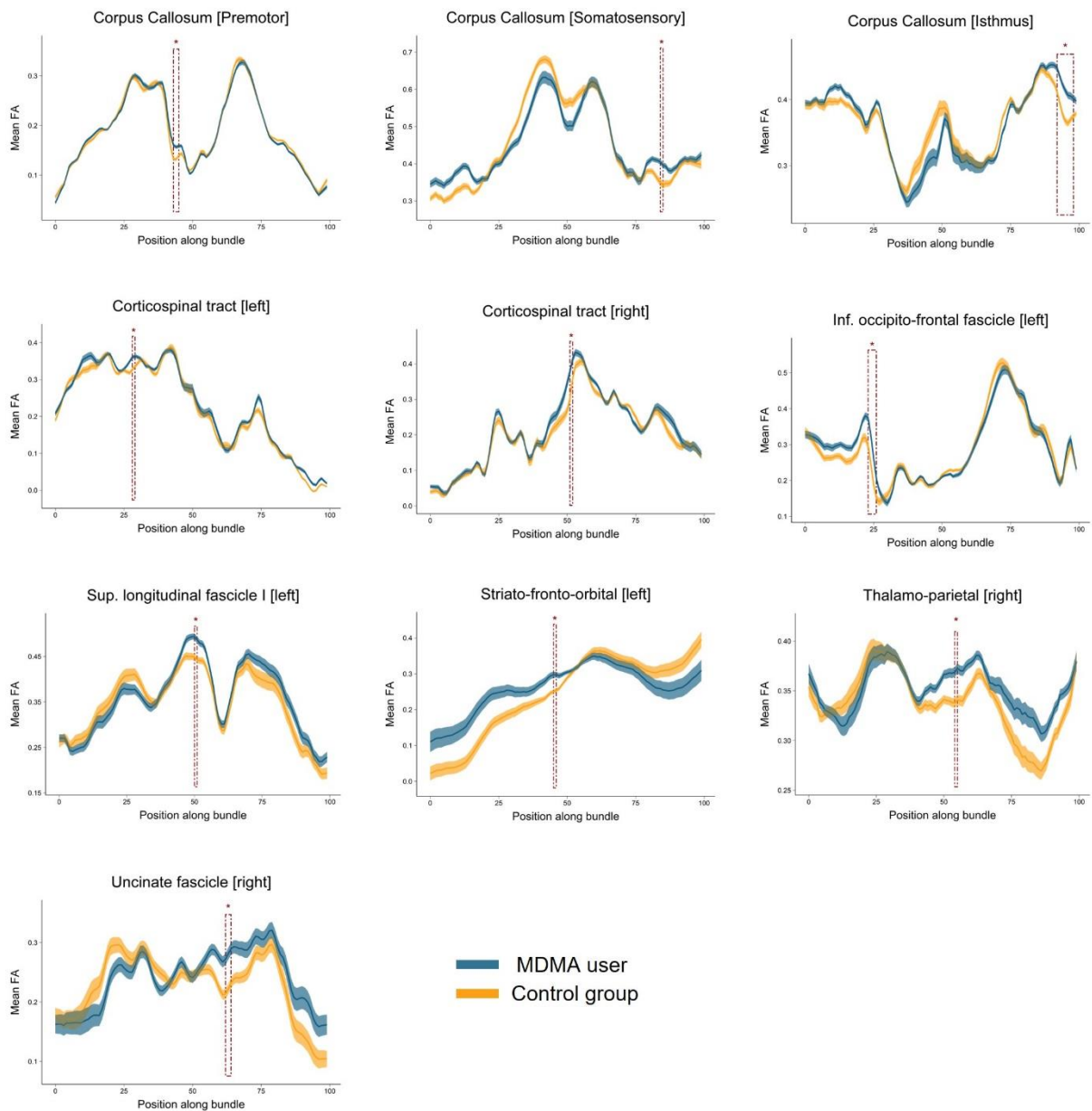- Striato-fronto-orbital (left)

Figure S1: Mean FA per segment and group for all bundles showing significant areas. Mean FA per group and respective standard error are displayed for MDMA user (blue) and control participants (yellow). Areas marked with a red dashed line are regions where MDMA users showed significantly increased FA values compared to the control group.

Neurofilament light chain analysis

Group difference

Analysis of neurofilament light chain (NfL) concentration in blood serum revealed no significant group difference when using two sample t-test (t(80)=0.06, p=0.95) or when including covariates in a linear regression model (β=-0.56, t(72)=-0.25, p=0.80, see Table S1). Accordingly, chronic MDMA users do not display elevated NfL levels compared to MDMA-naïve control participants. Age, gender and body-mass-index (BMI) were included as covariates as these factors have been previously shown to affect NfL concentration (Barro et al., 2020). Further, we controlled for co-use of other substances such as alcohol (self-reported use during last six months), cocaine, amphetamine, cannabis and ketamine (hair residuals reflecting use intensity over last four months) as it has been shown that alcohol (Li et al., 2021), cocaine (Bavato et al., 2022) and ketamine (Liu et al., 2021) use lead to increased NfL concentrations.

Table S1: Regression table for neurofilament light chain analysis

Predictors	Coefficients	Confidence interval	p-value
(Intercept)	25.08	11.13 – 39.04	
Group [MDMA user]	-0.56	-4.98 – 3.87	0.80
Age	0.09	-0.15 – 0.34	0.45
Gender [Male]	1.01	-2.30 – 4.31	0.55
Body-Mass-Index	-0.05	-0.52 – 0.41	0.82
Cocaine use[1]	0.50	-0.22 – 1.21	0.17
Cannabis use[1]	-0.46	-1.75 – 0.83	0.48
Amphetamine use[1]	-0.80	-1.62 – 0.03	0.06
Ketamine use[1]	-0.08	-0.97 – 0.80	0.85
Alcohol use[2]	0.58	-0.09 – 1.25	0.09
Observations	82		
R^2 / R^2 adjusted	0.14 / 0.02		

[1] hair concentration in pg/mg from toxicological analysis
[2] estimated alcohol use (in gram) within 6 months prior to study according to self-report

Correlation with FA values

To test if increased FA values in MDMA users are reflected in NfL concentrations, we correlated mean FA within areas per bundle showing significant group differences with NfL concentrations. However, no significant correlation was observed before and after Bonferroni correction (see Table S2).

Table S2: Correlation of NfL and mean FA within significant areas

White matter tract	Pearsons' R	df	p-value uncorrected	p-value corrected
Corpus Callosum [Genu]	-0.05	72	0.68	1.00
Corpus Callosum [anterior midbody]	-0.10	72	0.41	1.00
Corpus Callosum [posterior midbody]	-0.06	72	0.64	1.00
Corpus Callosum [Isthmus]	0.11	72	0.37	1.00
Corticospinal tract [left]	-0.09	72	0.47	1.00
Corticospinal tract [right]	-0.14	72	0.25	1.00
Inf. occipito-frontal fascicle [left]	-0.05	72	0.68	1.00
Sup. longitudinal fascicle I [left]	0.06	72	0.59	1.00
Uncinate fascicle [right]	-0.02	72	0.89	1.00
Thalamo-parietal tract [right]	-0.08	72	0.52	1.00
Striato-fronto-orbital tract [left]	-0.01	72	0.92	1.00

Supplementary References

Barro, C., Chitnis, T., & Weiner, H. L. (2020). Blood neurofilament light: a critical review of its application to neurologic disease. *Annals of Clinical and Translational Neurology*, *7*(12), 2508–2523. https://doi.org/10.1002/acn3.51234

Bavato, F., Kexel, A. K., Kluwe-Schiavon, B., Maceski, A., Baumgartner, M. R., Seifritz, E., Kuhle, J., & Quednow, B. B. (2022). *A longitudinal investigation of blood neurofilament light chain levels in chronic cocaine users* (p. 2022.02.03.22270384). medRxiv. https://www.medrxiv.org/content/10.1101/2022.02.03.22270384v1

Li, Y., Duan, R., Gong, Z., Jing, L., Zhang, T., Zhang, Y., & Jia, Y. (2021). Neurofilament Light Chain Is a Promising Biomarker in Alcohol Dependence. *Frontiers in Psychiatry*, *12*, 754969. https://doi.org/10.3389/fpsyt.2021.754969

Liu, Y.-L., Bavato, F., Chung, A.-N., Liu, T.-H., Chen, Y.-L., Huang, M.-C., & Quednow, B. B. (2021). Neurofilament light chain as novel blood biomarker of disturbed neuroaxonal integrity in

patients with ketamine dependence. *The World Journal of Biological Psychiatry*, *22*(9), 713–721. https://doi.org/10.1080/15622975.2021.1907709

Wasserthal, J., Neher, P., & Maier-Hein, K. H. (2018). TractSeg - Fast and accurate white matter tract segmentation. *NeuroImage*, *183*, 239–253. https://doi.org/10.1016/j.neuroimage.2018.07.070