Supplementary File 1

1. Detailed Materials and Methods

1.1 Subjects and Procedures

Subjects in this study were recruited for a study of a phenotyping battery that was designed to provide behavioral and brain imaging phenotypes for future OUD medication development research. An initial phone interview screened potential participants for study inclusion. Subjects who passed the phone screening were invited for a more thorough in-person screening f1nch. An initial

diagnosed severe Alcohol Use Disorder. For the HC group, the only inclusion criterion was age 18 to 70. Exclusion criteria were any history of substance use disorder, any history of schizophrenia, seizure disorder, significant head trauma, or any changes to psychoactive medications within 30 days of the study period.

Subjects who qualified for the study completed three additional visits: a visit in which they completed study behavioral measures and questionnaires (visit 2), a visit in which they completed safety screening for the MRI scan and a mock MRI session (visit 3), and a visit in which they completed an MRI scanning session (visit 4). All the fMRI scans in this study were resting-state fMRI scans, and thus none of the fMRI scans in this study involved tasks inside the scanner. The timing of the visits was as follows: after the initial in-person screening visit, the behavioral assessment visit was scheduled to take place within about 14 days (1-2 weeks). The MRI screening and mock scan visit typically occurred within 1-3 weeks after behavioral assessment. The MRI scan occurred within 30 days of the MRI screening visit. Participants were asked to refrain from smoking 1 hour and drinking caffeine 3 hours before their MRI scan. Urine drug screens (UDS) and breath alcohol screens were collected at each visit. A clinical assessment by a physician or nurse practitioner was performed during the MRI visit before scanning to ensure that subjects did not meet DSM-5 criteria for drug intoxication at the time of the scan. Subjects were also assessed for gross signs of drug withdrawal by a physician or nurse practitioner during the MRI visit before scanning. None of the UDS on the day of scanning were positive for HC subjects. None of the breath alcohol screens on the day of scanning were positive from HC or OUD subjects.

80 subjects (33 OUD, 47 HC) completed the MRI scanning session. In our final analyses, we only included subjects who met strict criteria for head motion (Parkes et al., 2018), had all physiological data needed to correct for physiological noise, and for OUD subjects had at least one UDS positive for

Dependence (Heatherton et al., 1991). All behavioral and demographic data were analyzed using JMP (JMP, Version 14. SAS Institute Inc., Cary, NC, 1989-2019).

A two-sample T-test was performed to test for statistical significance between groups with respect to age, NU, and all other sub-scores and the total score from the UPPS-P. An unequal variance two-sample T-test was performed for mean relative framewise displacement (mFD; a measure of head motion) because a Brown-Forsythe test determined the variances between groups were significantly different ($p<0.02$). The median and interquartile range are reported for time since last opioid use

Number of erosions = 2; Erosion neighborhood = 0. Also using CONN, 5 principal components were extracted from an unsmoothed twice eroded cerebrospinal fluid region of interest generated from FSL FAST tissue segmentation with 0.99 probability CSF and using the FSL MNI152_T1_2mm_VentricleMask. Subsequent multiple regression general linear model was used to remove these white matter and CSF components from the fMRI timeseries.

The FSL applywarp command was used to transform the ICA-AROMA-and-aCompCor-denoised

Dual regression analysis was then performed in FSL (Nickerson et al., 2017; https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/DualRegression) to obtain subject-specific component maps. The FSL technique of Group Independent Component Analysis (GICA) (Bijsterbosch et al., 2017, p. 59) derives network maps that are common to all the subjects (i.e., fMRI data from all subjects in both groups combined are included in the GICA). After the GICA procedure is complete, stage 1 of the FSL dual regression (Nickerson et al., 2017) uses the GICA generated network maps to estimate a subject-specific time-course (timeseries) for each network. Each subject-specific time-course essentially reflects the average time-course across voxels in the corresponding network map (after taking into account the contributions of the other networks) (Nickerson et al., 2017). Those subject-specific time-courses were then normalized by their amplitude (represented by the standard deviation of the time-course) to allow for measurement of network-wide and localized signal amplitude differences, in addition to differences in the spatial distribution of connectivity strength across subjects (Nickerson et al., 2017). In stage 2 of dual regression, the subject-specific time-course for each network is then used as a template to generate a subject-specific spatial map for that network. Specifically, in stage 2 of dual regression, a linear regression analysis is conducted within each voxel, where the dependent variable

amplitude of the resting fMRI signal is believed to reflect an important aspect of functional connectivity (reviewed in Nickerson et al., 2017).

Non-parametric permutation tests were then performed via the Permutation Analysis of Linear Models (PALM) program (Winkler et al., 2014; https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/PALM) using the subject-specific spatial maps of parameter estimates output of stage 2 of the dual regression to compare the within-

To test for associations between hypothesized functional connectivities and behavioral data, PALM was used to perform a voxel-wise regression analysis of the subject-specific SN functional connectivity on the mean-centered NU scores for both subject groups testing for both main and group interaction effects of NU. We first tested for group x NU interaction effects, and then assessed the main effects of NU if there were no statistically significant group x NU interaction effects (FWE-corrected for voxels $p<0.05$) per the recommendation of the authors of the FSL neuroimaging analysis software that we used (General Linear Model for neuroimaging guide https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/GLM) as well as Kutner et al. (2005; pp. 306-308, 312-313, 326-327, 921-925, 932-933). For exploratory analyses, using the same procedure, the regressions of the within-network functional connectivities of the DMN, LECN, and RECN on mean-centered NU scores were computed. For the analysis of the effects of mFD on functional connectivity and post-hoc analysis of time since last opioid use, the same procedure was followed. Once significant group interaction effects were ruled out, the regressions of the within-network functional connectivity of the DMN, SN, LECN, and RECN on mean-centered mFD and hours since last opioid use were computed.

The anatomical location of the only significant cluster reported in section 3.2 of the manuscript was determined by visually ie-

In order to examine heterogeneity of our OUD sample, we also performed two post-hoc analyses. In the first analysis, we compared OUD subjects with at least one UDS positive for buprenorphine or methadone to OUD subjects with UDS positive for only illicit opioids. In the second analysis, we regressed the subject-specific component maps onto mean-centered self-reported time since last opioid use, measured in hours.

We had planned to perform an ANCOVA with NU as a covariate if the main effect of NU in the preregistered regression analysis of functional connectivity on NU was statistically significant, but the regression results were not statistically significant. Based on recommendations from a standard statistics textbook (Kutner et al., 2005, pp 347, 919, 940), for a concomitant variable to be included as a covariate in ANCOVA, there should be a statistically significant regression relationship of the concomitant variable with the response variable. If potential covariates have no relation to the response variable, then nothing is to be gained by including them in ANCOVA (Kutner et al., 2005, p. 919), and in our case, the reduction in the degrees of freedom from adding such covariates to the model may be detrimental, given the relatively small sample size in our study. Furthermore, a worsening of the

lated to the response

variable, or if there is no regression relationship to allow for extrapolation of the regression line of the covariate between the means of the two groups (Kutner et al., 2005, pp. 347, 940). None of the NU regression results for any of the networks were statistically significant. We also performed a regression analysis of functional connectivity on head motion (mFD) and education for all four networks examined to determine whether to include head motion or education as a covariate in ANCOVA. None of the head motion or education regression results for any of the networks were statistically significant and therefore mFD and education were not included as covariates in ANCOVA. Given that tobacco

use was imbalanced between groups, we compared the functional connectivity of tobacco using OUD to non-tobacco using OUD to investigate the effects of tobacco use on functional connectivity.

1.6 ROI Group Differences

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