Brain Volume Correlates with Duration of Abstinence from Substance Abuse in a Region-Specific and Substance-Specific Manner

Supplemental Information

Supplementary Methods

Psychopathy Assessment

Psychopathy was assessed with the Psychopathy Checklist Revised (PCL-R) conducted by trained research assistants (1). The PCL-R is a 20-item scale completed based on a semi-structured interview and file review. Each item is scored as 0, 1, or 2 based on the severity of each trait. Inter-rater reliability (intraclass correlation) for total PCL-R score was 0.98 based on 10 dual ratings. In addition to total PCL-R scores, Factor 1 (interpersonal/affective traits) scores, and Factor 2 (lifestyle/antisocial traits) scores were also recorded (2).

Selection of "Other Substance" Covariates

FRU vs. FLU Between-Group Models

In addition to the covariates listed above, all between-group models comparing gray matter volumes between FRUs and FLUs of a particular substance included covariates to rule out the influence of other substances. These covariates were coded as binary variables marking each subject as either a FRU or non-FRU (defined as either a FLU or non-user) of each other substance. In addition to the three substances of interest here, the ASI includes data on subjects' use of at least eight other substances. Thus, in order to simplify the model we used a statistical diagnostic to select covariates marking FRU or non-FRU only for substances that were most likely to influence group differences. Fisher’s Exact Test was used to examine whether the FRU and FLU groups for the substance of interest consisted of a different number of FRUs and former

non-regular users of each other substance (see **Table S1**). For any other substance for which Fisher's Exact Test yielded a trending ($p<0.1$) or significant group difference, a covariate marking FRU or non-FRU of the substance was included in the model.

Thus, for the analysis comparing FRUs and FLUs of alcohol, covariates for former regular use of cocaine ($p=0.030$), cannabis ($p=0.055$), nicotine ($p=0.006$), and polysubstance ($p<0.001$) were also included in the model (see **Table S1**). For the analysis comparing FRUs and FLUs of cocaine, a covariate for former regular use of polysubstance ($p=0.063$) was included in the model. For the analysis comparing FRUs and FLUs of cannabis, covariates for former regular use of alcohol ($p=0.089$), cocaine ($p=0.038$), nicotine ($p=0.097$), and polysubstance ($p=0.002$) were included in the model. Furthermore, abstinence duration only from the substance of interest was included in each model.

Within-Group Models

Within-group models evaluating the relation between gray matter volume and abstinence duration also included binary FRU/non-FRU covariates to rule out the influence of other substances. However, in order to choose the relevant covariates, a different statistical diagnostic than was used for the between-group analyses was needed to determine which substances were most likely to influence individual differences within each group. Here, any other substance for which the cell counts of both FRUs and non-FRUs of that substance were above the expected count according to a Chi-squared test was included in the model (see **Table S1**). For example, in the within-group model for FRUs of cannabis, covariates indicating FRU or non-FRU of alcohol (30 non-FRUs, 50 FRUs), cocaine (57 non-FRUs, 23 FRUs), nicotine (14 non-FRUs, 66 FRUs) and polysubstance (38 non-FRUs, 42 FRUs) were included in the model. However, for the within-group model for FLUs of cannabis, only covariates indicating FRUs or non-FRUs of

alcohol (10 non-FRUs, 7 FRUs) and nicotine (6 non-FRUs, 11 FRUs) were included in the model, as cocaine (16 non-FRUs, 1 FRU) and polysubstance (15 non-FRUs, 2 FRUs) had less than the expected cell count in FRUs (see **Table S1**). Furthermore, for within-group models with FRUs, the duration of abuse of the substance of interest was also included as a covariate.

Supplementary Results

Whole-Brain "Slimmed Model" Results

Whole-brain analyses were conducted with the "slimmed" models to determine the sensitivity of the results to the particular covariates included in the model (**Table S4**). Even using this model, alcohol abstinence duration positively correlated with volume of a cluster including the right hippocampus, amygdala, and putamen, while cannabis abstinence duration correlated with volume of a cluster including left putamen and globus pallidus. However, there were no significant correlations between brain volume and cocaine abstinence duration.

Disentangling Effects Related to Alcohol from Effects Related to Cannabis

We performed whole-brain analyses, using the slimmed model, on a "clean" alcohol FRU group (n=9) and a "clean" cannabis FRU group (n=25) that had non-overlapping subjects; the same cluster thresholding procedure to correct for multiple comparisons was used as in the main analyses.

The "clean" alcohol FRU group displayed only one significant cluster, with peak coordinates in the right thalamus and also including parts of the right globus pallidus and right putamen, where volume positively correlated with abstinence duration. Though it did not survive cluster thresholding, the largest cluster in the "clean" cannabis FRU group had peak coordinates

in the left putamen and extended into the left insula, also closely mirroring the cluster in the main cannabis FRU group findings. The findings from the "clean" alcohol FRU group are generally consistent with the main alcohol FRU group findings (and distinct from the main cannabis FRU findings) and the findings from the "clean" cannabis FRU group are generally consistent with the main cannabis FRU findings (and distinct from the main alcohol FRU findings). For instance, both the main and "clean" alcohol FRU groups, but neither the main nor "clean" cannabis FRU groups, display a positive correlation between volume and abstinence duration in the thalamus. And both the main and "clean" cannabis FRU groups, but neither the main or "clean" alcohol FRU groups, display a positive correlation between volume and abstinence duration in the insula.

In comparing these "clean" groups of FRUs with FLUs, the "clean" alcohol FRU group had lower volume in a posterior/temporal cluster than FLUs of alcohol who were not FRUs of cocaine or cannabis (n=14). The "clean" cannabis FRU group had lower volume in a large prefrontal cluster than FLUs of cocaine who were not FRUs of alcohol or cocaine (n=10); there were no posterior or temporal clusters. These "clean" group findings also mirror the main findings. Both the "clean" and main alcohol FRU groups display clusters of lower volume in posterior/temporal regions, whereas neither the "clean" or main cannabis FRU groups display cluster of lower volume in posterior/temporal regions. And both the "clean" and main cannabis FRU groups display a more extensive area of lower prefrontal volume compared to FLUs than either the "clean" or main alcohol FRU groups.

Disentangling Effects Related to Cocaine from Effects Related to Nicotine

Among FRUs of cocaine, nearly all (24/25) subjects were also FRUs of nicotine. In order to ensure that the observed effects were indeed attributable to the effects of cocaine and not

nicotine, we performed a separate set of analyses on FRUs and FLUs of nicotine (*n*=83 and *n*=12, respectively) and found evidence to suggest that effects of nicotine were not appreciably influencing the cocaine findings. Specifically, a whole-brain analysis in FRUs of nicotine found only one significant cluster – in the occipital lobe – where there was a positive correlation between volume and abstinence duration. Thus, it does not appear that the positive correlations between volume and abstinence duration in the subcortical and prefrontal regions found in FRUs of cocaine can be attributed to abstinence from nicotine. Furthermore, a whole-brain analysis comparing volume between FRUs and FLUs of nicotine did not find any significant differences. Thus, it does not appear that former abuse of nicotine would have influenced volumetric differences between FRUs and FLUs of cocaine in this sample.

Table S1: Determining Inclusion of "Other Substance" Covariates

Alcohol

	Cocaine		**Cannabis**		**Nicotine**		**Polysubstance**	
	Non-FRUs	*FRUs*	*Non-FRUs*	*FRUs*	*Non-FRUs*	*FRUs*	*Non-FRUs*	*FRUs*
FLU	39	6	14	31	16	29	38	7
FRU	43	19	10	52	8	54	24	38
Fisher's Exact Test	**0.030**		**0.055**		**0.006**		**<0.001**	

Cocaine

	Alcohol		**Cannabis**		**Nicotine**		**Polysubstance**	
	Non-FRUs	*FRUs*	*Non-FRUs*	*FRUs*	*Non-FRUs*	*FRUs*	*Non-FRUs*	*FRUs*
FLU	8	12	3	17	0	20	11	9
FRU	6	19	1	24	1	24	7	18
Fisher's Exact Test	0.204		0.224		0.556		**0.063**	

Cells with less than expected count underlined.

Cannabis

	Alcohol		**Cocaine**		**Nicotine**		**Polysubstance**	
	Non-FRUs	*FRUs*	*Non-FRUs*	*FRUs*	*Non-FRUs*	*FRUs*	*Non-FRUs*	*FRUs*
FLU	10	7	16	1	6	11	15	2
FRU	30	50	57	23	14	66	38	42
Fisher's Exact Test	**0.089**		**0.038**		**0.097**		**0.002**	

Cells with less than expected count underlined.

Table S2: Multicollinearity and Covariate Statistics

FRUs of Alcohol: Regressing R Putamen Volume on Abstinence Duration

Variable	**Tolerance**	**VIF**	**Std. Beta**	***p*-value**
Age	.405	2.469	-.439	.020
Race	.528	1.895	.442	.008
Factor 2	.742	1.348	.137	.314
Intracranial Volume	.911	1.097	.140	.255
Duration of Regular Use of Alcohol	.620	1.612	.160	.284
Age of First Use of Alcohol	.791	1.263	.006	.961
Former Regular User of Cocaine?	.675	1.481	-.021	.881
Former Regular User of Nicotine?	.652	1.533	.116	.423
Former Regular User of Cannabis?	.485	2.061	-.093	.579
Former Regular Polysubstance User?	.713	1.403	-.084	.546
Duration of Abstinence from Alcohol	.481	2.080	.411	.018

FRUs of Cocaine: Regressing R Putamen Volume on Abstinence Duration

Variable	**Tolerance**	**VIF**	**Std. Beta**	***p*-value**
Age	.508	1.967	-.125	.593
Race	.466	2.146	.442	.083
Factor 2	.772	1.295	.061	.746
Intracranial Volume	.691	1.447	-.160	.427
Duration of Regular Use of Cocaine	.492	2.032	-.108	.649
Age of First Use of Cocaine	.676	1.480	-.292	.161
Former Regular User of Alcohol?	.671	1.491	-.540	.016
Former Regular Polysubstance User?	.709	1.411	.523	.016
Duration of Abstinence from Cocaine	.321	3.120	.790	.015

FRUs of Cannabis: Regressing L Putamen Volume on Abstinence Duration

Variable	**Tolerance**	**VIF**	**Std. Beta**	***p*-value**
Age	.270	3.709	-.198	.328
Race	.667	1.499	.342	.009
Factor 2	.791	1.264	.147	.216
Intracranial Volume	.784	1.275	.234	.051
Duration of Regular Use of Cannabis	.299	3.349	-.013	.946
Age of First Use of Cannabis	.765	1.308	.114	.344
Former Regular User of Alcohol?	.696	1.436	-.089	.477
Former Regular User of Cocaine?	.635	1.574	-.018	.889
Former Regular User of Nicotine?	.716	1.396	-.016	.898
Former Regular Polysubstance User?	.648	1.543	-.008	.950
Duration of Abstinence from Cannabis	.379	2.636	.290	.091

Table S3. Whole-Brain Group-by-abstinence Duration Interaction Analyses: Regions Where the Positive Correlation Between Volume and Abstinence Duration Was Stronger in FRUs than FLUs

Cocaine	**Region at Peak Coordinates**	**Other Regions in Cluster**	**Peak Coordinates**	**Cluster Size**
	R Putamen	R Amygdala R Hippocampus R Globus Pallidus R Accumbens R Caudate R Parahippocampal Gyrus R Insula R Subcallosal Cortex L Subcallosal Cortex	(27, 2, -10)	2792
Cannabis	**Region at Peak Coordinates**	**Other Regions in Cluster**	**Peak Coordinates**	**Cluster Size**
	R Anterior Insula	R Putamen R Globus Pallidus R Inferior Frontal Gyrus R Precentral Gyrus R Posterior Orbitofrontal Cortex	(28, 27, 14)	3000

Table S4: Whole-Brain Analyses Using "Slimmed" Models

Alcohol

Regions of decreased volume in Former Regular Users compared to Former Light Users

Region at Peak Coordinates	**Other Regions in Cluster**	**Peak Coordinates**	**Cluster Size**
Cerebellum	L Lingual Gyrus	(2, -50, -10)	790
R Precuneus	R Posterior Cingulate Cortex	(8, -54, 22)	894

Positive associations between volume and abstinence time within Former Regular Users

Region at Peak Coordinates	**Other Regions in Cluster**	**Peak Coordinates**	**Cluster Size**
R Inferior Temporal Gyrus	R Middle Temporal Gyrus	(50, -36, -12)	940
R Hippocampus	R Parahippocampal Gyrus R Amygdala R Putamen	(33, -20, -9)	1263

Cocaine

Regions of decreased volume in Former Regular Users compared to Former Light Users

No suprathreshold clusters.

Positive associations between volume and abstinence time within Former Regular Users

No suprathreshold clusters.

Cannabis

Regions of decreased volume in Former Regular Users compared to Former Light Users

No suprathreshold clusters.

Positive associations between volume and abstinence time within Former Regular Users

Region at Peak Coordinates	**Other Regions in Cluster**	**Peak Coordinates**	**Cluster Size**
L Central Opercular Cortex	L Insula L Putamen L Globus Pallidus	(-34, 3, 21)	1240

Table S5. Positive Associations Between Volume and Abstinence Duration in All Former Regular Users (whole-brain analysis)

	Region at Peak Coordinates	**Other Regions in Cluster**	**Peak Coordinates**	**Cluster Size**
All FRUs	L Superior Temporal Gyrus	L Middle Temporal Gyrus L Inferior Temporal Gyrus	(-50, -34, 2)	1384
	L Putamen	L Globus Pallidus L Insula	(-33, -4, 8)	1151

Table S6. Clean Sub-Samples: Regions of Less Volume in Former Regular Users Compared to Former Light Users (whole-brain analysis)

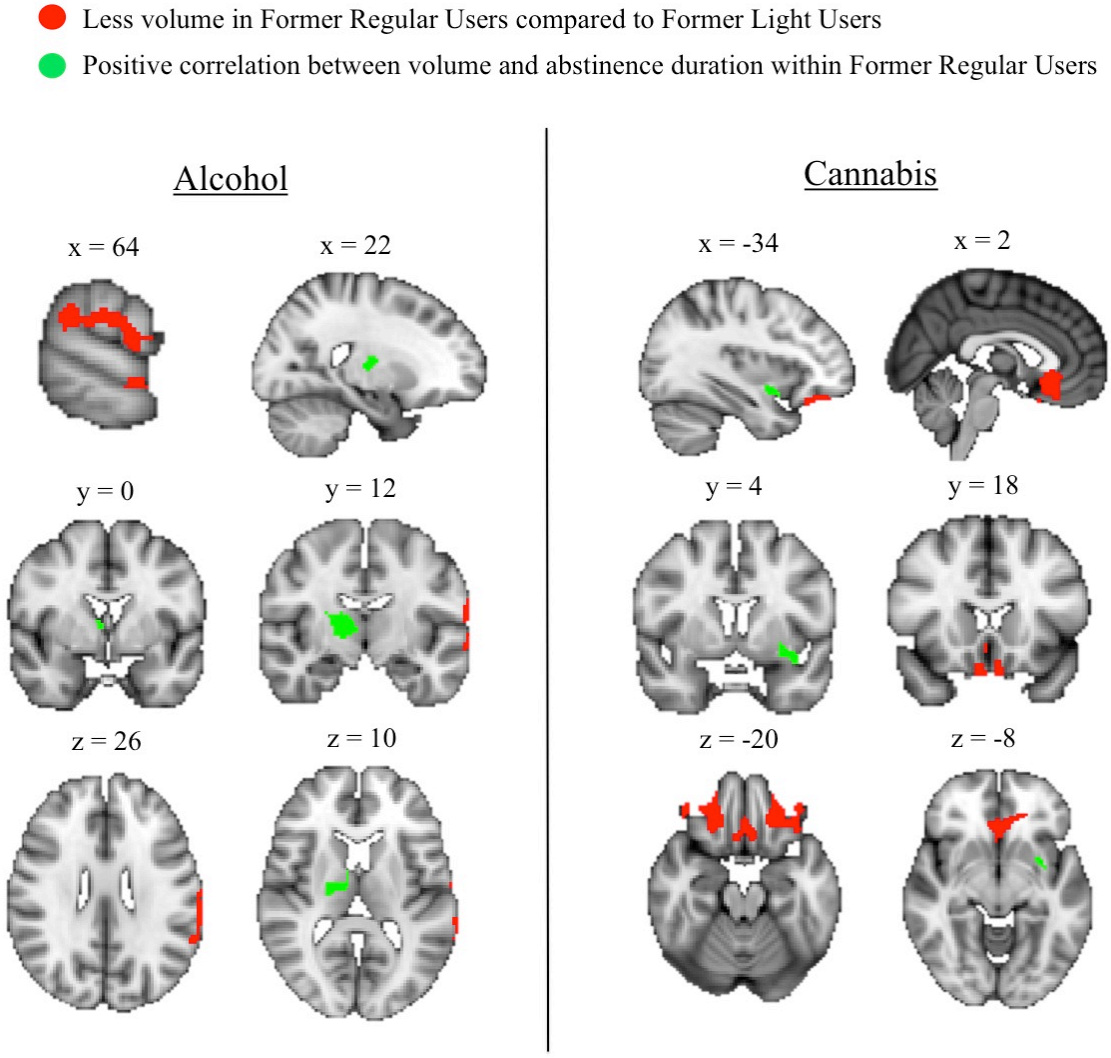
	Region at Peak Coordinates	**Other Regions in Cluster**	**Peak Coordinates**	**Cluster Size**
Alcohol	L Supramarginal Gyrus	L Postcentral Gyrus L Superior Temporal Gyrus	(-58, -48, 28)	765
	Region at Peak Coordinates	**Other Regions in Cluster**	**Peak Coordinates**	**Cluster Size**
Cannabis	R Medial Orbitofrontal Cortex	L Medial Orbitofrontal Cortex L Paracingulate Gyrus R Subcallosal Cortex L Subcallosal Cortex R Lateral Orbitofrontal Cortex L Lateral Orbitofrontal Cortex R Anterior Cingulate Cortex L Anterior Cingulate Cortex	(18, 34, -20)	3585

Table S7. Clean Sub-Samples: Positive Associations Between Volume and Abstinence Duration Within Former Regular Users (whole-brain analysis)

Alcohol	**Region at Peak Coordinates**	**Other Regions in Cluster**	**Peak Coordinates**	**Cluster Size**
	R Thalamus	R Globus Pallidus R Putamen	(4, -2, 6)	779
Cannabis	**Region at Peak Coordinates**	**Other Regions in Cluster**	**Peak Coordinates**	**Cluster Size**
	L Putamen	L Insular Cortex	(-30, 4, -9)	295*

*Does not meet cluster threshold

Figure S1. Clean Sub-samples



Supplementary References

1. Hare RD. *The Hare psychopathy checklist-revised (2nd ed.).* Toronto: Multi-Health Systems; 2003.
2. Harpur T, Hare R, Hakstian A. Two-factor conceptualization of psychopathy: Construct validity and assessment implications. *Psychological Assessment: A Journal of consulting and clinical Psychology.* 1989;1(1).