SUPPLEMENTARY INFORMATION

Imaging Nociceptive Opioid Peptide Receptors in Alcohol Use Disorders With [^{11}C]NOP-1A and Positron Emission Tomography: Findings From a Second Cohort

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Contents

Table S1. MRI based volume of regions of interest (mm^3)

	Alcohol use disorders (n=27)		Healthy controls (n=27)	
	Mean	SD	Mean	SD
Amygdala	2092	308	2126	307
Hippocampus	4258	593	4437	609
Midbrain	6216	822	6568	935
Ventral Striatum	825	164	777	106
Caudate	7002	960	7267	859
Putamen	7962	1008	8259	684
Dorsolateral Prefrontal Cortex	74462	9769	77715	11058
Orbital Frontal Cortex	45077	5769	44692	6931
Medial Prefrontal Cortex	19005	3873	20164	3906
Anterior Cingulate Cortex	17674	4376	16592	3326
Cerebellum	71432	9532	71246	10251

Table S2. Effect of tobacco use status on [^{11}C]NOP-1A V_T in Alcohol use disorders and Healthy controls

Region	Tobacco	Alcohol use disorders 12 Yes/15 No		Healthy controls 15 Yes/12 No	
		Mean	SD	Mean	SD
Amygdala	Yes	16.46	4.21	15.55	2.13
	No	14.60	3.20	14.80	2.37
Hippocampus	Yes	12.23	2.85	11.31	1.41
	No	10.45	2.21	10.82	1.73
Midbrain	Yes	9.77	2.22	8.95	0.95
	No	8.33	1.77	8.62	1.39
Ventral striatum	Yes	15.70	3.75	14.30	1.79
	No	13.54	2.81	14.10	2.00
Caudate	Yes	12.90	3.04	11.96	1.62
	No	11.03	2.14	11.58	1.39
Putamen	Yes	15.39	3.64	13.70	1.66
	No	12.81	2.66	13.28	1.94
Dorsolateral prefrontal cortex	Yes	14.22	3.26	13.31	1.68
	No	12.51	2.76	12.76	1.91
Orbitofrontal cortex	Yes	14.58	3.38	13.81	1.80
	No	12.87	2.86	13.27	2.03
Medial prefrontal cortex	Yes	14.21	3.23	13.36	1.63
	No	12.47	2.74	12.77	1.69
Anterior cingulate cortex	Yes	14.78	3.31	13.80	1.62
	No	12.97	2.77	13.27	1.94
Cerebellum	Yes	8.76	1.97	7.97	0.88
	No	7.49	1.58	7.89	1.25

Unpaired t-tests in all regions comparing V_T in tobacco users vs. non-users were $p > 0.05$ in both the AUD and HC groups. However, differences in V_T between tobacco users vs. non-users in the AUD group were at a statistical trend ($p < 0.1$) in the hippocampus, midbrain, caudate, putamen and cerebellum.

Table S3. Clinical characteristics of AUD subjects based on follow-up outcome

	Abstained (n=6)	Relapsed (n=8)	Dropped out (n=8)
Males (n)	1	1	1
Tobacco use (n)	5	3	3
Comorbid psychiatric disorders (n) (GAD or PTSD)	2	1	1
Comorbid pain Disorders (n)	1	1	2
Psychotropic Medications (n)	2	3	3
Hair ETG ≥ 30 pg/mg (n)	2	7†	4
Hair ETG < 30 pg/mg (n)	4	0	4
Alcohol Dependence Scale (range 0 to 47)	23 ± 10	18 ± 6	19 ± 3
Penn Alcohol Craving Scale (range 0 to 30)	17 ± 8	14 ± 7	16 ± 10
Duration abstinent from alcohol before PET (days)	47 ± 32	18 ± 12	25 ± 12

† Hair from one subject was unavailable

Table S4. Pearson correlation coefficient (r) for relationships between ROI V_T and total money earned in contingency management paradigm (data is from n=19 subjects as three AUD are excluded for completing part of the follow-up in an honor system via phone due to COVID19 pandemic).

Region		
Amygdala	r	0.620
	p	0.00462
Hippocampus	r	0.695
	p	< 0.001
Midbrain	r	0.686
	p	0.001
Ventral Striatum	r	0.686
	p	0.001
Caudate	r	0.621
	p	0.00453
Putamen	r	0.627
	p	0.004
Dorsolateral prefrontal cortex	r	0.699
	p	< 0.001
Orbitofrontal cortex	r	0.719
	p	< 0.001
Medial prefrontal cortex	r	0.664
	p	0.002
Anterior Cingulate Cortex	r	0.674
	p	0.002
Cerebellum	r	0.659
	p	0.002

p is the p-value for r

p < 0.00454 is significant after Bonferroni correction for n=11 ROIs (0.05/11)

Table S5. Partial correlations (r) for relationships between ROI V_T and total money earned in contingency management paradigm after controlling for heavy drinking status (hair ETG +/-) **(data is from n=18 subjects,** three AUD were excluded for completing part of the follow-up in an honor system via phone due to COVID19 pandemic and one AUD was excluded for not having hair ETG).

Region		
Amygdala	r	0.562
	p	0.019
Hippocampus	r	0.641
	p	0.006
Midbrain	r	0.628
	p	0.007
Ventral Striatum	r	0.628
	p	0.007
Caudate	r	0.564
	p	0.018
Putamen	r	0.560
	p	0.019
Dorsolateral prefrontal cortex	r	0.643
	p	0.005
Orbitofrontal cortex	r	0.666
	p	0.004
Medial prefrontal cortex	r	0.609
	p	0.010
Anterior cingulate cortex	r	0.609
	p	0.009
Cerebellum	r	0.602
	p	0.011

p is the p-value for r

$p < 0.00454$ is significant after Bonferroni correction for n=11 ROIs (0.05/11)

Table S6. Partial correlations (r) for relationships between ROI V_T and total money earned in contingency management paradigm after controlling for the number of abstinent days before PET (data is from n=19 subjects as three AUD are excluded for completing part of the follow-up in an honor system via phone due to COVID19 pandemic).

Region		
Amygdala	r	0.574
	p	0.013
Hippocampus	r	0.611
	p	0.007
Midbrain	r	0.609
	p	0.007
Ventral Striatum	r	0.610
	p	0.007
Caudate	r	0.537
	p	0.021
Putamen	r	0.546
	p	0.019
Dorsolateral prefrontal cortex	r	0.632
	p	0.005
Orbitofrontal cortex	r	0.652
	p	0.003
Medial prefrontal cortex	r	0.594
	p	0.009
Anterior cingulate cortex	r	0.593
	p	0.010
Cerebellum	r	0.590
	p	0.010

p is the p-value for r

$p < 0.00454$ is significant after Bonferroni correction for n=11 ROIs (0.05/11)

Table S7. Partial correlations (r) for the relationships between V_T and self-reported alcohol use measures after controlling for tobacco use and sex

		Controlling r for					
		Tobacco use			Sex		
Region V_T		Abstinent Days	Drinking days in past 30 days	Drinks/ drinking day	Abstinent days	Drinking days in past 30 days	Drinks/ drinking day
Amygdala	r	.191	-.563	-.560	.369	-.568	-.579
	p	.349	.003	.003	.064	.002	.002
Hippocampus	r	.360	-.574	-.594	.521	-.552	-.592
	p	.071	.002	.001	.006	.003	.001
Midbrain	r	.301	-.499	-.539	.475	-.480	-.540
	p	.136	.009	.005	.014	.013	.004
Ventral Striatum	r	.316	-.596	-.564	.488	-.581	-.570
	p	.116	.001	.003	.011	.002	.002
Caudate	r	.289	-.502	-.525	.468	-.483	-.527
	p	.153	.009	.006	.016	.012	.006
Putamen	r	.271	-.566	-.554	.467	-.535	-.549
	p	.181	.003	.003	.016	.005	.004
Dorsolateral prefrontal cortex	r	.276	-.548	-.548	.462	-.551	-.566
	p	.173	.004	.004	.017	.004	.003
Orbitofrontal cortex	r	.289	-.618	-.594	.469	-.620	-.612
	p	.152	.001	.001	.016	.001	.001
Medial prefrontal cortex	r	.267	-.548	-.542	.450	-.547	-.558
	p	.188	.004	.004	.021	.004	.003
Anterior cingulate cortex	r	.311	-.568	-.557	.480	-.561	-.568
	p	.122	.002	.003	.013	.003	.002
Cerebellum	r	.255	-.470	-.535	.427	-.451	-.535
	p	.208	.015	.005	.029	.021	.005

p is p-value for r

Table S8. Basic studies that that have investigated N/OFQ and NOP in human and rodent models of AUD

Reference	Species/model	Study design	Region	ppN/OFQ	NOP
(1)	Wistar rats	*<u>Compared alcohol dependent vs. vehicle treated rats</u>* - 7 days after alcohol was withdrawn - 21 days after alcohol was withdrawn Ethanol dependent rats were administered alcohol (10g/Kg) four times daily for six days.	Bed nucleus of the stria terminalis	No difference	**Higher** at 7 and 21 days after alcohol withdrawn
			Central nucleus of the amygdala	**Higher** at 21 days, but not 7-days after alcohol withdrawn	No difference
			Lateral Hypothalamus	No difference	**Higher** at 7 and 21 days after alcohol withdrawn
(2)	Sprague-Dawley rats	<u>Compared alcohol administered vs. control rats</u> Alcohol (4.5 g/Kg/day) or water (control), administered for 1 day or 5-days. Rat brains examined 30 min after alcohol/water, or 1-, 3- or 7-days of withdrawal	Amygdala	**Higher** during alcohol administration (1- and 5-day groups), that persisted during withdrawal on day 1, but not days 3 and 7	No difference
			Prefrontal cortex	No difference	No difference
(3)	C57BL/6J mice	<u>Alcohol self-administration (SA) vs. controls (water)</u> - 4 weeks following alcohol SA	Ventral Tegmental Area	**Lower** in the 5-day, but not 21-day withdrawn	Not measured

		- 5 days after alcohol SA was withdrawn - 21 days after alcohol SA was withdrawn			
			Frontal cortex	**Lower** in the 21-day, but not 5-day withdrawn	Not measured
			Striatum, Amygdala, Periaqueductal gray	No difference	Not measured
(4)	Wistar rats bred for high and low ethanol drinking in adolescence (second filial generation)	<u>High vs. low ethanol drinking rats</u> High ethanol drinkers 7.7 to 9.2 g/Kg/day Low ethanol drinkers 0.9 to 1.2 g/Kg/day Postmortem brains examined 40 days after last alcohol use	Prefrontal cortex	**Lower** (males > females)	**Lower** (males > females)
			Nucleus accumbens	No difference	**Lower** only in males, and not in females
			Ventral tegmental area	No difference	No difference
(5)	Marchigian Sardinian alcohol-preferring rats (msP) Wistar rats (controls)	<u>Alcohol preferring msP vs Wistar control rats</u> Alcohol self-administration following which in situ hybridization and NOP receptor autoradiography were performed	Bed nucleus of the stria terminalis	**Higher**	**Higher** (binding, but not mRNA)
			Central nucleus of the amygdala	**Higher**	**Higher** (binding and mRNA)
			Medial amygdaloid nucleus	No difference	**Higher** (binding, but not mRNA)
			Basolateral amygdaloid nucleus	Not measured	**Higher** (in mRNA, but not binding)
			Cingulate cortex	No difference	**Higher**

					(binding, but not mRNA)
			Motor cortex	**Higher**	**Higher** (binding and mRNA)
			Nucleus accumbens	Not measured	**Higher** (binding; mRNA not measured)
			Ventral tegmental area	Not measured	**Lower** (binding; mRNA not measured)
(6)	mSP rats	<u>Chronic intermittent exposure to alcohol for 30 days Vs. water exposed controls</u> Postmortem brains were examined 24-hours after last alcohol use	Amygdala	No difference *(Trend lower)*	**Lower**
			Bed nucleus of the stria terminalis	**Lower**	**Higher**
	Wistar rats	<u>Chronic intermittent exposure to alcohol for 30 days Vs. water exposed controls</u> Postmortem brains were examined 24-hours after last alcohol use	Amygdala	No difference	No difference
			Bed nucleus of the stria terminalis	No difference	No difference
(7)	Postmortem humans	15 Alcohol use disorder 15 healthy controls (Tobacco use in 75% in healthy controls and 83% in AUD) RT-PCR analysis	Hippocampus	**Lower**	No difference *(Trend lower)*
			Central nucleus of the amygdala	No difference	**Lower**
			Prefrontal cortex	No difference	No difference
			Orbitofrontal cortex	No difference	No difference
			Motor cortex	No difference	No difference

(8)	Postmortem humans	27 substance use disorders (56% AUD, 22% AUD + another SUD and 22% other substance use disorder) 53 healthy controls	Anterior insula	No difference	No difference
			Mediodorsal thalamus	No difference	No difference
			Dorsal anterior cingulate cortex	No difference	No difference

Supplement analyses

1. Effect of sex on [^{11}C]NOP-1A V_T

[^{11}C]NOP-1A V_T was not significantly different between AUD and HC when sex was included as a factor in the linear mixed model (effect of diagnosis, $F(1, 50) = 0.01$, $p = 0.98$; effect of sex, $F(1, 50) = 5.32$, $p = 0.03$; effect of sex X diagnosis, $F(1, 50) = 0.25$, $p = 0.62$). Females had significantly lower V_T compared to males, as previously reported in (9), but not in (10).

2. Effect of tobacco use status on [^{11}C]NOP-1A V_T

[^{11}C]NOP-1A V_T was not significantly different between AUD and HC when tobacco use status was included as a factor in the linear mixed model (effect of diagnosis, $F(1, 50) = 0.26$, $p = 0.62$; effect of tobacco use, $F(1, 50) = 3.29$, $p = 0.08$; effect of tobacco use X diagnosis, $F(1, 50) = 1.22$, $p = 0.28$). Higher V_T in tobacco users compared to non-users, which is at trend-level in the LMM is shown in **Table S2**.

3. Effect of comorbid psychiatric/chronic pain disorders on [^{11}C]NOP-1A V_T in AUD

[^{11}C]NOP-1A V_T was not significantly different between AUD with (n=7) and without (n=20) comorbid psychiatric/chronic pain disorders (effect of comorbid disorders, $F(1, 25) = 0.03$, $p = 0.86$; effect of region, $F(10, 250) = 168.47$, $p < 0.001$; effect of region X comorbid disorders, $F(10, 250) = 0.83$, $p = 0.60$).

4. Effect of psychotropic medications on [^{11}C]NOP-1A V_T in AUD

[^{11}C]NOP-1A V_T was not significantly different between AUD off- (n=19) and on- (n=8) and psychotropic medications (effect of psychotropic medications, $F(1, 25) = 0.01$, $p = 0.92$; effect of region, $F(10, 250) = 190.90$, $p < 0.001$; effect of region X psychotropic medications, $F(10, 250) = 0.63$, $p = 0.79$).

Supplement Figures

Figure S1 shows the positive relationship (which failed to survive a multiple comparison correction) between number of abstinent days prior to the scan and ventral striatum V_T in n= 27 AUD subjects.

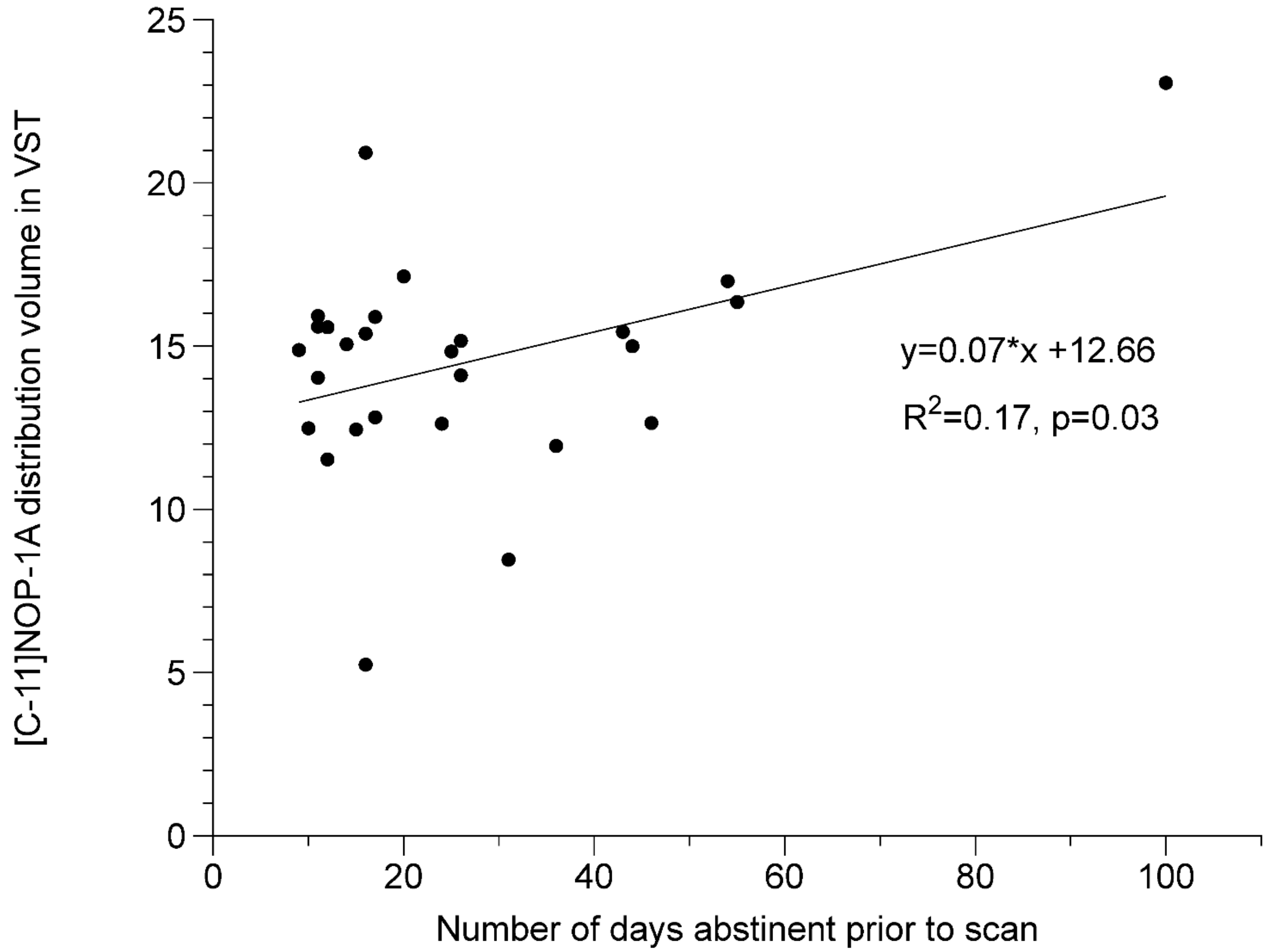


Figure S2 shows the negative association between the number of drinking days in the month prior to enrollment and orbitofrontal cortex V_T in n=27 AUD subjects.

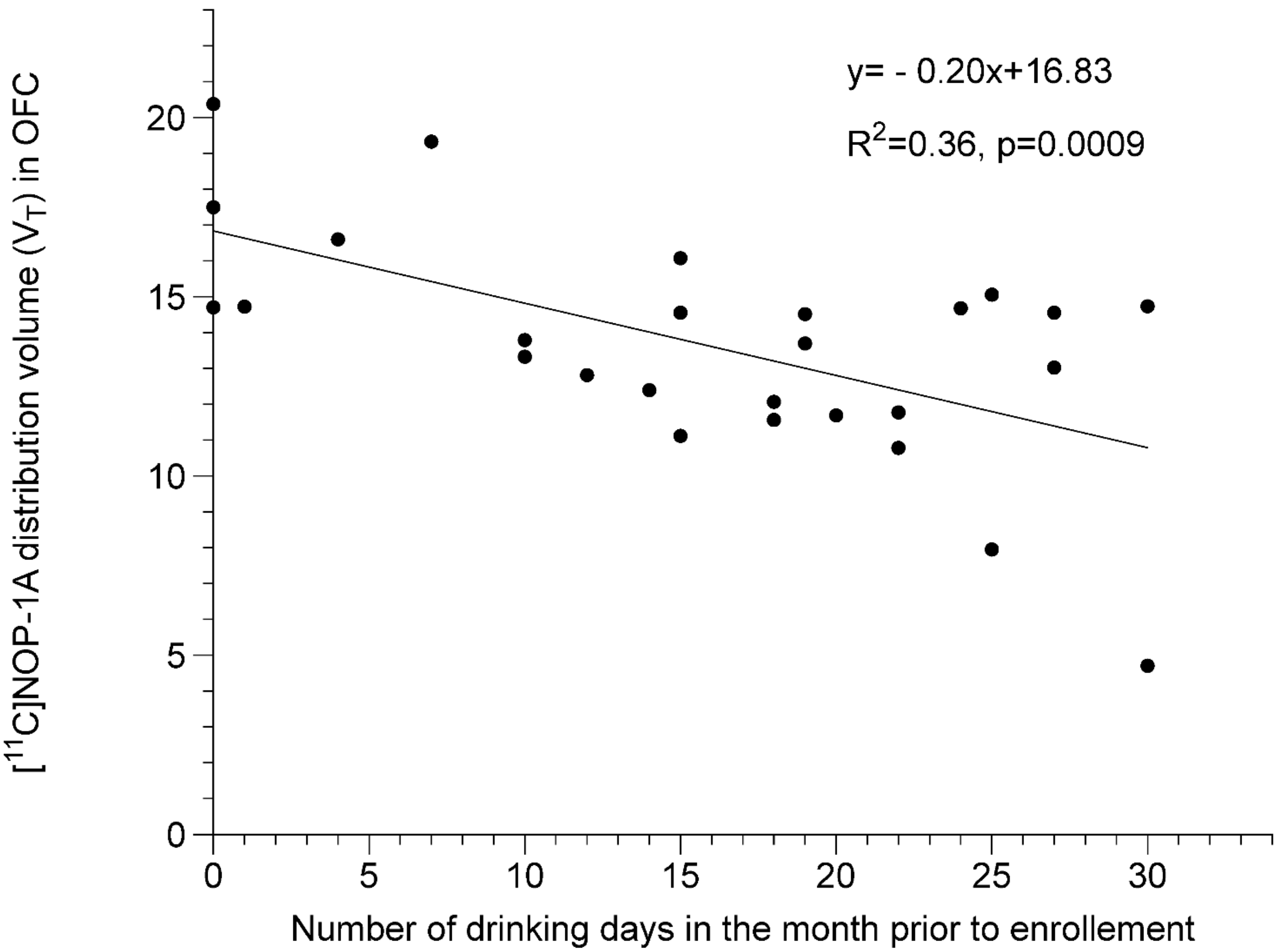
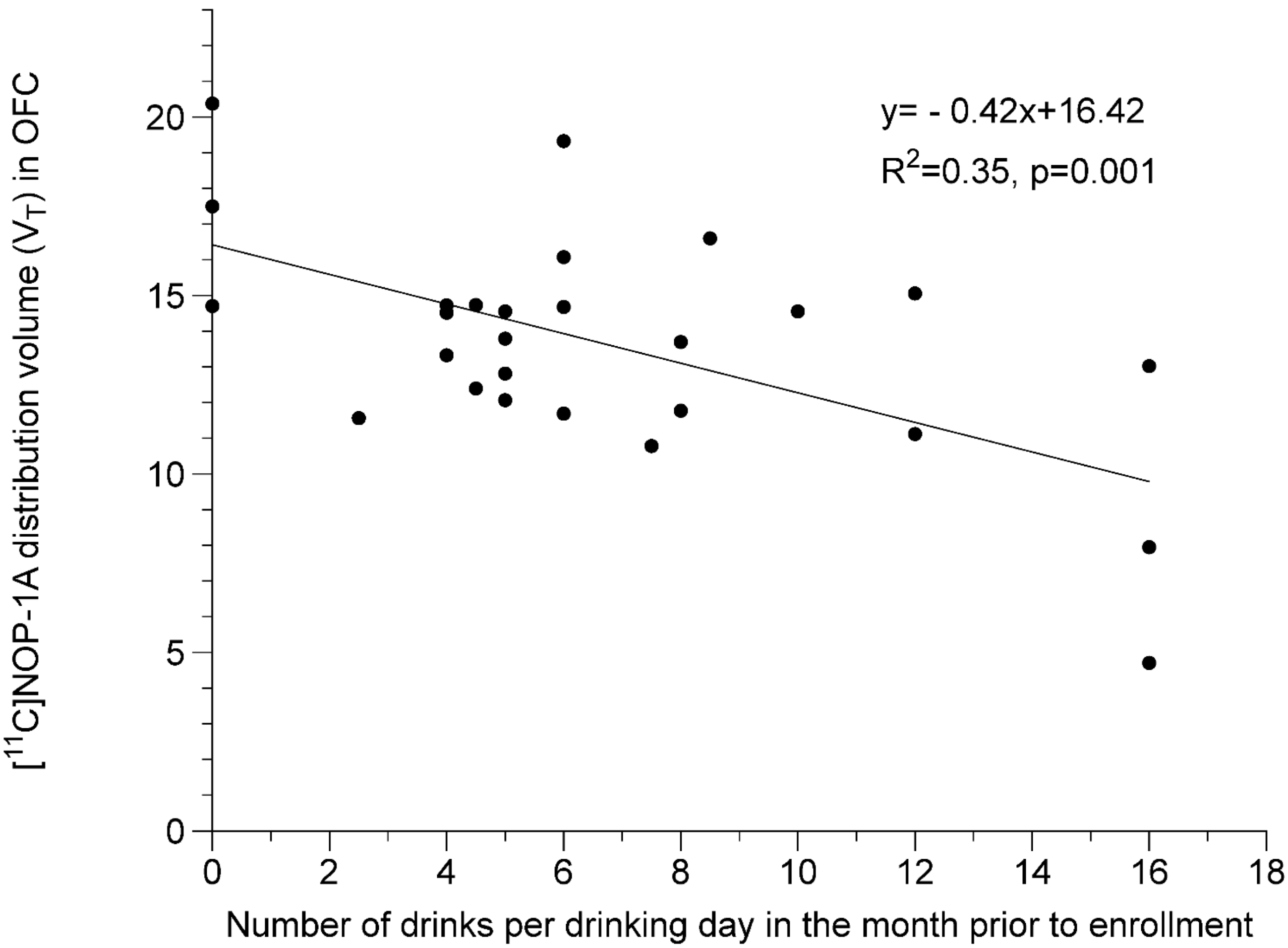


Figure S3 shows the negative association between the mean number of drinks consumed per drinking day in the month prior to enrollment and the orbitofrontal cortex V_T in n=27 AUD subjects.



Supplement References

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