Psychopharmacology

Electronic Supplementary Material 1

Orbitofrontal and caudate volumes in cannabis users: a multi-site mega-analysis comparing dependent versus non-dependent users

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Assessment instrument by imaging site

Differences in assessment instruments by imaging site are presented in Supplementary Table 1 below. Participants from each site were scanned on different scanners. Particularly, all participants except those from Barcelona were scanned on 3T scanners (Barcelona = 1.5T), which may have resulted in the observed site difference wherein ROI volumes from participants in Barcelona are significantly smaller (see Supplementary Tables 2 and 3). While cannabis use across the sites were quantified in either of joints, grams, or cones, units were converted to cones prior to analysis (https://ncpic.org.au/static/pdfs/ assessment-tools/timeline-followback.pdf).

Supplementary Table 1 Assessment instruments by imaging site

Participants		*Amsterdam* *N = 76*	*Barcelona* *N = 55*	*Wollongong* *N = 30*	*Melbourne* *N = 100*
Inclusion criteria	CB group	>/= 10 days/mth, >/= 240 days over last 2 yrs	First use age < 16, 14-28 joints/week over last 2 yrs	>/= 3-4 times/wk	>/= 2 days/mth for at least 2 yrs
	HC group	< 50 joints/lifetime, did not use in last yr	< 15 times/lifetime, did not use in last mth	No lifetime history of regular CB use, did not use in last yr	</= 10 times/lifetime, did not use in last yr
Exclusion criteria	IQ	< 80	</= 90	-	< 80
	Axis I psychiatric disorders	Assessed via MINI, medical history	Assessed via PRISM, medical history	SCID (psychotic screening), medical history	SCID (depression and psychotic screening), medical history
	Illicit substance use except CB	> 25 times/lifetime	> 5 times/lifetime	-	> 50 times/past 10 yrs
	Urine test	Positive for alcohol and other illicit drugs except CB	Positive for other illicit drugs except CB	Positive for other illicit drugs except CB	Positive for other illicit drugs except CB
	Others	General MRI contraindication, use of psychoactive medication	Left-handed, female, general MRI contraindication, use of psychoactive medication	Left-handed, general MRI contraindication, use of psychoactive medication	General MRI contraindication, use of psychoactive medication

Scanner	Model	Phillips Intera	General Electic Signa Excite	Phillips Intera	Siemens Team Trio
	Strength	3T	1.5T	3T	3T
Measures		Amsterdam	Barcelona	Wollongong	Melbourne
IQ		Dutch version of NART (DART)	WAIS-III vocabulary subscore [a]	NART	WASI
Alcohol use		StDr/mth, AUDIT	Age first drink, StDr/wk	StDr/wk	Age first drink, Age regular drink, StDr/mth, AUDIT
Tobacco use		Age regular use, cig/day, cig/mth	Age first use, age regular use, cig/day, cig/mth	Age regular use, cig/day, cig/mth (extrapolated from cig/day)	Age first use, age regular use, cig/day, cig/mth
Cannabis use		*Amsterdam* *N = 33*	*Barcelona* *N = 29*	*Wollongong* *N = 15*	*Melbourne* *N = 63*
Onset first use		Yes	Yes	Yes	Yes
Onset regular use (criteria)		> weekly use	>/= 4 times/wk	>/= 2 times/mth	>/= 2 times/mth
Frequency		Days/wk	-	Days/mth	Days/mth
Quantity [b]	Current use	Grams/wk	Joints/mth	Cones/mth	Cones/mth
	Lifetime use	Self report estimate	Extrapolated from onset age and use	Self report estimate, including any heavier or lighter use period	Self report estimate, including any heavier or lighter use period
Dependence		MINI – score >/= 3 as dependent	SDS – score >/= 4 as dependent	-	SDS – score >/= 4 as dependent

[a] WAIS-III vocabulary subscale converted to an estimated full scale IQ by standardizing to the average IQ of CB users and HC respectively of the three other sites.

[b] CB use quantity (joints or grams) was converted to cones (https://ncpic.org.au/static/pdfs/ assessment-tools/timeline-followback.pdf).

*CB = cannabis, HC = healthy controls; wk = week, mth = month, yrs = years, MINI = Mini Neuropsychiatry International Interview (Lecrubier et al. 1997; Sheehan et al. 1997; Swift et al. 1998), PRISM = Psychiatric Research Interview for Substance and Mental Disorders (http://www.columbia.edu/~dsh2/prism/, Hasin et al. 1996), SCID = Structured Clinical Interview for DSM Disorders (Spitzer et al. 1994; First et al. 2001), DART = Dutch version of the National Adult Reading Test (Schmand et al. 1991), WAIS-III = Wechsler Adult Intelligence Scale – Third Edition (Wechsler 1997), NART = National Adult Reading Test (Nelson 1982), WASI = Wechsler Abbreviated Scale of Intelligence (Wechsler 1999), StDr = standard drinks, AUDIT = Alcohol Use Disorder Identification Test (Saunders et al. 1993), cig = cigarette, SDS = Severity of Dependence Scale (Gossop et al. 1995; van der Pol et al. 2013).

Demographic and ROI volumes for HC and CB by imaging site

Demographic and substance use characteristics of participants by imaging site, along with any significant site effects, are presented in Supplementary Table 2. Amsterdam and Barcelona had the youngest samples (all male in Barcelona) and used significantly less cannabis (monthly and lifetime), followed by Melbourne, and then Wollongong. Participants from Wollongong meanwhile have the highest IQ, and used the most alcohol and cigarettes of all sites. CB users from Melbourne started using CB at a significantly younger age than those from Amsterdam and Wollongong. The only significant site by group effects in terms of demographic were found for IQ (p = .023) and monthly cigarette uss (p = .002). CB users had a significantly lower IQ than HC in Barcelona (p = .023) and Melbourne (p < .001) samples only. Meanwhile, HC from all four sites used fewer cigarettes than CB users, but did not differ between each other in monthly cigarette use; while CB users from Wollongong smoked the most cigarettes, followed by CB users from Melbourne and Amsterdam, and finally Barcelona.

Site effect for the ROI comparisons from the main analysis are reported in Supplementary Table 2 as well. Participants from Barcelona had the smallest OFC (both medial and lateral), while participants from Amsterdam had the largest caudate. The larger ROI volume in Amsterdam likely reflects the younger age of participants (Walhovd et al. 2005), while the smaller ROI volume in Barcelona may represent differences in scanner field strength (higher scanner field strength is associated with larger volume (Han et al. 2006)). However, no site by group interaction effect was found, suggesting that site effect did not skew or mask any HC vs. CB effects.

Supplementary Table 2 Demographic, substance use characteristics, and ROI volumes of healthy controls (HC) and cannabis (CB) users by imaging site (Mean (SD); ROI volumes in mm^3)

	HC				*CB*				*Site Effect*	
	Amsterdam N = 43	*Barcelona N = 26*	*Wollongong N = 15*	*Melbourne N = 37*	*Amsterdam N = 33*	*Barcelona N = 29*	*Wollongong N = 15*	*Melbourne N = 63*	*F/X²*	*p*
Age	21.98 (2.46)	22.57 (3.36)	35.00 (10.08)	29.95 (11.29)	21.32 (2.39)	21.03 (2.34)	38.99 (9.24)	32.67 (11.07)	49.88	<.001; Amst < Melb***, Amst < Woll***, Barc < Melb***, Barc < Woll***, Melb < Woll***[a]
Gender (% M / F)	62.79 / 37.21	100 / 0	93.33 / 6.67	48.65 / 51.35	66.67 / 33.33	100 / 0	93.33 / 6.67	46.03 / 53.97	55.94	<.001

IQ		104.86 (7.25)	109.26 (10.47)	113.45 (8.07)	112.32 (12.72)	104.18 (5.44)	103.58 (10.71)	109.37 (6.16)	101.83 (12.96)	3.22	.023; Amst < Woll**, Barc < Woll*, Melb < Woll**
Alcohol (StDr/mth)[a]		21.44 (26.76)	12.43 (10.52)	21.79 (5.63)	26.72 (4.39)	23.64 (23.85)	21.14 (15.38)	26.37 (6.81)	28.06 (3.51)	3.28	.022; Amst < Woll*, Barc < Woll**, Melb < Woll*
Tobacco (Cig/mth)[a]		41.73 (106.41)	24.22 (103.97)	100.20 (23.62)	77.77 (12.79)	211.99 (218.91)	168.41 (152.35)	274.73 (68.68)	233.14 (29.14)	4.34	.005; Amst < Woll**, Barc < Woll**, Barc < Melb*
Cannabis Use											
Onset Regular Use (years)		-	-	-	-	18.85 (2.26)	18.12 (2.05)	20.14 (5.25)	16.72 (3.27)	5.81	.001; Melb < Amst**, Melb < Woll**
Current Use (cones/month)		-	-	-	-	158.51 (115.95)	224.13 (138.20)	634.13 (545.45)	404.97 (308.94)	12.33	<.001; Amst < Melb***, Amst < Woll***, Barc < Melb*, Barc < Woll***, Melb < Woll**
Lifetime Use (cones)		-	-	-	-	4,738.64 (4,275.06)	15,611.28 (12,577.01)	177,772.50 (205,672.08)	74,579.21 (75,833.71)	26.53	<.001; Amst < Melb***, Amst < Woll***, Barc < Melb**, Barc < Woll***, Melb < Woll***
Intracranial Cavity (10^6)		1.44 (0.22)	1.62 (0.12)	1.72 (0.15)	1.56 (0.15)	1.36 (0.18)	1.59 (0.13)	1.65 (0.10)	1.54 (0.14)		
Lateral OFC	Left	8238.56 (905.83)	7691.00 (819.29)	8771.20 (882.19)	7858.86 (871.29)	8273.09 (1012.49)	7728.59 (877.69)	8031.00 (905.51)	7644.71 (844.76)	12.44[b]	<.001; Barc < Amst***, Barc < Woll*, Barc < Melb**, Melb < Amst*
	Right	7191.09 (902.04)	8031.69 (731.90)	8751.33 (1083.36)	7757.41 (815.22)	6839.64 (978.39)	7706.69 (925.15)	7926.07 (849.01)	7518.59 (921.74)		
Medial OFC	Left	4712.93 (611.28)	5502.23 (604.62)	5921.67 (803.90)	5375.95 (607.90)	4570.42 (692.96)	5496.90 (777.61)	5700.47 (594.56)	5106.84 (744.48)	5.85[b]	.001; Barc < Amst**; Barc < Melb*

	Right	5782.37	5295.23	6091.73	5397.16	5364.55	5385.93	5504.80	5132.95		
		(600.14)	(535.52)	(797.08)	(518.76)	(687.07)	(751.12)	(725.33)	(652.93)		
Caudate	Left	3977.13	3868.95	4303.71	3817.29	3793.47	3893.28	3880.48	3676.41	18.68 [b]	<.001; Barc < Amst***, Melb < Amst***, Woll < Amst**
		(405.45)	(502.19)	(569.12)	(488.19)	(473.73)	(558.45)	(608.80)	(577.91)		
	Right	4332.80	4077.08	4237.88	3720.92	4135.25	4087.04	3781.87	3588.19		
		(459.78)	(547.87)	(634.89)	(469.49)	(503.70)	(613.56)	(565.86)	(611.47)		

[a] StDr/mth = standard drinks per month, Cig/mth = cigarettes smoked per month, Amst = Amsterdam, Barc = Barcelona, Melb = Melbourne, Woll = Wollongong

[b] repeated-measures analysis of covariance (ANCOVA) – hemisphere = within subjects measure, imaging site, gender, group = between-subject factors, age, IQ, monthly alcohol and tobacco use = covariates – as per main analysis.

* $p < .05$, ** $p < .01$, *** $p < .001$

Demographic and ROI volumes for CB-nondep and CB-dep by imaging site

Demographic, substance use characteristics, and ROI volumes of CB-nondep and CB-dep group by imaging site, along with any significant site effects, are presented in Supplementary Table 3. Similar to the previous table, Amsterdam and Barcelona had younger participants who used significantly less cannabis (monthly and lifetime) than Melbourne. CB users from Melbourne also started using cannabis at a significantly younger age than CB users from Amsterdam. The only significant site by dependence group interaction effect was found for monthly CB use ($p = .009$), whereby CB-nondep users from all sites, and CB-dep users from Amsterdam and Barcelona did not differ in monthly CB use, but CB-dep users from Melbourne used significantly more cannabis per month than all other groups and sites.

Site effect for the ROI comparisons from the main analysis are reported in Supplementary Table 3 as well. Participants from Barcelona had a smaller lateral OFC than participants from Amsterdam. While the primarily larger volume of CB-nondep users from Amsterdam (compared to every other group) may have driven the dependence-related effect on lateral OFC volume, there was no site by dependence interaction effect. There was no significant site, or site by dependence interaction effect on medial OFC volume. The trend of CB-dep users having smaller medial OFC than CB-nondep users was present in all sites (when analysed separately), suggesting that the smaller medial OFC in CB-dep users in the main analysis is robust across sites. Finally, participants from Amsterdam had a larger caudate than participants from Barcelona and Melbourne. There is also a site by dependence interaction effect for caudate volume ($p = .047$), with CB-nondep Amsterdam users having the largest caudate.

Supplementary Table 3 Demographic, substance use characteristics, and ROI volumes of non-dependent (CB-nondep) and dependent (CB-dep) cannabis users by imaging site (Mean (SD); ROI volumes in mm^3)

	CB-nondep			*CB-dep*			*Site effect*	
	Amsterdam N = 21	*Barcelona N = 7*	*Melbourne N = 22*	*Amsterdam N = 12*	*Barcelona N = 18*	*Melbourne N = 40*	F/X^2	*p*
Age	20.87 (2.16)	20.14 (1.35)	36.04 (11.51)	22.10 (2.66)	20.78 (2.16)	30.83 (10.25)	31.58	<.001; Amst < Melb***, Barc < Melb***[a]
Gender (% M / F)	61.90 / 38.10	100 / 0	45.45 / 54.55	75.00 / 25.00	100 / 0	45.00 / 55.00	23.20	<.001
IQ	103.10 (4.70)	98.95 (10.33)	104.48 (14.86)	106.08 (6.30)	104.21 (10.79)	100.00 (11.63)	0.64	.528
Alcohol (StDr/mth)[a]	21.05 (24.48)	27.14 (26.28)	20.22 (26.10)	28.17 (23.02)	17.94 (9.85)	21.76 (26.59)	0.15	.859
Tobacco (Cig/mth)[a]	202.05 (207.92)	131.43 (82.55)	298.43 (299.15)	229.39 (245.52)	158.56 (178.88)	244.34 (189.20)	2.58	.080
Cannabis Use								
Onset Regular Use (years)	18.54 (1.96)	17.43 (1.99)	17.26 (3.22)	19.39 (2.72)	18.33 (2.06)	16.53 (3.42)	6.06	.003; Melb < Amst**
Current Use (cones/month)	177.03 (125.56)	286.00 (220.90)	255.87 (244.34)	126.10 (92.96)	201.91 (98.25)	479.20 (312.64)	12.32	<.001; Amst < Melb***, Barc < Melb**
Lifetime Use (cones)	4350.00 (4038.32)	12,534.00 (8,406.45)	69,214.13 (58,145.03)	5418.75 (4766.87)	18,004.17 (14,755.26)	78,527.13 (85,791.12)	20.23	<.001; Amst < Melb***, Barc < Melb***
Intracranial Cavity (10^6)	1.35 (0.20)	1.62 (0.13)	1.51 (0.14)	1.38 (0.16)	1.58 (0.14)	1.56 (0.13)		
Lateral Left	8426.19	7687.43	7568.86	8005.17	7699.33	7633.20	7.96[b]	.001; Barc < Amst**

OFC		(1115.85)	(848.72)	(758.93)	(771.57)	(916.47)	(839.46)		
	Right	6980.86	7884.43	7365.27	6592.50	7615.39	7570.43		
		(1006.88)	(855.05)	(744.94)	(975.15)	(991.34)	(996.26)		
Medial	Left	4660.05	5602.86	5174.09	4413.58	5367.22	5052.63	1.32 [b]	.271
OFC		(720.33)	(563.20)	(816.76)	(641.39)	(901.95)	(695.87)		
	Right	5435.10	5479.14	5321.32	5241.08	5266.39	5013.23		
		(791.09)	(704.24)	(718.69)	(457.49)	(767.34)	(595.17)		
Caudate	Left	3854.74	3883.84	3519.06	3686.24	3842.11	3771.25	6.31 [b]	.003; Barc < Amst*, Melb <
		(478.93)	(459.47)	(526.95)	(464.87)	(570.97)	(596.16)		Amst**
	Right	4205.93	4049.37	3360.55	4011.54	4064.21	3728.42		
		(523.80)	(576.73)	(565.50)	(461.51)	(632.87)	(602.94)		

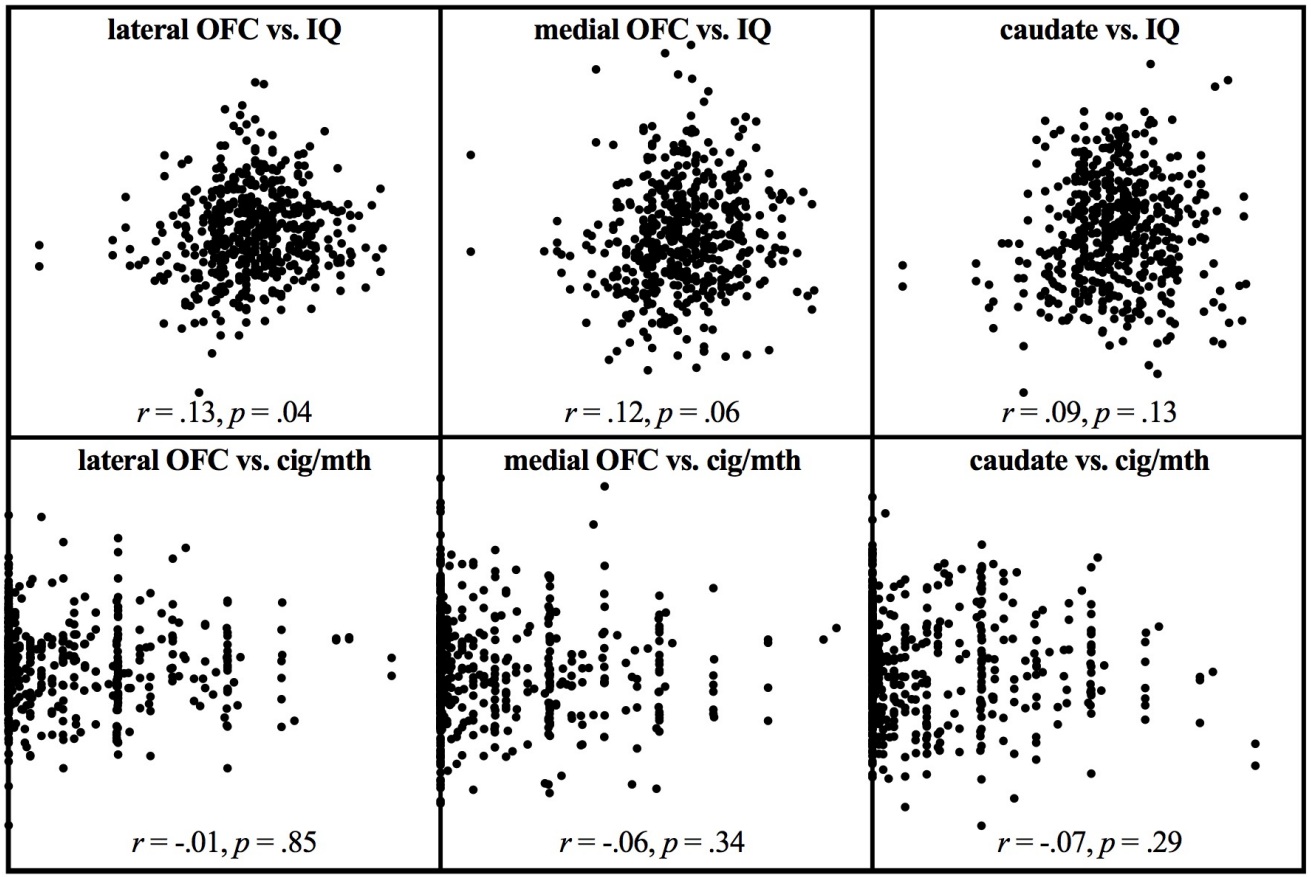
[a] StDr/mth = standard drinks per month, Cig/mth = cigarettes smoked per month, Amst = Amsterdam, Barc = Barcelona, Melb = Melbourne

[b] repeated-measures analysis of covariance (ANCOVA) – hemisphere = within subjects measure, imaging site, gender, group = between-subject factors, age, IQ, monthly alcohol and tobacco use = covariates – as per main analysis.

* $p < .05$, ** $p < .01$, *** $p < .001$

Correlation of ROI against IQ, monthly tobacco use

Preliminary correlations between ROI (lateral OFC, medial OFC, caudate) volumes and IQ in all sites combined (range of $r = .09 – 13$, $p \geq .04$, Supplementary Fig. 1) suggest they were not associated after correcting for multiple comparison (critical value = .027, Benjamini and Yekutieli's modified FDR method (Benjamini and Yekutieli 2001). Similarly, when correlations were split across individual sites, IQ was not correlated with any of the ROI volumes (range of $r = -.13 – 29$, $p \geq .08$). Monthly cigarette use was also not correlated with ROI volumes, whether in all sites combined (range of r = -.07 – -.01, p ≥ .29, Supplementary Fig. 1), or in individual sites (range of $r = -.26 – .03$, $p \geq .14$).



Supplementary Fig. 1. Scatterplot of correlation of regions of interest, ROI volumes (lateral orbitofrontal cortex (OFC), medial OFC, caudate) against IQ and monthly cigarette use (cig/mth). Volumes are averaged across hemispheres, adjusted for intracranial volume and age. Critical value after FDR correction = .027 (Benjamini and Yekutieli 2001).

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