

# Supplementary Materials

## The role of sex in the association between cannabis use and working memory related brain activity

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**Data Accessibility:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**Study 1**

A total of 25 cannabis users and 24 controls between 18 and 25 years old participated in the study. The data included was collected during a 3-year follow-up session of the original project and inclusion criteria described applied on the baseline session. Cannabis users were included if they used cannabis a minimum of 10 times per month for at least the previous 18 months, while controls were not allowed to have used cannabis over 50 times in their life and not during the last year. Exclusion criteria were substance use other than cannabis over a hundred times, excessive alcohol use, smoking over 20 cigarettes a day, history of major psychological or medical problems. Included participants were requested to abstain from using drugs or alcohol 24 hours before the start of the session. A urine screening was conducted to assess recent drug use and all that tested positive for a drug other than THC in the cannabis group (i.e. alcohol, amphetamines, benzodiazepines, cocaine, or opiates) were excluded.

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**Study 2**

A total of 34 cannabis users and 31 controls between 18 and 25 years old participated in the study. Cannabis users were included if they used cannabis a minimum of 10 times per month for at least the last 2 years, while controls were not allowed to have used cannabis over 50 times in their life and not during the last year. Exclusion criteria were substance use other than cannabis over a hundred times, excessive alcohol use, smoking over 20 cigarettes a day, current use of prescription or illicit psychoactive drugs besides cannabis, history of major psychological or medical problems, leaving school before age 16, and treatment for cannabis use disorder. Included participants were requested to abstain from using drugs or alcohol 24 hours before the start of the session. A urine screening was conducted to assess recent drug use and all that tested positive for a drug other than THC in the cannabis group (i.e. amphetamines, benzodiazepines, cocaine, methamphetamines, or opiates) were excluded.

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**Study 3**

A total of 45 cannabis users and 30 controls between 18 and 30 years old participated in the study. Cannabis users were included if they used cannabis a minimum of 6 times a week for at least the past year, while controls were not allowed to have used cannabis over 25 times in their life and not more than 5 times during the last year. Exclusion criteria were regular use of substances other than cannabis, excessive alcohol use, current use of prescription or illicit psychoactive drugs besides cannabis, history of major psychological or medical problems, and treatment for cannabis use disorder. Included participants were requested to abstain from using drugs or alcohol 24 hours before the start of the session. A urine screening was conducted to assess recent drug use and all that tested positive for a drug other than THC in the cannabis group (i.e. amphetamines, barbiturates, benzodiazepines, buprenorphine, cocaine, ecstasy (MDMA), methamphetamines, methadone, morphine/opiates, phencyclidine (PCP), oxycodone) were excluded.

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**Figure S1. Study specific information and exclusion criteria.**

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Table S1 Overview of linear mixed model results assessing the effect of group, sex and their interaction on the included continuous outcome variables						
Model	Model coefficients					
	Fixed effects					Random effects
Measure	<i>B</i>	95% <i>CI</i> ( <i>B</i> )	<i>SE</i> ( <i>B</i> )	<i>t</i>	<i>p</i>	<i>SD</i>
<b>Age</b>						
Intercept	21.74	20.76 – 22.71	0.50	43.48	<0.001	1.72
Group	0.84	-0.52 – 2.20	0.70	1.20	0.23	
Sex	0.49	-0.73 – 1.72	0.63	0.78	0.44	
Group x Sex	-0.69	-2.48 – 1.10	0.92	-0.75	0.45	-
<b>Estimated IQ<sup>1</sup></b>						
Intercept	-0.21	-0.52 – 0.10	0.16	-1.31	0.19	0.55
Group	0.27	-0.16 – 0.71	0.22	1.22	0.22	
Sex	0.08	-0.31 – 0.47	0.20	0.41	0.68	
Group x Sex	0.16	-0.41 – 0.73	0.29	0.54	0.59	-
<b>Depression (BDI)</b>						
Intercept	8.24	6.08 – 10.40	1.11	7.44	<0.001	3.81
Group	-2.39	-5.40 – 0.63	1.55	-1.54	0.12	
Sex	0.16	-2.55 – 2.87	1.39	0.11	0.91	
Group x Sex	-1.65	-5.62 – 2.31	2.03	-0.81	0.42	-
<b>State Anxiety (STAI)<sup>2</sup></b>						
Intercept	34.26	31.45 – 37.06	1.44	23.80	<0.001	4.46
Group	-1.52	-5.53 – 2.48	2.05	-0.74	0.46	
Sex	-1.34	-4.94 – 2.26	1.85	-0.73	0.47	
Group x Sex	-0.30	-5.68 – 5.09	2.76	-0.11	0.92	-
<b>Trait Anxiety (STAI)<sup>2</sup></b>						
Intercept	38.97	35.87 – 42.07	1.59	24.52	<0.001	4.92
Group	-4.87	-9.29 – -0.45	2.27	-2.15	<b>0.03</b>	
Sex	-2.10	-6.09 – 1.90	2.05	-1.02	0.31	
Group x Sex	1.29	-4.67 – 7.24	3.06	0.42	0.67	-
<b>Alcohol use and related problems (AUDIT)</b>						
Intercept	5.42	4.32 – 6.52	0.57	9.59	<0.001	1.95
Group	-0.85	-2.39 – 0.70	0.79	-1.07	0.29	
Sex	1.38	-0.00 – 2.77	0.71	1.95	0.05	
Group x Sex	0.04	-1.98 – 2.07	1.04	0.04	0.97	-

<b>Nicotine Dependence (FTND)</b>						
Intercept	2.43	1.65 – 3.20	0.40	6.09	<0.001	1.17
Group	-0.90	-2.16 – 0.36	0.65	-1.39	0.17	
Sex	-0.29	-1.29 – 0.71	0.51	-0.56	0.57	
Group x Sex	-0.14	-1.67 – 1.95	0.93	-0.16	0.88	
<b>Cigarettes/Day</b>						
Intercept	10.15	7.62 – 12.68	1.31	7.78	<0.001	3.21
Group	-2.36	-6.49 – 1.78	2.13	-1.11	0.27	
Sex	-1.45	-4.64 – 1.74	1.65	-0.88	0.38	
Group x Sex	1.86	-3.94 – 7.67	2.99	0.62	0.54	
<b>Other substance use</b>						
Intercept	24.68	12.04 – 37.32	6.48	3.81	<0.001	22.29
Group	-21.63	-39.29 – -3.98	9.04	-2.39	<b>0.02</b>	
Sex	10.85	-5.02 – 26.71	8.13	1.33	0.18	
Group x Sex	-8.83	-32.04 – 14.38	11.89	-0.74	0.46	
<b>Cannabis use and related problems (CUDIT-R)</b>						
Intercept	13.68	11.80 – 15.57	0.96	14.23	<0.001	3.31
Sex	-0.20	-2.57 – 2.17	1.21	-0.17	0.87	
<b>CUD symptoms – Study 2<sup>3</sup></b>						
Intercept	5.60	4.46 – 6.74	0.58	9.67	<0.001	1.24
Sex	-0.50	-1.90 – 0.90	0.71	-0.70	0.48	
<b>CUD symptoms – Study 3<sup>3</sup></b>						
Intercept	3.38	2.57 – 4.18	0.41	8.32	<0.001	0.89
Sex	0.18	-0.92 – 1.28	0.56	0.32	0.75	
<b>Gram/Week</b>						
Intercept	3.30	1.92 – 4.68	0.70	4.69	<0.001	2.43
Sex	1.69	-0.05 – 3.44	-0.89	1.91	0.06	
<b>Age of Onset</b>						
Intercept	15.47	14.91 – 16.03	0.29	54.26	<0.001	0.98
Sex	-0.40	-1.10 – 0.31	0.36	-1.11	0.27	
<b>Days Since Last Use</b>						
Intercept	18.76	-9.85 – 47.36	14.56	1.29	0.20	49.48
Sex	-3.51	-39.34 – 32.32	18.24	-0.19	0.85	

Note: AUDIT: alcohol use disorder identification test; BDI: Beck's depression inventory; CUD: cannabis use disorder; CUDIT-R: cannabis use disorder identification test; FTND: Fagerström test for nicotine dependence; STAI: state trait anxiety inventory; <sup>1</sup> Using standardized (Z) scores to compare studies; <sup>2</sup> STAI State & STAI Trait only assessed in study 2 and 3; <sup>3</sup> CUD scores separate for study 2 (SCID) and 3 (MINI) due to different measures used to assess DSM-5 CUD symptoms, study 1 did not assess CUD; Significant results are presented in bold.

**Table S2 Overview of model selection to assess accuracy during the N-back task as a function of working memory (WM)-load, group, sex and their interaction**

Model		Model coefficients					Model comparison			
		Fixed effects			Random effects		AIC	$\chi^2$	p	
Accuracy		B	95% CI (B)	SE (B)	t	p				SD
BM	(Intercept)	94.42	93.93 – 94.90	0.25	381.92	0.00	4.74	3379.58	-	-
1	(Intercept)	96.32	95.30 – 97.34	0.52	184.64	<0.001	2.36	3221.90	165.67	<.001
	WM-load: 0-back - 1-back	-1.97	-2.80 – -1.15	0.42	-4.69	<0.001	3.74			
	WM-load: 0-back - 2-back	-5.88	-6.71 – -5.06	0.42	-13.97	<0.001	-			
	Group	0.53	-0.45 – 1.50	0.50	1.06	0.289	-			
	Sex	0.80	-0.19 – 1.78	0.50	1.58	0.115	-			
2	(Intercept)	96.58	95.48 – 97.69	0.57	170.85	<0.001	2.38	3217.01	8.90	.01
	WM-load: 0-back - 1-back	-1.85	-2.95 – -0.75	0.56	-3.28	0.001	3.69			
	WM-load: 0-back - 2-back	-6.80	-7.90 – -5.70	0.56	-12.04	<0.001	-			
	Group	-0.05	-1.40 – 1.31	0.69	-0.07	0.95	-			
	Sex	0.79	-0.19 – 1.78	0.50	1.58	0.12	-			
	WM-load: 0-back - 1-back x Group	-0.28	-1.91 – 1.35	0.84	-0.34	0.74	-			
	WM-load: 0-back - 2-back x Group	2.01	0.37 – 3.64	0.84	2.40	0.02	-			
3	(Intercept)	96.84	95.58 – 98.10	0.65	149.48	<0.001	2.39	3220.34	.67	.72
	WM-load: 0-back - 1-back	-2.23	-3.76 – -.70	0.78	-2.85	0.01	3.67			
	WM-load: 0-back - 2-back	-7.18	-8.71 – -5.65	0.78	-9.15	<0.001	-			
	Group	-0.09	-1.45 – 1.27	0.70	-0.13	0.90	-			
	Sex	0.39	-0.98 – 1.77	0.70	0.56	0.58	-			
	WM-load: 0-back - 1-back x Group	-0.21	-1.86 – 1.43	0.84	-0.24	0.80	-			
	WM-load: 0-back - 2-back x Group	2.07	0.43 – 3.72	0.84	2.46	0.01	-			
	WM-load: 0-back - 1-back x Sex	0.60	-1.06 – 2.27	0.85	0.71	0.48	-			
	WM-load: 0-back - 2-back x Sex	0.59	-1.07 – 1.26	0.85	0.70	0.49	-			
4	(Intercept)	96.55	95.15 – 97.94	0.72	134.88	<0.001	2.38	3221.42	.92	.34
	WM-load: 0-back - 1-back	-2.23	-3.76 – -.70	0.79	-2.84	0.01	3.69			
	WM-load: 0-back - 2-back	-7.18	-8.71 – -5.65	0.79	-9.14	<0.001	-			
	Group	0.47	-0.31 – 2.25	0.91	0.51	0.60	-			
	Sex	0.85	-0.81 – 2.51	0.85	1.00	0.32	-			
	WM-load: 0-back - 1-back x Group	-0.21	-1.86 – 1.43	0.84	-0.25	0.80	-			
	WM-load: 0-back - 2-back x Group	2.07	0.43 – 3.72	0.84	2.46	0.02	-			
	WM-load: 0-back - 1-back x Sex	0.60	-1.06 – 2.27	0.85	0.71	0.48	-			
	WM-load: 0-back - 2-back x Sex	0.59	-1.07 – 2.26	0.85	0.70	0.49	-			
	Group x Sex	-0.96	-2.93 – 1.01	1.01	-0.95	0.34	-			

<b>5</b>	(Intercept)	96.84	95.34 – 98.35	0.77	125.02	<0.001	2.39	3220.68	4.74	.09
	WM-load: 0-back - 1-back	-2.18	-4.00 – -0.36	0.94	-2.33	<b>0.02</b>	3.66			
	WM-load: 0-back - 2-back	-8.11	-9.93 – -6.29	0.94	-8.67	<b>&lt;0.001</b>				
	Group	-0.09	-2.19 – 2.00	1.07	-0.09	0.93	-			
	Sex	0.39	-1.50 – 2.28	0.97	0.40	0.69	-			
	WM-load: 0-back - 1-back x Group	-0.32	-2.85 – 1.10	1.30	-0.25	0.80	-			
	WM-load: 0-back - 2-back x Group	3.87	1.34 – 6.39	1.20	2.98	<b>0.00</b>	-			
	WM-load: 0-back - 1-back x Sex	0.52	-1.75 – 2.79	1.17	0.44	0.66	-			
	WM-load: 0-back - 2-back x Sex	2.06	-0.22 – 4.34	1.17	1.76	0.08	-			
	Group x Sex	0.01	-2.74 – 2.76	1.41	0.01	1.00	-			
	WM-load: 0-back - 1-back x Group x Sex	0.18	-3.12 – 3.49	1.70	0.11	0.91	-			
	WM-load: 0-back - 2-back x Group x Sex	-3.09	-6.40 – 0.22	1.70	-1.82	0.07	-			

*Linear mixed model results using random intercept and maximum likelihood estimation; BM: baseline model; CI: Confidence Interval; SE: Standard Error; SD: Standard deviation; AIC: Akaike information criterion. Note: final models as presented in the manuscript are presented in italic and significant results are presented in bold.*

Table S3 Overview of model selection to assess reaction time during the N-back task as a function of working memory (WM)-load, group, sex and their interaction										
Model		Model coefficients						Model comparison		
		Fixed effects				Random effects				
Reaction Time		B	95% CI (B)	SE (B)	t	p	SD	AIC	$\chi^2$	P
BM	(Intercept)	549.31	535.67 – 562.96	6.94	79.11	0.00	90.22	6910.46	-	-
1	(Intercept)	492.12	465.28 – 518.96	13.71	35.89	<0.001	89.18	6593.68	324.78	<0.001
	WM-load: 0-back - 1-back	51.81	39.03 – 64.59	6.53	7.94	<0.001	56.25			
	WM-load: 0-back - 2-back	146.23	133.47 – 158.98	6.52	22.44	<0.001				
	Group	-4.53	-32.50 – 23.43	14.24	-0.02	0.75	-			
	Sex	-10.42	-38.69 – 17.84	14.39	-0.72	0.47	-			
2	(Intercept)	483.94	456.38 – 511.51	14.11	34.30	<0.001	89.10	6591.09	6.59	<b>0.04</b>
	WM-load: 0-back - 1-back	61.94	44.78 – 79.11	8.78	7.05	<0.001	55.78			
	WM-load: 0-back - 2-back	160.96	143.86 – 178.07	8.75	18.39	<0.001				
	Group	13.61	-17.89 – 45.10	16.07	0.85	0.40	-			
	Sex	-10.49	-38.70 – 17.72	14.39	-0.73	0.47	-			
	WM-load: 0-back - 1-back x Group	-22.35	-47.81 – 3.12	13.03	-1.71	0.09	-			
	WM-load: 0-back - 2-back x Group	-32.55	-57.98 – -7.13	13.01	-2.50	<b>0.01</b>	-			
3	(Intercept)	486.17	456.99 – 515.34	14.96	32.50	<0.001	89.15	6594.88	0.21	0.90
	WM-load: 0-back - 1-back	58.78	34.93 – 82.63	12.23	4.81	<0.001	55.75			
	WM-load: 0-back - 2-back	157.40	133.56 – 181.24	12.23	12.88	<0.001				
	Group	13.21	-18.33 – 44.76	16.12	0.82	0.41	-			
	Sex	-13.95	-45.85 – 17.95	16.30	-0.86	0.39	-			
	WM-load: 0-back - 1-back x Group	-21.77	-47.39 – 3.86	13.14	-1.66	0.10	-			
	WM-load: 0-back - 2-back x Group	-31.90	-57.49 – -6.30	13.12	-2.43	<b>0.02</b>	-			
	WM-load: 0-back - 1-back x Sex	4.92	-21.00 – 30.83	13.29	0.37	0.71	-			
	WM-load: 0-back - 2-back x Sex	5.54	-21.35 – 31.43	13.27	0.42	0.68	-			
4	(Intercept)	487.62	453.96 – 521.28	17.27	28.23	<0.001	89.14	6596.85	0.03	0.87
	WM-load: 0-back - 1-back	58.78	34.93 – 82.63	12.24	4.80	<0.001	55.75			
	WM-load: 0-back - 2-back	157.40	133.56 – 181.24	12.23	12.87	<0.001				
	Group	10.38	-35.10 – 55.86	23.26	0.45	0.66	-			
	Sex	-16.24	-57.70 – 25.23	21.21	-0.77	0.45	-			
	WM-load: 0-back - 1-back x Group	-21.76	-47.39 – 3.86	13.15	-1.65	0.10	-			
	WM-load: 0-back - 2-back x Group	-31.89	-57.48 – -6.30	13.13	-2.43	<b>0.02</b>	-			
	WM-load: 0-back - 1-back x Sex	4.92	-21.00 – 30.83	13.30	0.37	0.71	-			
	WM-load: 0-back - 2-back x Sex	5.54	-20.35 – 31.43	13.28	0.42	0.68	-			
	Group x Sex	4.89	-51.67 – 61.44	28.93	0.17	0.87	-			

5	(Intercept)	488.94	454.10 – 523.78	17.91	27.30	<0.001	89.18	6599.31	1.55	.46
	WM-load: 0-back - 1-back	61.54	33.04 – 90.05	14.65	4.20	<b>&lt;0.001</b>	55.62			
	WM-load: 0-back - 2-back	150.64	122.13 – 179.14	14.65	10.28	<b>&lt;0.001</b>				
	Group	7.85	-40.81 – 56.52	24.94	0.31	0.75	-			
	Sex	-18.29	-62.07 – 25.49	22.43	-0.82	0.42	-			
	WM-load: 0-back - 1-back x Group	-27.12	-66.53 – 12.29	20.26	-1.34	0.18	-			
	WM-load: 0-back - 2-back x Group	-18.91	-58.32 – 20.51	20.26	-0.93	0.35	-			
	WM-load: 0-back - 1-back x Sex	0.48	-35.18 – 36.13	18.33	0.03	0.98	-			
	WM-load: 0-back - 2-back x Sex	16.14	-19.45 – 51.72	18.30	0.88	0.38	-			
	Group x Sex	9.22	-54.67 – 73.11	32.74	0.28	0.78	-			
	WM-load: 0-back - 1-back x Group x Sex	9.41	-42.38 – 61.21	26.63	0.35	0.72	-			
	WM-load: 0-back - 2-back x Group x Sex	-22.45	-74.20 – 29.29	26.60	-0.84	0.40	-			
<p><i>Linear mixed model results using random intercept and maximum likelihood estimation; BM: baseline model; CI: Confidence Interval; SE: Standard Error; SD: Standard deviation; AIC: Akaike information criterion. <b>Note:</b> final models as presented in the manuscript are presented in italic and significant results are presented in bold.</i></p>										



**Table S4 Activation overview for the effect of WM and WM-load**

			MNI coordinates				
	Cluster size (voxels)	Brain regions	Hemisphere	X	Y	Z	Zmax
<b>WM</b>							
<b>2 &gt; 0</b>	30508	Insula	Right	34	22	0	14.40
		Paracingulate cortex	Right	6	20	46	13.40
		SFG	Left	-4	18	52	12.80
		MFG	Right	30	4	54	12.60
	14499	SMG	Right	38	-46	42	12.90
		Angular gyrus	Right	46	-48	50	12.40
		SMG	Left	-38	-48	42	12.10
		Angular gyrus	Left	-40	-54	48	11.70
	1274	MTG	Right	66	-32	-10	7.68
		ITG	Right	56	-44	-12	6.80
<b>0 &gt; 2</b>	43724	Precuneus	Left	-8	-54	18	13.70
		PCC	Left	-8	-54	24	12.90
		PCC	Right	2	-50	26	12.50
	479	Lateral occipital lobe	Left	-54	-68	34	7.22
		Angular gyrus	Left	-44	-60	28	4.33
<b>WM-load</b>							
<b>2 &gt; 1</b>	34408	Paracingulate gyrus	Left	-6	22	48	11.80
		Insula	Left	-36	22	-2	11.80
		Paracingulate gyrus	Right	8	24	40	11.70
		Insula	Right	-32	22	0	11.40
		Frontal pole	Right	38	50	16	11.10
	17425	Sup. Lateral occipital lobe	Right	32	-76	54	9.78
		Sup. Lateral occipital lobe	Left	-34	-60	42	9.23
		SMG	Right	42	-46	44	9.19
		SPL	Left	-34	-54	40	9.16
	896	MTG	Right	64	-46	-10	6.33
<b>1 &gt; 2</b>	1536	Central operculum	Left	-40	0	14	7.32
		Parietal operculum	Left	-42	-24	18	6.77
	1474	Parietal operculum	Right	54	-24	24	6.54
		Central operculum	Right	38	4	14	6.10
		Insula	Right	42	-12	20	5.77
	1016	ACC	Left	-12	34	-2	6.63
		Subcallosal area	Right	2	30	-2	6.18
		Subcallosal area	Left	-4	30	-2	5.90
		ACC	Right	10	36	2	4.70
		PCC	Left	-6	40	-10	4.32
	829	Precuneus	Left	-8	-52	18	5.38
	502	SMA	Right	6	-10	58	4.24
		SMA	Left	-2	-12	52	4.23
	479	PHG	Left	-20	-38	-12	5.78
		Hippocampus	Left	-26	-16	-14	5.08
		Pallidum	Left	-22	-8	-8	3.83
	314	CWM	Right	16	-30	26	5.23
	170	Precuneus	Right	16	-50	10	4.77
		PCC	Right	6	-50	24	3.78

MNI = Montreal Neurological Institute; MNI coordinates and Z-scores of separate local maxima for each cluster (whole-brain cluster-corrected at  $p < 0.05$ ,  $Z > 3.1$ ); 1 = 1-back, 2 = 2-back; ACC: anterior cingulate cortex, CWM: cerebral white matter, ITG: inferior temporal gyrus, MFG: medial frontal gyrus, MTG: medial temporal gyrus, PCC: posterior cingulate cortex, SFG: superior frontal gyrus, SMG: supramarginal gyrus, Sup: superior, PHG: parahippocampal gyrus.