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

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Abstract

Although cannabis use patterns differ between men and women, studies on sex differences on the effects of cannabis on the brain and cognitive control are largely lacking. Working memory (WM) is a component of cognitive control believed to be involved in the development and maintenance of addiction. In this study, we evaluated the association between cannabis use and WM (load) related brain activity in a large sample, enabling us to assess sex effects in this association. The brain activity of 104 frequent cannabis users (63% men) and 85 controls (53% men) was recorded during an N-back WM task. Behavioral results showed a significant interaction between WM load and group for both accuracy and reaction time, with cannabis users showing a relatively larger decrease in performance with increasing WM load. Cannabis users compared to controls showed a relatively smaller reduction in WM (load) related activity in the precuneus and posterior cingulate cortex at higher WM load. This WM (load) related activity was not associated with performance nor cannabis use and related problems. An exploratory analysis showed higher WM-related activity in the superior frontal gyrus in men compared to women. While cannabis users showed higher WM (load) related activity in central nodes of the default mode network, this was not directly attributable to group specific worsening of performance under higher cognitive load. Further research is necessary to assess whether observed group differences increase with higher cognitive load, how group differences relate to measures of cannabis use, and how sex affects these group differences.

1 | INTRODUCTION

Although gender and sex differences in cannabis use are well-documented with twice as many men using as women (UNODC, 2019), sex differences in the association between cannabis use and the brain are rarely investigated. Cannabis is the most used illicit drug worldwide with about 192 million users in 2018 (UNODC, 2020). Since both animal and human research is primarily conducted with male animals and men, we are largely uncertain about the effects of cannabis on the approximately 64 million women users every year.

As cannabis use among women is increasing, it is crucial to look into potential sex differences in the effects of cannabis (Colell et al., 2013). Research shows sex differences in the preferred route of administration (Cuttler et al., 2016), physiological effects of THC (Sholler et al., 2020), self-reported intoxication (Cuttler et al., 2016; Fogel et al., 2017; Matheson et al., 2020), and type of withdrawal

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symptoms (Cuttler et al., 2016; Schlienz et al., 2017). Also, comorbidities in individuals with a cannabis use disorder (CUD) differ between men and women (Bassir Nia et al., 2018; Khan et al., 2013) and women transition from first use to CUD faster (Khan et al., 2013), which could warrant different prevention and treatment approaches.

Differences in the development of CUD may be partially guided by biological sex differences in the endocannabinoid system (Bassir Nia et al., 2018; Calakos et al., 2017; Laurikainen et al., 2019). Although the direction of the effect is inconsistent and highly dependent on study design, CB1 receptor density and availability differ between males and females. For example, Laurikainen et al. (2019) found higher CB1 receptor availability in males, with higher availability associated with lower visuospatial working memory (WM) performance in both males and females (Laurikainen et al., 2019). Nevertheless, studies on sex differences in the association between cannabis and cognition are sparse and a sample bias toward men remains prominent in brain research.

Theories of addiction highlight the importance of cognitive control, including WM, in the development and maintenance of substance use disorders (Bickel et al., 2018). WM is the short-term memory storage that enables us to flexibly use, update, and manipulate information needed to make decisions, and is reliant on fronto-parietal brain activation (Owen et al., 2005). The N-back task is commonly used to assess WM related brain activity but results regarding the effects of cannabis therein are inconsistent. Hatchard et al. (2020) found increased activity in the right superior frontal gyrus (SFG) and temporal regions in cannabis users during a letter N-back task, but found no behavioral differences in performance (Hatchard et al., 2020). On the other hand, Owens et al. (2019) found a positive urine test for THC to negatively relate to performance on the N-back task (using picture stimuli). Also, task-related brain activation mediated the association between a positive test and task performance, while general measures of cannabis use were unrelated to performance and brain activity (Owens et al., 2019). These inconsistencies are also reflected in earlier research (Solowij & Battisti, 2008), in which some studies found associations between cannabis use and WM related brain activity (Kanayama et al., 2004; Padula et al., 2007; Schweinsburg et al., 2008) or connectivity (Ma et al., 2018), while others did not (Cousijn et al., 2014; Jager et al., 2006). In studies that do find an association, increased activity in WM related regions in cannabis users is often observed despite no performance difference (e.g., Cousijn et al., 2014; Hatchard et al., 2020; Jager et al., 2006). This increase in activation is commonly interpreted as a compensation mechanism indicative of increased effort to maintain performance in cannabis users.

A primary concern with previous fMRI WM studies is small sample sizes, with most lacking balanced and sizable samples to assess sex differences, which could partly explain inconsistent finding between studies. To our knowledge, there are currently no studies that investigated the role of sex in WM performance and related brain activity in cannabis users, while sex differences in fronto-parietal functioning could play a considerable role in the faster transition from use to abuse in women (Calakos et al., 2017; Khan et al., 2013). A recent study did assess the role of sex in neuropsychological functioning in cannabis users, showing that sex differences could be domain specific with women outperforming men on visual recognition, but the reverse being true for attention and executive functions including spatial working memory (Savulich et al., 2021). Furthermore, a study in cocaine users examined the effect of sex on the association between use and WM performance and related activity in the prefrontal cortex (PFC; Cousijn et al., 2021). While they found no effect of sex or group on WM performance, both sex and group moderated PFC activity. Specifically, cocaine using women showed more WM related middle frontal gyrus (MFG) activation than cocaine using men and non-drug using women showed less WM related MFG activation than non-drug using men. Also, WM related activity in multiple fronto-limbic areas was negatively associated with cocaine use in women only. These results are partially in line with an earlier neuroimaging meta-analysis suggesting women generally recruit more frontal and limbic structures during classic WM tasks (Hill et al., 2014), providing evidence of sex-dependent PFC alterations in substance users.

In the current study, we combined three data sets with identical N-back tasks allowing us to evaluate the association between cannabis use and WM related brain activity, with sufficient power to detect potential sex differences in this association. While we did not expect the employed N-back task to reveal behavioral differences between the cannabis and control group, nor between men and women, we expected cannabis users to show increased WM related activation in fronto-parietal regions compared to controls. This hypothesis is in line with suggested compensatory mechanisms of increased effort in cannabis users. Expectations regarding the role of sex in the association between cannabis use and WM-related activity are highly speculative. Based on limited earlier research (Cousijn et al., 2021; Hill et al., 2014) we expected women to show increased WM related activation in frontal regions with a more prominent effect in cannabis users.

Significance

This study assesses the role of sex in the association between cannabis use and cognitive control and thereby contributes to the small but increasing literature base assessing sex differences in the neural processes underlying substance use disorders. Although sex differences in substance use are very common, differences in underlying neural processes are rarely studied. Expanding the knowledge on this topic is crucial to inform clinical practice on how sex differences could affect prevention and intervention outcomes.

2 | MATERIALS AND METHODS

The current study combined data from three different fMRI studies using an identical letter N-back task to assess how cannabis use influences WM performance and related brain activity (see

Figure S1 for additional study-specific information). Procedures were approved by the medical ethical committee of the Academic Medical Centre of the University of Amsterdam (study 1, data also used in Cousijn et al., 2014) and the ethical committee of the department of psychology of the University of Amsterdam (study 2: 2015-DP-6387, unpublished data; study 3: 2018-DP-9616, unpublished data). All participants provided informed consent before the start of the session and were financially compensated for their participation.

2.1 | Participants

A total of 104 frequent cannabis users (63% men) and 85 never to sporadic using controls (53% men) were included. Cannabis users used 10–31 times per month for at least the previous year, while the controls used 0–50 times in their life with at maximum of five uses in the last year. Additional exclusion criteria were excessive other substance use, excessive alcohol use, and a history of major psychological or medical problems (see Figure S1 for additional study-specific exclusion criteria). Participants were requested to abstain from using drugs or alcohol 24 hr before the session. A urine screening was conducted to assess recent substance use and all who tested positive for a substance other than THC in the cannabis group were excluded.

2.2 | Assessments

2.2.1 | Cannabis use and cannabis use disorder severity

In all studies, severity of cannabis use was assessed using the cannabis use disorder identification test-revised (CUDIT-R; Adamson et al., 2010) and heaviness of use was assessed as grams of cannabis used per week. Furthermore, self-reported age of onset and last use was recorded. DSM-5 CUD severity was assessed in study 2 and 3 only, using the cannabis section of the Structured Clinical Interview for the DSM (First, 2014; study 2) or the CUD section of the Mini International Neuropsychiatric Interview 7.0.2 (Sheehan et al., 1997; study 3). As both measures reflect DSM-5 symptoms but are not measured using the same methods and scale, scores will be analyzed separately for these studies.

2.2.2 | Other substance use

In all studies, alcohol use and related problems were assessed with the alcohol use disorder identification test (AUDIT; Saunders et al., 1993). Average number of cigarettes per day was assessed and nicotine dependence was assessed using the Fagerström test for nicotine dependence (FTND; Heatherton et al., 1991). A substance use history questionnaire was used to measure self-reported lifetime use of other substances.

2.2.3 | Sex

In study 1 and 2, sex was assessed with the question "are you a man or a woman?" during a pre-inclusion phone screening. In study 3, participants were asked the following two questions: "What is your gender?" (answers: man, woman, other) and "What biological sex were you identified with at birth?" (answers: male, female, intersex/undetermined). Individuals with non-binary gender or a gender identification not matching their biological sex at birth were not included in any of the studies to clarify grouping criteria. As gender (identity) was not specifically assessed in all studies, the term *sex* will be used throughout this article. However, we must note that the reported difference between men and women may reflect biological as well as gender-related influences.

2.2.4 | Other assessments

IQ was estimated using different methods: study 1 used the Dutch reading test for Adults (Schmand et al., 1991), study 2 used the matrix reasoning and similarities subscales of the fourth edition of the Wechsler Adult Intelligence Scale (WAIS-IV; Wechsler, 2012), and study 3 used the matrix reasoning and vocabulary subscale of the WAIS-IV. Scores were standardized before combining the data. The Beck's depression inventory (BDI-II; Beck et al., 1961) was used to assess depressive symptoms in all studies. Symptoms of trait and state anxiety were assessed using the State Trait Anxiety Inventory (STAI; Spielberger & Sydeman, 1994) in study 2 and 3 only.

2.3 | N-back task

Blood oxygen level dependent (BOLD) signals were recorded during a letter N-back task. Blocks with three different N-back levels were included: 0-back (recognition), 1-back (low WM load), and 2-back (high WM load). During each trial, a capital letter was presented in the middle of the screen requiring a response: press the target button when the letter is a target in the current block, otherwise press the non-target button. In the 0-back blocks, participants were instructed to press the target button when the letter "X" was presented (recognition). In the 1-back blocks, participants were instructed to press the target button when the letter presented was the same as the letter in the last trial (low WM load). In the 2-back blocks, participants were instructed to press the target button when the letter presented was the same as the letter presented before the previous trial (high WM load). All blocks were repeated four times in a fixed order (2-back–0-back–1-back) resulting in a total of 12 blocks. Each block included 15 2-s trials (block duration 30 s) followed by a 5-s break with instructions for the next block (task duration 7 min). No feedback was provided during or after the task. The difference between 2-back trials and 0-back trials was used as a measure of the effect of WM and the difference between 2-back trials and 1-back trials was used as a measure of the effect of WM load.

2.4 | fMRI data acquisition

2.4.1 | Study 1

Scanning was performed at the University Medical Center Amsterdam, using a 3T Intera MRI scanner (Philips Intera, Best, The Netherlands) with 8-channel SENSE head coil. High resolution structural scans were acquired for anatomical reference (T1 turbo field echo, TR = 9.60 s, TE = 4.60 ms, 182 slices, slice thickness = 1.20 mm, field of view [FOV] = 256 × 256 mm, voxel size = 1 × 1 mm, flip angle = 8°). During the N-back task, BOLD responses were recorded using a T2* single-shot echo-planar imaging (EPI) sequence (TR = 2.30 s, TE = 30 ms, 38 slices, slice thickness = 3 mm, inter slice gap = 0.30 mm, FOV = 220 × 220 mm, voxel size = 2.30 × 2.30 mm, flip angle = 80°).

2.4.2 | Study 2

Scanning was performed at the Spinoza Centre for Neuroimaging at the University Medical Center Amsterdam, using a 3T Intera MRI scanner (Philips Intera, Best, The Netherlands) with 32-channel SENSE head coil. High resolution structural scans were acquired for anatomical reference (T1 turbo field echo, TR = 8.20 s, TE = 3.80 ms, 220 slices, slice thickness = 1 mm, FOV = 240 × 188 mm, voxel size = 1 × 1 mm, flip angle = 8°). During the N-back task, BOLD responses were recorded using a T2* single-shot EPI sequence (TR = 2 s, TE = 28 ms, 37 slices, slice thickness = 3 mm, inter slice gap = 0.30 mm, FOV = 240 × 240 mm, voxel size = 3 × 3 mm, flip angle = 76°).

2.4.3 | Study 3

Scanning was performed at the Spinoza Centre for Neuroimaging at the University of Amsterdam, using a 3T Achieva MRI scanner (Philips Intera, Best, The Netherlands) with 32-channel SENSE head coil. High resolution structural scans were acquired for anatomical reference (T1 fast field echo, TR = 8.20 s, TE = 3.70 ms, 220 slices, slice thickness = 1 mm, FOV = 240 × 188 mm, voxel size = 1 × 1 mm, flip angle = 8°). During the N-back task, BOLD responses were recorded using a T2* single-shot multiband accelerated (MB4) EPI sequence (TR = 0.55 s, TE = 30 ms, 36 slices, slice thickness = 3 mm, interslice gap = 0.30 mm, FOV = 240 × 240 mm, voxel size = 3 × 3 mm, flip angle = 55°).

2.5 | fMRI data preprocessing

Preprocessing was conducted using FSL FEAT (FMRIB's Software Library version 5.0.6, part of fMRI Expert Analysis Tool version 6.0) and non-brain tissue was removed using BET (Brain Extraction Tool). Preprocessing settings included regular-up slice timing correction, high-pass filtering (90 s), MCFLIRT motion correction, spatial smoothing (5 mm FWHM Gaussian kernel), and prewhitening. Functional scans were registered to the participants high resolution T1-weighted scan (BBR, 12DOF) and transformed to standard space (MNI-152) using FNIRT (FMRIB's non-linear registration tool). None of the participants showed excessive motion (max residual motion = 0.20 mm).

2.6 | Data analysis

2.6.1 | Sample characteristics

For all included questionnaire, means and standard deviations or medians (in case of violation of assumption of normality) per group and per sex within group were calculated using R (version 4.1.2; R Core Team, 2021) in RStudio (version 2021.9.2.382; RStudio Team, 2022). (Pairwise) Chi-square tests (or Fisher's exact test when sample size was below five for any of the included cells) were used to compare group and sex differences in categorical variables. Additionally, the effect of group, sex, and their interaction on the included questionnaires with a continuous outcomes was assessed using a linear mixed model approach with maximum likelihood estimation, random intercept and subject, sex, and group as random variables to account for the grouping structure of the data.

2.6.2 | N-back task performance

The effects of WM load, group, sex, and their interactions on N-back task performance (accuracy (% correct) and reaction time (on accurate trials)) were assessed using a linear mixed model approach with maximum likelihood estimation, random intercept and subject, and WM load as random variables to incorporate repeated measures. All potential models (including at minimum WM load, group, and sex) were run and the model with the best fit was selected based on Akaike information criterion (AIC; lower AIC reflecting relatively better fit and $\Delta AIC > 2$ [between models] indicating substantial support for relatively better fit; Burnham & Anderson, 2002).

2.6.3 | fMRI data

As described in the preregistration (https://aspredicted.org/blind.php?x=uh4t82), first, a general linear model (GLM) analysis was conducted in FSL's FEAT adding the three different trial types, 0-back, 1-back, and 2-back, as regressors convolving them with a double gamma hemodynamic response function, which incorporates the undershoot before oxygen rich blood flow increases in a specific area into each regressor (Lindquist et al., 2009), and adding temporal derivates to improve model fit. The effect of WM (2-back–0-back) and the effect of WM load (2-back–1-back) on brain activity (BOLD response) were the primary contrasts of interest.

Second, whole-brain mixed effects analyses (FLAME 1) were ran in FSL FEAT, using cluster-wise multiple comparison correction ($Z > 3.10$, cluster-based $p < 0.05$) to assess the effects of group, sex, and their interaction on WM and WM load related brain activity, while controlling for scanner differences by adding study as a regressor to the model.

Third, mean activations in significant clusters were extracted using FSL featquery to visualize the direction of the effects. Additionally, separate regression analyses were conducted to assess whether extracted activation within the significant clusters could be explained by accuracy (% correct) and reaction time (on accurate trials) on the N-back task or whether extracted activation (within the cannabis group) could be explained by severity or cannabis use (CUDIT-R score), heaviness of cannabis use (grams/week), or age of onset. Also, the moderating role of sex in these associations was assessed.

3 | RESULTS

3.1 | Sample characteristics

Sex distribution ($\chi_1^2 = (2.14)$ ($n = 189$), $p = 0.14$) and handedness ($p = 0.41$; Table 1) did not differ between groups, but the cannabis group included more daily smokers than the control group ($\chi_1^2 = (13.19)$ ($N = 189$), $p < 0.001$; Table 1). The number of daily smokers ($\chi_1^2 = (0.04)$ ($n = 189$), $p = 0.84$) did not differ between men and women, but there were more left-handed women than men ($p = 0.04$).

Cannabis users scored higher than the controls on trait anxiety ($B = -4.87$, 95% CI = −9.29: −0.45, $p = 0.03$) and other substance use ($B = -21.63$, 95% CI = −39.29: −3.98, $p = 0.02$; Table 1). No other effects of group, sex, nor their interaction were observed for any of the outcomes (Table S1).

3.2 | Behavioral N-back results

As expected, accuracy decreased with increasing WM load (Tukey post hoc: 0-back–1-back: $p < 0.01$, 0-back–2-back: $p < 0.001$, 1-back–2-back: $p < 0.001$; Figure 1), but no main effect of group or sex was found (Table 2). However, there was a significant interaction between WM level and group (Table 2). Post hoc simple effects *t* tests showed lower 2-back accuracy in cannabis users versus controls ($t[189] = -2.04$, $p = 0.04$). Adding the interactions of sex with WM load and group, as well as the three-way interaction did not reveal additional significant effects and did not improve model fit (Table S1).

Similar results were found for reaction time (RT) on accurate trials, where performance was found to be WM load dependent with RT increasing with increasing difficulty (Tukey post hoc: 0-back–1-back: $p < 0.01$, 0-back–2-back: $p < 0.001$, 1-back–2-back: $p < 0.001$; Figure 1; Table 2). No effect of sex or group was found, but there was an interaction between group and WM load (Table 2). However, while the pattern was similar to the interaction effect found for accuracy, the post hoc simple effects *t* tests showed that there were no significant group differences on any of the WM levels (lowest *p* value = 0.18). Adding the interactions of sex with WM load and group as well as the three-way interaction did not reveal additional significant effects and did not improve model fit (Table S1).

3.3 | fMRI N-back results: WM(-load) effects

Whole-brain analysis revealed a clear pattern of WM (2 > 0 and 0 > 2; Figure 2a) and WM load (2 > 1 and 1 > 2; Figure 2b)-related activation. Higher WM load was associated with relatively higher activation in fronto-parietal regions known to be part of the central executive network and a relatively lower activation in default mode network regions including the precuneus and posterior cingulate cortex (PCC; Table S1).

3.4 | fMRI N-back results: The effects of group, sex, and their interaction

Cannabis users showed relatively higher WM related and WM load-related activity than controls in a cluster including the precuneus and PCC (Table 3; Figure 2c). Further inspection of the mean WM related activation extracted from this cluster showed that while activation in these regions was lower during 2-back trials than 0-back trials in both groups, this difference was smaller in the cannabis group (Figure 2d). A similar but less pronounced pattern was observed for WM load related activity, where the cannabis group showed similar activation for both trials types, but controls showed relatively lower activity in these regions on the more difficult 2-back trials compared to 1-back trials (Figure 2e). No effects of sex or the interaction between group and sex on WM (load) related activation were found.

3.5 | Within cannabis group association between measures of cannabis use and WM(load) related activity

Mean WM (load) related activation was not associated with cannabis use and related problems (CUDIT-R; WM: $R^2 = -0.00$, $F_{1,102} = 0.79$; $n = 103$, $\beta = -0.01$, $p = 0.38$; WM load: $R^2 = -0.01$, $F_{1,102} = 0.01$; $n = 103$, $\beta < 0.001$, $p = 0.92$), grams of cannabis use per week (WM: $R^2 = -0.01$, $F_{1,101} = 0.58$; $n = 102$, $\beta = -0.01$, $p = 0.58$; WM load: $R^2 = -0.01$, $F_{1,101} = 0.08$; $n = 102$, $\beta = 0.00$, $p = 0.78$), or age of onset (WM: $R^2 = 0.001$, $F(1,102) = 0.29$, $\beta = 0.04$, $p = 0.29$; WM load: $R^2 = -0.01$, $F(1,101) = 0.26$, $\beta = 0.01$, $p = 0.61$). Similarly, no association between activation and accuracy (WM, 2–0 accuracy: $R^2 = 0.01$, $F_{1,97} = 1.95$; $n = 98$, $\beta = -0.01$, $p = 0.17$; WM load, 2–1 accuracy: $R^2 = 0.00$, $F_{1,97} = 1.40$; $n = 98$, $\beta = -0.01$, $p = 0.24$) or RT (WM, 2–0 RT: $R^2 = -0.01$, $F_{1,99} = 0.28$; $n = 100$, $\beta < 0.001$, $p = 0.60$; WM load, 2–1 RT: $R^2 = 0.01$, $F_{1,98} = 1.63$; $n = 99$, $\beta < 0.001$, $p = 0.21$)

TABLE 1 Sample characteristics

		Cannabis group			Control group		
Measures	**Unit**	**Total**	**Men**	**Women**	**Total**	**Men**	**Women**
N (% of group)		104	66 (63%)	38 (37%)	85	45 (53%)	40 (47%)
Handedness	L/R	2/101	0/66	2/35[a]	4/81	1/44	3/37
Age	Med	22	22	21	22.50	22	22
Estimated IQ[b]	Mean (*SD*)	−0.16 (0.96)	−0.13 (0.95)	−0.21 (0.99)	0.19 (1.01)	0.30 (1.03)	0.06 (0.98)
Depression (BDI)	Med	6	6	6	4	4	4.50
State anxiety (STAI)[c]	Mean (*SD*)	33.44 (9.08)*	32.92 (9.43)	34.26 (8.59)	31.90 (6.31)*	31.10 (7.25)	32.73 (5.15)
Trait anxiety (STAI)[c]	Med	37	36	38	34	33	34
Alcohol use and related problems (AUDIT)	Med	6	6	5	5	6	3
Smoking	*N* (%)	54 (52%)	34 (52%)	20 (53%)	22 (26%)	10 (22%)	12 (30%)
Nicotine dependence (FTND)	Med	2	2	2.50	0.50	0	1
Cigarettes/day	Med	9	8	10	6	8	5
Other substance use	Med	12.50*	12.50	12.50	0*	0	0
Cannabis use and related problems (CUDIT-R)	Mean (*SD*)	13.56 (5.90)	13.48 (5.95)	13.68 (5.89)	–	–	–
CUD symptoms[d]							
Study 2	Mean (*SD*)	3.47 (1.60)	3.56 (1.65)	3.38 (1.59)	–	–	–
Study 3	Mean (*SD*)	5.27 (2.23)	5.10 (2.16)	5.60 (2.41)	–	–	–
Gram/week	Med	3	3	2.5	–	–	–
Age of onset	Med	15	15	15.5	–	–	–
Days since last use	Med	1	1	1	–	–	–

Abbreviations: 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.

[a]Missing handedness data for one participant.

[b]Using standardized (*Z*) scores to compare studies.

[c]STAI State and STAI Trait only assessed in study 2 and 3.

[d]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; Medians are reported when assumptions of normality were violated (as assessed using Shapiro–Wilk normality tests).

*$p < 0.05$.

on the N-back task was found in the cannabis group. In the control group, higher WM related activation in these regions was associated with lower performance (WM, 2–0 accuracy: $R^2 = 0.05$, $F_{1,81} = 5.04$; $n = 82$, $\beta = -0.03$, $p = 0.03$). However, these results were no longer significant (Table 1; ($R^2 = 0.01$, $F_{4,44} = 1.08$; $n = 48$, $\beta = -0.03$, $p = 0.05$) after correcting for the variables that differed across groups (trait anxiety, smoking, and other drug use). Additional analyses showed that sex did not moderate any of the associations between extracted activity and any of the cannabis or performance-related variables (lowest uncorrected p value = 0.11).

3.6 | fMRI N-back results: Exploratory analysis of sex effects within the cannabis group

Non-planned exploratory whole-brain analyses were performed to assess whether the effect of WM and WM load related brain activity differed between men and women within the cannabis group. Analyses revealed that men (2 > 0; $M = 0.74$, $SD = 0.53$) show relatively higher WM related activation in the superior frontal gyrus (SFG) compared to women (2 > 0; $M = 0.38$, $SD = 0.36$), while there was no effect for WM load related activation (Table 4). The increased activation could not be explained by cannabis use variables or performance (lowest uncorrected p value = 0.25).

4 | DISCUSSION

The aim of the current study was to assess the effects of cannabis on WM and WM load related brain activity and the potential role of sex in these effects. Results showed no sex effect on WM or WM load related brain activity. However, cannabis users showed higher WM as well as WM load related activity in the precuneus and PCC compared to controls. This relative over recruitment of regions known to be central nodes of the default mode network could be indicative of a relatively smaller shift from default mode to executive

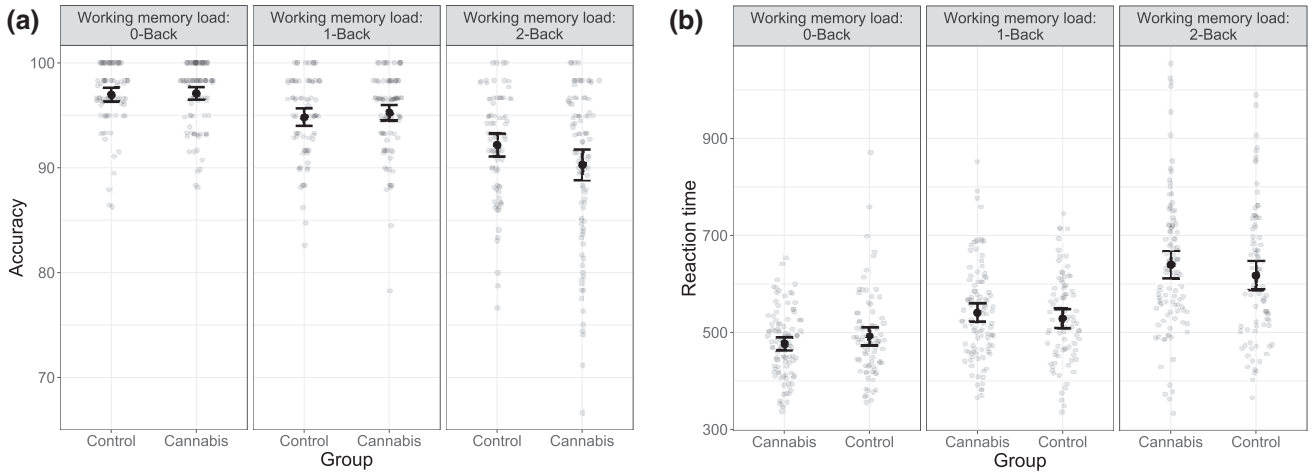

FIGURE 1 N-back task performance. (a) No group differences in mean accuracy on 0-back, 1-back, and 2-back trials. Accuracy decreased with increasing working memory load and an interaction between group and working memory load was found. (b) No group differences in mean reaction times on 0-back, 1-back, and 2-back trials. Reaction time increased with increasing working memory load and an interaction between group and working memory load was found. Error bars reflect standard error (*SE*) of the mean

TABLE 2 Final models showing the effect of working memory (WM) load on accuracy and reaction time during the N-back task

	Model coefficients					
	Fixed effects					Random effects
Model	*B*	95% CI (*B*)	*SE* (*B*)	*t*	*p*	*SD*
Accuracy						
(Intercept)	96.58	95.48–97.69	0.57	170.85	<0.001	2.38
WM: 0-back—1-back	−1.85	−2.95 to −0.75	0.56	−3.28	0.00	3.69
WM: 0-back—2-back	−6.80	−7.90 to −5.70	0.56	−12.04	<0.001	
Group	−0.05	−1.40 to 1.31	0.69	−0.07	0.95	
Sex	0.79	−0.19 to 1.78	0.50	1.58	0.12	
WM: 0-back—1-back * Group	−0.28	−1.91 to 1.35	0.84	−0.34	0.74	
WM: 0-back—2-back * Group	2.01	0.37–3.64	0.84	2.40	0.02	
Reaction time						
(Intercept)	483.94	456.38–511.51	14.11	34.30	<0.001	89.10
WM: 0-back—1-back	61.94	44.78–79.11	8.78	7.05	<0.001	56.78
WM: 0-back—2-back	160.96	143.86–178.07	8.75	18.39	<0.001	
Group	13.60	−17.89 to 45.10	16.07	0.85	0.40	
Sex	−10.49	−38.70 to 17.72	14.39	−0.73	0.47	
WM: 0-back—1-back * Group	−22.35	−47.81 to 3.12	13.03	−1.71	0.09	
WM: 0-back—2-back * Group	−32.55	−57.98 to −7.13	13.01	−2.50	0.01	

Note: Mixed model results using random intercept and maximum likelihood estimation. Other models ran as part of the model selection process can be found in Tables S1 and S1. Accuracy: ΔAIC = 3.33; Reaction time: ΔAIC = 2.59.

Abbreviations: CI, confidence interval; *SD*, standard deviation; *SE*, standard error; WM, working memory.

control network activation with increasing difficulty (e.g., Bossong et al., 2013; Danckert & Merrifield, 2018; Raichle, 2015).

Based on previous inconsistencies in the effect of cannabis use on WM performance, we hypothesized that there would be a general effect of WM level but no effects of group on performance. Results showed a clear effect of WM level with accuracy going down and reaction time going up with increasing difficulty. However, there was also an interaction between group and WM level on performance, with more pronounced reduction in performance with increasing difficulty in cannabis users compared to controls. Although inconsistent with previous studies (e.g., Cousijn et al., 2014; Hatchard et al., 2020), this is in line with the general expectation that current

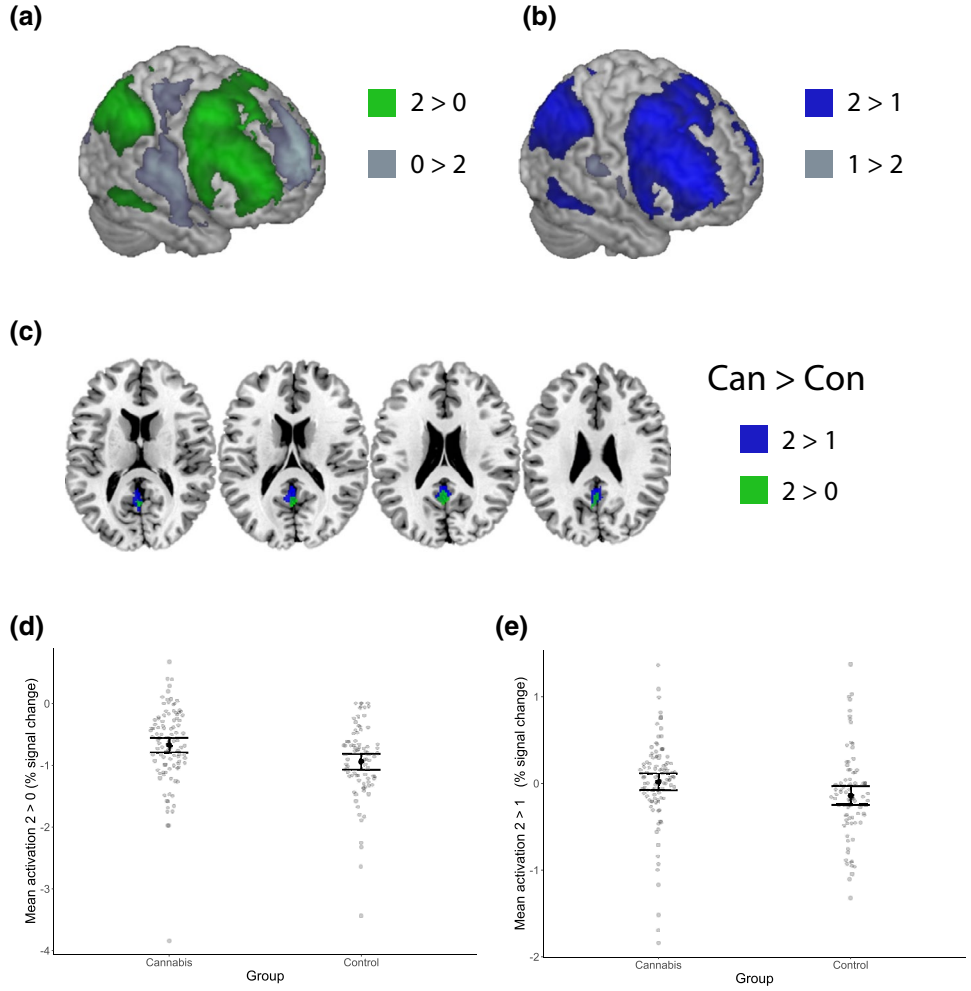

FIGURE 2 fMRI results. (a) WM related activation (2 > 0) across groups; (b) WM load-related activation (2 > 1) across groups; (c) group differences (Can > Con) in WM (2 > 0) and WM load related (2 > 1) activation. (d) Mean WM related activation (2 > 0) extracted from the group difference cluster (e) mean WM load-related activation (2 > 1) extracted from the group difference cluster. Error bars reflect standard error (*SE*) of the mean. Can, cannabis group; Con, control group; WM, working memory; 0: 0-back, 1: 1-back, 2: 2-back

TABLE 3 Group differences in WM and WM load related activation

					MNI coordinates			
	Comparison	Cluster size (voxels)	Brain regions	Hemisphere	X	Y	Z	Zmax
WM effect								
2 > 0	Con > Can	ns	ns	ns	ns	ns	ns	ns
2 > 0	Can > Con	164	Precuneus	Mid	0	−60	16	4.18
			PCC	Left	−2	−50	24	4.09
WM load effect								
2 > 1	Con > Can	ns	ns	ns	ns	ns	ns	ns
2 > 1	Can > Con	404	PCC	Mid	0	−50	22	4.75
			Precuneus	Left	−2	−58	14	4.53
			Lingual gyrus	Left	−4	−60	4	3.34

Note: MNI coordinates and *Z*-scores of separate local maxima for each cluster (whole-brain cluster corrected at $p < 0.05$, $Z > 3.10$).

Abbreviations: Can, cannabis group; Con, control group; MNI, Montreal Neurological Institute; PCC, posterior cingulate cortex; WM, working memory; 0, 0-back; 1, 1-back; 2, 2-back.

TABLE 4 Sex differences in WM and WM load related activation in the cannabis group only

	Comparison	Cluster size (voxels)	Brain regions	Hemisphere	MNI coordinates			Zmax
					X	Y	Z	
WM effect								
2 > 0	Female > Male	ns	ns	ns	ns	ns	ns	ns
2 > 0	Male > Female	181	SFG	Right	26	2	64	4.00
WM load effect								
2 > 1	Female > Male	ns	ns	ns	ns	ns	ns	ns
2 > 1	Male > Female	ns	ns	ns	ns	ns	ns	ns

Note: MNI coordinates and *Z*-scores of separate local maxima for each cluster (whole-brain cluster corrected at $p < 0.05$, $Z > 3.10$).

Abbreviations: Can, cannabis group; Con, control group; MNI, Montreal Neurological Institute; SFG, superior frontal gyrus; 0, 0-back; 1, 1-back; 2, 2-back.

cannabis users experience problems with cognitive control related functions such as WM (e.g., Crean et al., 2011; Scott et al., 2018), which could be more pronounced when cognitive load increases. These results also indicate previous studies with smaller sample sizes (e.g., Hatchard et al., 2020; Jager et al., 2006; Kanayama et al., 2004) may have been underpowered to detect subtle 2-back group differences. As performance on 2-back trials is often close to ceiling, as can also be seen in the current study, it is also important to assess the effects of current cannabis use on performance under higher cognitive load.

The fMRI results showed a group difference in WM (2 > 0) and WM load (2 > 1)-related activation in the precuneus and PCC, with cannabis users showing relatively higher activation than controls. Both groups show higher activation in these regions on 0-back trials than on 2-back trials, but the relative reduction in activation as cognitive load increases is less pronounced in the cannabis group. The direction of the group difference was the same for WM load related activity, but controls showed higher activation for 1-back than 2-back trials while activation was similar for both trial types in the cannabis group. While we expected relatively higher WM and WM load related activation in the cannabis group, the specific regions in which these activation differences were found do not match our hypotheses. Cannabis users were expected to show increased fronto-parietal and not precuneus or PCC, activity as a compensatory mechanism to maintain performance (as proposed in e.g., Cousijn et al., 2014; Hatchard et al., 2020; Jager et al., 2006). The precuneus and PCC are well-known nodes of the default mode network in which activity is expected to go down with increased cognitive effort (Raichle, 2015). Indeed, activity was relatively lower for 2-back than 0-back trials and also lower for 2-back trials than 1-back trials in controls. However, in the cannabis group, activity was comparable for 2-back and 1-back trials and the relative reduction in activity with increasing cognitive effort was less pronounced. While higher default mode network activity during higher cognitive load could indicate reduced attention or effort (Danckert & Merrifield, 2018) and thereby potentially affect performance, activity was not predictive of performance in the cannabis group. Nevertheless, higher activity in these regions was associated with lower accuracy in the control group (before adding multiple control variables). This is in line with earlier results on executive functioning by Bossong et al. (2013) in which task performance was negatively affected by THC and associated with reduced deactivation in regions of the default mode network. However, the THC induced reduction in performance was not associated with activation of fronto-parietal regions (Bossong et al., 2013). Although results are not consistent across groups and findings should be treated with caution, it is worth investigating to what extent higher default mode network activation during cognitively demanding tasks, rather than altered fronto-parietal activation, affects performance.

No sex differences or interactions between sex and group in WM and WM load related activation or performance were found. While using a different task, these results are in line with a recent study on response inhibition in cannabis users, where group differences in activity but no sex or group–sex interaction effects were found (Wallace et al., 2020). Although speculative, this lack of sex effects may indicate that the sex differences in cannabis use patterns and the development of CUD are not directly related to differences in cognitive control related processes. Nevertheless, evidence is limited, and research is warranted to replicate these findings and assess how sex differences in motivational processes rather than cognitive control related processes might relate to sex differences in cannabis use. However, it could also be the case that we were underpowered to detect more subtle interaction effects using a relatively strict whole-brain threshold. Hence, an additional whole-brain analysis was conducted to assess sex differences within the cannabis group. Men showed higher WM related activation than women in the SFG, a frontal region important in higher cognitive functions like WM (e.g., Ranganath et al., 2003; Rypma et al., 1999), while no sex difference was found for WM load related activation. The direction of the observed effect is opposite from our expectations that WM related frontal activation would be higher in women than men (Cousijn et al., 2021; Hill et al., 2014) and differences in activity did not relate to cannabis use or performance. These results should be treated with caution due to the exploratory nature of this analysis. While studies with sex comparisons in cannabis users focusing on cognitive control are largely lacking, activation in the SFG has regularly been

found to differ between substance users and controls during cognitive tasks. For example, previous studies showed increased activation in the right SFG in cannabis users compared to controls during WM tasks (Kanayama et al. 2004; Hatchard et al., 2020), but another study found cannabis users to display relatively lower activation in the SFG during learning (Nestor et al., 2008) and mixed directions of these effects have also been identified for other addictive behaviors (García-García et al., 2014; Hester & Garavan, 2004; Moreno-López et al., 2012). The SFG is apparently involved in cognitive functions including WM, but it is unclear in what way addictive behaviors, sex, and cognitive load affect its involvement.

While the sample size and mixed sex sample of this study are substantial advantages, there are several limitations that should be noted. First, cannabis users had higher anxiety scores than controls, but differences were relatively small and below clinical thresholds. Second, higher cigarette and substance use in the cannabis group could have affected the results; however, other drug use (Connor et al., 2014; UNODC, 2016) and mental health problems (e.g., Agosti et al., 2002) are more common among substance users than controls. Thus, a fully matched sample might not accurately reflect the cannabis using population. As there might also be substantial overlap in the underlying mechanisms and the causal effects of these substances on the brain, controlling for the existing differences in the analyses would also potentially obscure the effects of cannabis use. Third, while participants testing positive on other substances than cannabis were excluded, we were not able to verify the instructed 24 hr abstinence from alcohol and cannabis. Nevertheless, it seems unlikely that direct rather than indirect effects of cannabis would have affected the results as reaction times were similar between groups, which would not be expected in case of direct intoxication effects (Hartman & Huestis, 2013). Fourth, performance was relatively high on the most difficult 2-back trials and studies should be encouraged to increase WM load to assess whether WM (load) effects are more pronounced when cognitive demand increases. Fifth, in our study we were not able to differentiate between biological sex and gender effects. This is a clear limitation of most studies not initially designed for studying gender and sex effects and future studies should be specifically designed to make this differentiation. These studies should also aim to not exclude individuals with non-binary gender, but rather take gender into account as a more continuous measure (Heidari et al., 2016). Last, the design of our study is cross-sectional and longitudinal studies assessing the causal nature of the association between cannabis use and altered brain functioning are essential.

In conclusion, cannabis users showed poorer performance and a smaller reduction in activation in central nodes of the default mode network when cognitive load increased. Explorative analyses revealed higher WM related SFG activity in cannabis-using men compared to women; however, sex effects were non-significant when the cannabis and control groups were both included. To further unravel the impact of cannabis use on brain and behavior, studies investigating tasks requiring higher cognitive demands, clinical populations, and longitudinal effects are needed.

DECLARATION OF TRANSPARENCY

The authors, reviewers and editors affirm that in accordance to the policies set by the *Journal of Neuroscience Research*, this manuscript presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

All authors reviewed and approved the final version. *Conceptualization*, E.K., J.C., and A.M.K.; *Methodology*, J.C.; *Formal Analysis*, E.K. and J.C.; *Data Curation*, E.K. and J.C.; *Writing – Original Draft*, E.K.; *Writing – Review & Editing*, J.C., A.M.K., L.K., and F.F.; *Visualization*, E.K.; *Supervision*, J.C.; *Funding Acquisition*, J.C. and F.F.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

FIGURE S1 Study-specific information and exclusion criteria

TABLE S1 Overview of linear mixed model results assessing the effect of group, sex, and their interaction on the included continuous outcome variables

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

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

TABLE S4 Activation overview for the effect of WM and WM load

Transparent Science Questionnaire for Authors

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