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Cognitive stimulants: from caffeine to cannabinoids - current and future perspectives

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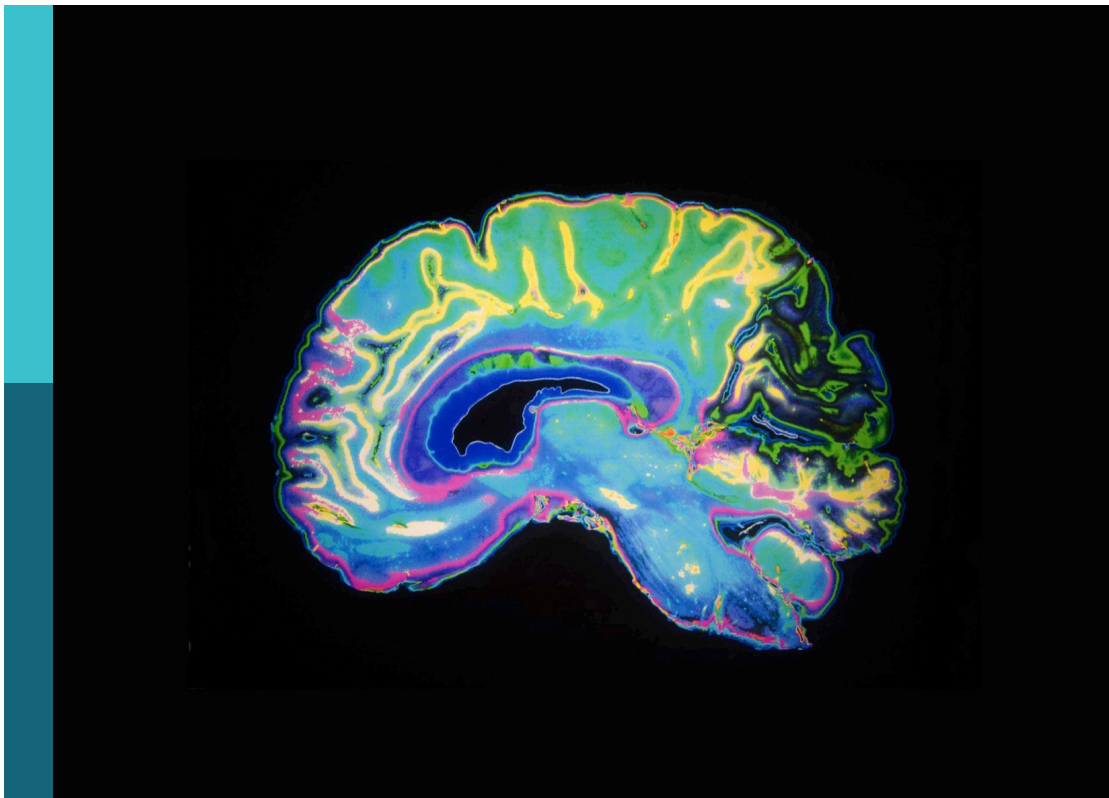

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Cognitive stimulants: from caffeine to cannabinoids - current and future perspectives

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Editorial: Cognitive stimulants: from caffeine to cannabinoids - current and future perspectives

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Editorial on the Research Topic
Cognitive stimulants: from caffeine to cannabinoids - current and future perspectives

There is an emerging demand for cognitive enhancement in recent years, led by increasing pressure to maintain high mental performance in academic, professional, and personal spheres. Cognitive stimulants have gained popularity as faster-acting alternatives in comparison to traditional and more challenging-to-follow strategies like a balanced routine of exercise, nutrition, and sleep. This Research Topic comprehensively explores recent studies that uncover the intricate interactions among cognitive enhancers, neural substrates, and pharmacological side effects. Widely used substances such as caffeine and cannabinoids were emphasized while intriguing findings on novel compounds, such as Icariin, were also discussed. Importantly, the collection recognizes that non-pharmacological factors, such as environmental enrichment, play a pivotal role in cognitive enhancement, underscoring the need for an integrated approach to optimizing cognitive function.

Caffeine: the everyday cognitive booster with a sharp edge

Caffeine is by far the most consumed cognitive stimulant worldwide, primarily due to its widespread availability in beverages like coffee, tea, and energy drinks. While caffeine is renowned for its ability to improve alertness and focus, there are growing concerns about its anxiogenic effects at higher doses. Consistent with previous data in the literature, Bao et al.'s study showed that moderate doses of caffeine (around 90 mg) may alleviate depressive symptoms. On the flip side, factors like sleep quality, education, and exercise may have influenced these outcomes, requiring further investigation. Liu et al. conducted a meta-analysis involving 546 healthy individuals across eight studies and found that caffeine intake, particularly in doses above 400 mg per day, significantly increased the risk of anxiety. This finding brings light to a concern of high epidemiological relevance, given

caffeine's popularity, and calls for moderation in its use, especially for individuals with a predisposition to anxiety disorders. Picó-Pérez et al.'s fMRI analysis further differentiates caffeine's effects from the overall coffee-drinking experience. They revealed that habitual coffee intake boosts connectivity in the higher visual and right executive control networks but reduces it in the posterior default mode network and somatosensory/motor networks. Caffeine alone, however, only affects the posterior default mode, suggesting additional bioactive components contribute to cognitive modulation. Altogether, these findings underscore the nuanced balance between cognitive benefits and potential psychological downsides of caffeine consumption in humans. Additional studies examining the effects of a wider range of doses of caffeine in different physiological and pathological contexts are needed to elucidate the intricacies of the dose-response relationship.

Cannabis and cannabinoids: dual roles in cognitive modulation

Similarly, cannabinoids, as plant-derived pharmaceutical agents, also exhibit a complex dose-response relationship in cognitive modulation, offering potential benefits but posing risks depending on dosage, individual health status, and context of use. Cannabinoids are chemical compounds found in plants such as *Cannabis sativa* and *Cannabis indica*, including tetrahydrocannabinol (THC), which is psychoactive, and cannabidiol (CBD), which is non-psychoactive and known for its therapeutic potential. Ognibene et al.'s study demonstrated that daily exposure to inhaled cannabis (containing 10.3% Δ9-THC) reduces brain sensitivity to Adderall, a drug commonly prescribed for narcolepsy and ADHD, particularly in dopaminergic pathways. Through detailed analysis using statistical heat maps and 3D reconstructions of 134 mouse brain regions, the study revealed that Adderall-induced activation patterns in reward and attention networks are suppressed in cannabis-exposed subjects. In the same direction, Beyer et al.'s article synthesized fMRI analysis on the brains of 534 individuals and noticed that the reward function is remarkably altered in cannabis users. Schouten et al.'s review on CBD highlights its therapeutic potential for neurological and psychiatric disorders, including epilepsy, Alzheimer's, and anxiety. CBD's ability to counteract the psychotic effects of THC illustrates the dual nature of cannabinoids. Altogether, these studies draw attention to the potential for cannabis to interfere with the effects of other cognitive stimulants and raise concerns about stimulant misuse.

Novel cognitive enhancers and environmental influences: balancing therapeutic potential and possible caveats

The field of cognitive enhancement offers a spectrum of possibilities, from well-established stimulants like caffeine and methylphenidate to emerging natural compounds such as Icariin. Findings by Wang et al. reveal that Icariin, derived from the Epimedium plant, shows promising therapeutic potential by mitigating surgery-induced memory impairment, reducing hippocampal inflammation, and protecting against neuronal injury in elderly individuals with postoperative cognitive dysfunction (POCD).

However, cognitive function is not solely determined by pharmacological interventions. Research by Herrera-Isaza et al. demonstrates that environmental enrichment—which includes cognitive, sensory, and social stimulation—can alleviate emotional and cognitive dysfunction caused by methylphenidate. This underscores the importance of combining pharmacological treatments with supportive environments to achieve optimal cognitive outcomes.

The need for careful, multifactor evaluation of cognitive enhancers is further emphasized by Marques et al.'s review of therapies for hypoxic-ischemic encephalopathy (HIE). While substances like erythropoietin and melatonin present promising potential in preclinical models, their efficacy in humans remains unconfirmed in the context of cognitive dysfunction. This gap highlights the challenge of translating animal research into clinical practice and the importance of assessing both the benefits and risks of cognitive enhancers, particularly their long-term implications.

Toward an integrated understanding of cognitive enhancement

The research presented in this collection illustrates the complexity of cognitive enhancement. From the cognitive-boosting potential of caffeine and methylphenidate to novel compounds like Icariin, these enhancers offer hope for improving cognitive function, especially for individuals under neuropathological conditions. Yet, studies on cannabinoids and environmental enrichment reveal that these substances do not act in isolation. Individual health status, dosage, and environmental factors all influence their effectiveness and safety.

This nuanced understanding is crucial for developing therapies that enhance cognition while protecting the brain from secondary psychological effects and avoiding unnecessary risks that could increase or exacerbate mental health disturbances. A multidimensional approach holds promise not only for individuals with cognitive impairments but also for those aiming to optimize their mental performance in a healthy, sustainable manner.

Author contributions

DM-S: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. MC-R: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. AS: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing.

PP: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Caffeine is negatively associated with depression in patients aged 20 and older

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Introduction: Previous studies have observed the association between caffeine intake and depression, but few have considered the potential threshold effect of this issue. Therefore, the study aimed to examine the association between caffeine consumption and depression in patients aged 20 years or older using curve fitting analysis.

Methods: The population was 3,263 patients from the 2017 to 2018 National Health and Nutrition Examination Survey (NHANES) with reliable answers to questions of caffeine intake and depression. Participants' depression levels were assessed using the 9-item Patient Health Questionnaire (PHQ-9) depression scale and the caffeine consumption were investigated in a private room of NHANES. The confounding variables of this study included level of education, monthly sleepiness, age, marital status, race, cigarette smoking, sex and recreational activities.

Results: In linear regression analysis, patients with a higher PHQ-9 score tend to have less caffeine intake. A similar conclusion was drawn in logistic regression model using PHQ-9 $\geq$ 10 as a cut-off score for depression. But when caffeine intake exceeded 90 mg, there was no significant association between caffeine intake and depression based on the curve fitting analysis.

Discussion: These results suggest that people can consume some caffeine to reduce depression. But further study is needed to examine the precise causal relationship between these factors.

KEYWORDS

caffeine intake, depression, NHANES, PHQ-9, cross-sectional study

Introduction

Depression, which has become one of the most common mental disorders, is experienced by a significant number of people globally and considered a primary care disease (1). It was found that one-third of adults in the United States will be affected by depression during their lives (1). According to the World Health Organization, nearly 350 million people suffer from depression globally (2). Based on data from the US. National Health and Nutrition Examination Survey (NHANES) study in 2005–2008, the prevalence of depressive symptoms and severe depression was 22 and 0.6%

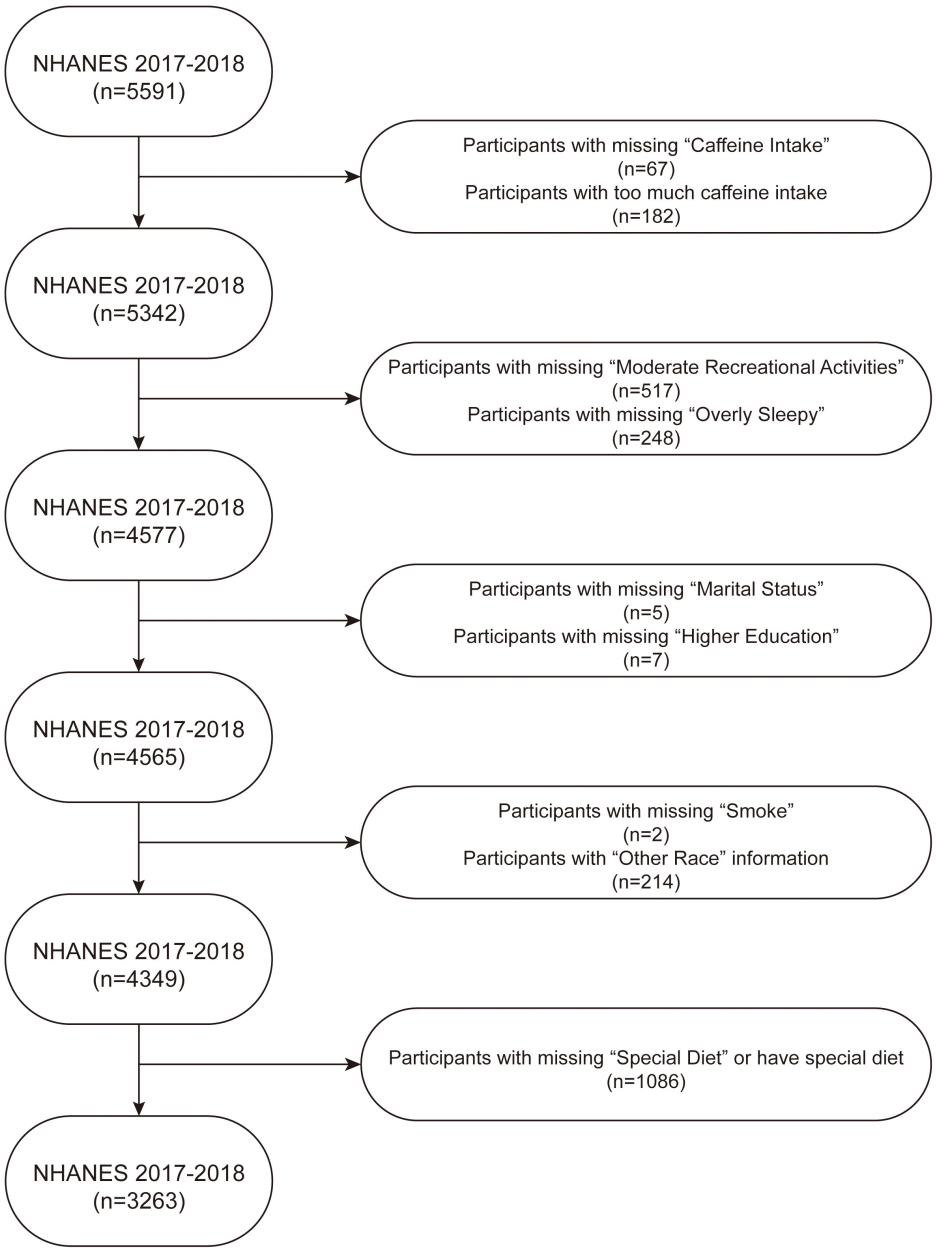

FIGURE 1
Flow chart of sample selection from the NHANES 2017–2018.

(a point prevalence), respectively (3). In recent years, some studies have focused on the association between caffeine intake and depressive symptoms (4–6). When focusing on diet-related factors, it was found that coffee was one of the most popular drinks worldwide. Several epidemiological studies have found that caffeine use has a protective effect against cognitive impairment/decline (7). Another study pointed out that combined caffeine and glucose could increase the efficiency of the attentional system (8). It is concluded that caffeine has an effect on depression.

Previous studies on this issue have not reached a consensus. An animal study had researched the causal association between caffeine consumption and depressive-like mood alterations, and found the caffeine-induced A2AR blockade has therapeutically effect on depression (4). A study based on data from NHANES from 2005 to 2006 found an inverse association between caffeine intake and depressive symptoms in US adults (6). This study was supported by a similar study conducted on 9,576 participants in Korea (5). Another case-control study conducted in 2021 found a positive association between caffeine and depression

in children (9). However, the samples used in these former studies were not nationally representative (9), or too old (6) to represent current conditions. Most importantly, the potential threshold effect of the association was of less concern in the former study (6).

Thus, the aim of this study was to examine the association between caffeine consumption and depression using data from the NHANES 2017–2018 database and curve fitting analysis. It is hypothesized that within a certain range greater caffeine intake could protect against depression.

Materials and methods

Study design

The NHANES program began in the early 1960s. The survey examines a nationally representative sample of about 5,000 people each year. The NHANES interview includes demographic, socioeconomic, dietary, and health-related questions. The examination component consists of medical, dental, and physiological measurements, as well as laboratory tests administered by trained medical personnel.

TABLE 1 The characteristics of the study participants in the NHANES 2017–2018.

	Non-depression (n = 3019)	Depression (n = 244)	P-Values
Caffeine intake (mg)	143.2 ± 122.1	126.3 ± 122.8	0.05
Moderate recreational activities (minutes per week)	93.0 ± 186.8	58.3 ± 128.2	0.006
Age (years)	48.1 ± 17.6	45.0 ± 17.5	0.01
Higher education* (%)	60.5	53.2	0.03
Marital status (%)			<0.0001
Current	63.4	44.0	
Past	17.9	27.5	
Never	18.7	28.5	
Monthly sleepiness (%)			<0.0001
Never (0)	13	5.1	
Rarely (1 time)	27	6.3	
Sometimes (2–4 times)	36	32.9	
Often (5–15 times)	18	34.9	
Always (16–30 times)	6	20.8	
Race (%)			0.15
Latin	16.5	17	
Non-Hispanic white	64.8	64	
African American	12.5	16	
Asian	6.2	3	
Cigarette smoking (%)	15.1	35.1	<0.0001
Male (%)	48.9	41.2	0.03

Mean ± SD for continuous variables; % for categorical variables. *"Higher Education" means whether or not patients have entered college.

Study participants

The study participants were based on the NHANES database 2017–2018 (Figure 1). Participants with missing caffeine intake information (n = 67), too much caffeine intake (>500 mg) (n = 182), missing moderate recreational activities information (n = 517), missing monthly sleepiness information (n = 248), missing marital status information (n = 5), missing higher education information (n = 7), missing smoking status information (n = 2), participants whose race information is "other race" (n = 214), and participants with special diets or missing answers of this issue (n = 1086) were excluded from the study. After exclusions a total of 3,263 participants were included in the analysis. Approval for this study was obtained from the ethics review board of the NHANES 2017–2018 (n = 5591) National Center for Health Statistics and written consent was obtained from every participant.

Variables

The exposure variable of the study is participants with caffeine intake. In-person interviews were conducted in a private room in NHANES to obtain detailed dietary intake information to estimate the types and amounts of food and beverage (including all types of water) consumed during the 24-h period prior to the interview. NHANES calculated the energy and 64 nutrients (including caffeine) of each food using the USDA's Food and Nutrient Database for Dietary Studies, and estimated patients' caffeine intake. After excluded those on a special diet, the present study used the data to represent the daily caffeine

TABLE 2 Association between caffeine intake and depression (linear regression model).

Exposure	Model 1 β (95% CI)	Model 2 β (95% CI)	Model 3 β (95% CI)
Caffeine	−0.002 (−0.003, −0.0006)	−0.002 (−0.003, −0.0003)	−0.002 (−0.003, −0.0005)
Caffeine consumption			
Q1	Reference	Reference	Reference
Q2	−0.66 (−1.05, −0.27)	−0.65 (−1.04, −0.26)	−0.67 (−1.03, −0.31)
Q3	−0.71 (−1.06, −0.36)	−0.63 (−1.00, −0.26)	−0.56 (−0.90, −0.21)
P for trend	0.002	0.02	0.004

Non-adjusted model (Model 1): None. Minimally adjusted model (Model 2): Age; sex; race. Fully adjusted model (Model 3): Higher education; marital status; monthly sleepiness; moderate recreational activities; race; cigarette smoking; sex and age.

TABLE 3 Association between caffeine intake and depression (logistic regression model).

	Model 1	Model 2	Model 3
Non-depression (Reference)	1	1	1
Depression			
OR (95% CI)	0.999 (0.997, 0.9996)	0.998 (0.9967, 0.9991)	0.998 (0.9967, 0.9991)
P	0.008	<0.001	<0.001

Non-adjusted model (Model 1): None. Minimally adjusted model (Model 2): Age; sex; race. Fully adjusted model (Model 3): Higher education; marital status; monthly sleepiness; moderate recreational activities; race; cigarette smoking; sex and age.

consumption of patients. In the linear regression model, caffeine intake was divided into three trisection parts: Q1 (≤39 mg), Q2 (≥39 mg and ≤144 mg), and Q3 (≥144 mg and <500 mg) to calculate the P for trend. The detailed data can be accessed in the Total Nutrient Intakes, First Day of Dietary Interview.

The outcome variable was depression. NHANES assessed the levels of depression using the 9-item Patient Health Questionnaire (PHQ-9) depression scale, which consists of nine questions based on symptoms of depression. Each answer to the nine questions is scored 0 to 3, with 0 (not at all), 1 (several days), 2 (more than half the days), and 3 (nearly every day). The scores are summed to a total score between 0 and 27. In the linear regression model and curve fitting analysis, the initial scores of PHQ-9 (a continuous variable), from 0 to 27 were used. The present study used an PHQ-9 score ≥10 as a cut-off score for depression, as it has a sensitivity of 88% and a specificity of 88% (10), and conducted the logistic regression analysis. These are contained in the Mental Health—Depression-screener data of Questionnaire Data section in NHANES.

Eight confounding factors were included in this study. The categorical variables included higher education, monthly sleepiness, marital status, race, cigarette smoking, and sex. The continuous variables included age and moderate recreational activities. The race consisted of four parts, with "Latin" including those of Hispanic white and Mexican background. Data for moderate recreational activities in a week was a product of "days recreational activities" and "minutes recreational activities" in NHANES. Higher education had two answers, "yes" which included college graduates and those with college degrees or Associates of Arts degrees, while "no" included those who had not entered college. Smoking status in the past 30 days was a continuous variable of frequency of smoking per day in the past 30 days and was categorized as "no" (= 0) and "yes" (>0).

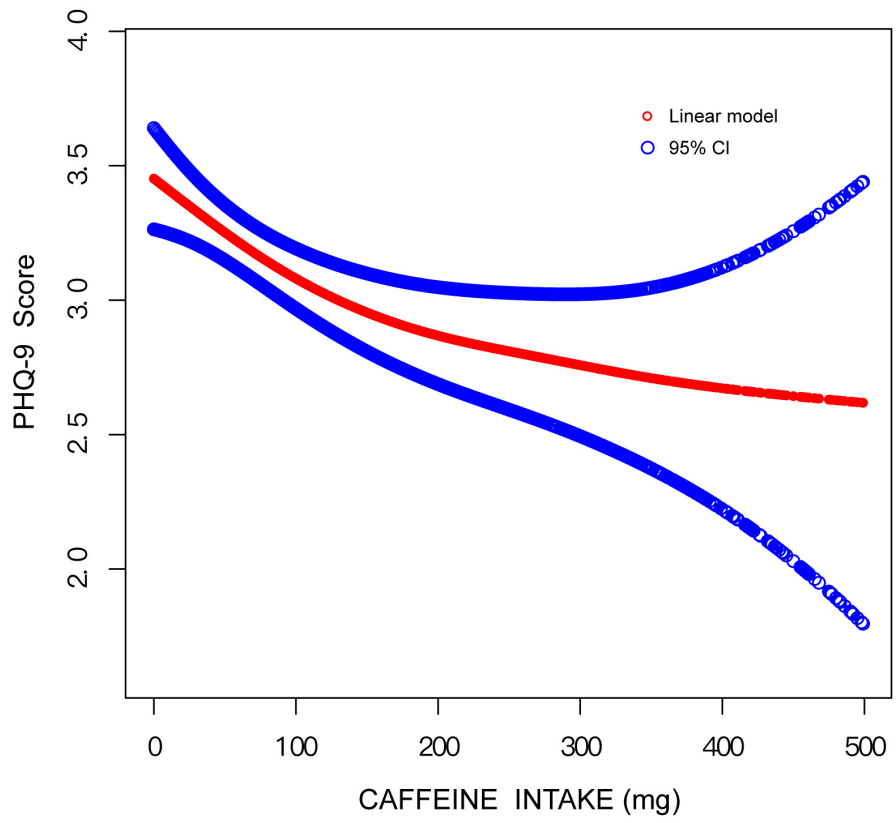

FIGURE 2
Curve fitting model on the association between caffeine intake and depression.

Monthly sleepiness is the frequency of overly sleepiness during the past month before patients answered the question. Marital status included "current" (married, living with partners), "past" (widowed, divorced, or separated), and "never" (never married). All these variables are available in NHANES.

Statistical analysis

The present study conducted the linear regression analysis and curve fitting analysis using the PHQ-9 score to examine the association between caffeine consumption and depression. Then the categorized depression (depression or non-depression) was used to conduct the logistic regression analysis. Subgroup analysis using linear regression analysis and curve fitting analysis were also performed. All analyses were conducted using EmpowerStats software (version 3.0) and the R Project for Statistical Computing (version 3.2.3), and $p < 0.05$ was considered statistically significant.

Results

A total of 3,263 participants (aged 20 years or older) were included in this analysis and divided into two groups: non-depression ($n = 3039$) and depression ($n = 244$), as shown in Table 1. There were statistically significant differences in caffeine intake, marital status, monthly sleepiness, moderate recreational activities, age, education level, smoking status, and sex between the groups. Compared to patients without depression, depressive patients are more likely to be female, younger, sleepier, have been married but broken up or never married, have not received higher education, have less than moderate recreational activities, tend to smoke, and have less caffeine intake.

Linear regression analysis was used to estimate the association between caffeine and depression and the results of which are presented in Table 2. In the non-adjusted model, patients with higher PHQ-9 score were more likely to have less caffeine intake (β: −0.002, 95% CI: −0.003 to −0.0006, *P* for trend: 0.002). The same conclusion was drawn according to the minimally adjusted model (β: −0.002, 95% CI: −0.003 to −0.0003, *P* for trend: 0.02) and fully adjusted model (β: −0.002, 95% CI: −0.003 to 0.0005, *P* for trend: 0.004). In the logistic regression model (as shown in Table 3), the results also indicated a negative association between caffeine consumption and depression (OR: 0.998, 95% CI: 0.9967 to 0.9991, $P < 0.001$).

More detailed results were found using curve fitting analyses (Figure 2). A negative relationship between caffeine and depression was found when caffeine intake was lower than 90 mg. However, when caffeine intake was greater than 90 mg, there was not a significant association between caffeine intake and depression.

In subgroup analyses, results of multivariate regression analysis stratified by education level, smoking status, sex, and marital status are presented in Table 4. The association between caffeine intake and depression only exists in those who do not smoke (β: −0.002, 95% CI: −0.003 to −0.0004, *P*: 0.01) and the "never" group in marital status (β: −0.004, 95% CI: −0.007 to −0.0004, *P*: 0.03). The association between caffeine consumption and depression was more apparent in those who have not received higher education (β: −0.002, 95% CI: −0.004 to −0.0003, *P*: 0.03) in subjects with high-level education level and in females (β: −0.002, 95% CI: −0.004 to −0.0001, *P*: 0.04). More detailed information could be seen in Table 4. In curve fitting analysis, for three kinds of patients (smoking, female or married in the past), the negative association between caffeine and depression was significant only when caffeine is lower than

TABLE 4 Association between caffeine intake and depression, stratified by higher education, sex, marital status, and cigarette smoking.

Caffeine	Model 1	Model 2	Model 3
Higher education			
Yes			
β (95% CI) *P*	−0.0024 (−0.004, −0.001) 0.001	−0.0021 (−0.004, −0.0005) 0.008	−0.0015 (−0.003, −0.0001) 0.03
No			
β (95% CI) *P*	−0.0006 (−0.0025, 0.0013) 0.55	−0.001 (−0.0027, 0.0013) 0.50	−0.002 (−0.0043, −0.0003) 0.03
Marital status			
Current			
β (95% CI) *P*	−0.002 (−0.004, −0.001) 0.001	−0.0006 (−0.002, 0.001) 0.43	−0.0010 (−0.002, 0.0004) 0.16
Past			
β (95% CI) *P*	−0.0029 (−0.006, −0.0002) 0.04	−0.0025 (−0.005, 0.0004) 0.09	−0.0015 (−0.004, 0.001) 0.29
Never			
β (95% CI) *P*	−0.0034 (−0.0066, −0.0002) 0.04	−0.0038 (−0.0072, −0.0004) 0.03	−0.0035 (−0.0066, −0.0004) 0.03
Sex			
Male			
β (95% CI) *P*	−0.0017 (−0.003, −0.0001) 0.04	−0.0013 (−0.003, 0.0003) 0.10	−0.0015 (−0.003, 0.0000) 0.052
Female			
β (95% CI) *P*	−0.0017 (−0.003, 0.0001) 0.06	−0.0017 (−0.004, 0.0002) 0.08	−0.0018 (−0.004, −0.0001) 0.04
Cigarette smoking			
Yes			
β (95% CI) *P*	−0.0027 (−0.004, −0.002) <0.0001	−0.0017 (−0.005, 0.002) 0.32	−0.0021 (−0.005, 0.001) 0.20
No			
β (95% CI) *P*	−0.0013 (−0.004, 0.002) 0.44	−0.0024 (−0.004, −0.001) 0.0002	−0.0016 (−0.003, −0.0004) 0.01

Non-adjusted model (Model 1): None. Minimally adjusted model (Model 2): Age; sex; race. Fully adjusted model (Model 3): Higher education; marital status; daily sleepiness; moderate recreational activities; race; cigarette smoking; sex and age.

respective certain doses (as showed in Figures 3A,C,D). But for Latin and non-Hispanic white, the situation is more complex (also showed in Figures 3B).

Discussion

The present study showed a negative association between depression and daily consumption of caffeine lower than 90 mg. Meanwhile, the results varied when considering factors as race, smoking status, sex, education level and marital status.

A previous study indicated that caffeine intake has a significant inverse association with depression, concluding that the caffeine's psychostimulant properties were able to protect against depressive symptoms (6). Some studies reiterated the complex association between caffeine intake and depression risk (11), and others have noticed the differences between males and females (12), so it is reasonable to include more confounders (variable Z) in future studies.

As for a mechanism, due to the similar structure, caffeine is able to compete with adenosine to combine with A1 receptors and A2A receptors (4, 13). When combining with A1 receptors, an increased basal transmission occurs (14). But the main mechanism is that after combining with A2A receptors, the synaptic plasticity decreased in excitatory synapses, which influences synaptic networks in the hippocampus to have the neuroprotective effects (15). And a cross-sectional population-based study shows that a single nucleotide polymorphism in A2A receptor gene has an association with depression (16), which also proves that A2A receptors play an important role in this negative association with depression. Besides, there exists two issues related to caffeine consumption. On the one hand, people often consume significant amounts of sugar with coffee, which causes the hyperinsulinemia and then stimulates plasminogen activator inhibitor (PAI)-1 production. So, the proteolytic cleavage of brain-derived neurotrophic factor (BDNF) precursor to mature BDNF is impaired by PAI-1 and brain remodeling in response to highly stressful situation or prolonged stress. On the other hand, sleep deprivation will be also influenced by the consumption of caffeine, resulting that cortisol levels and PAI-1 increase. Similarly, brain remodeling

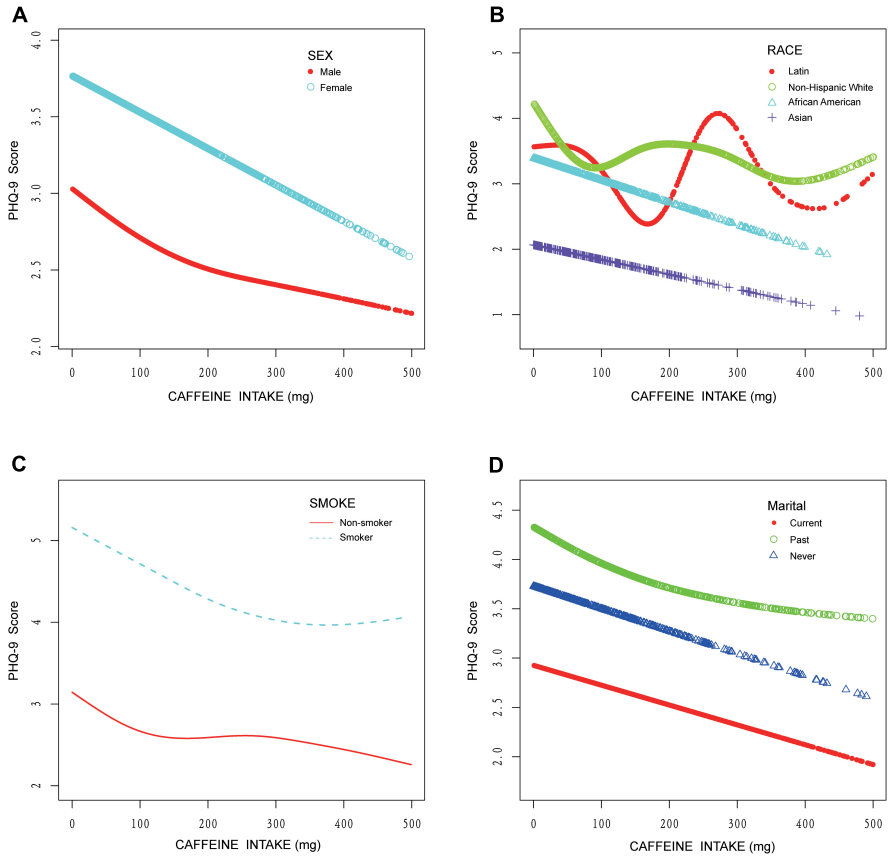

FIGURE 3
Curve fitting model on the association between caffeine intake and depression stratified by sex, race, cigarette smoking, and marital status. **(A)** Stratified by sex. **(B)** Stratified by race. **(C)** Stratified by cigarette smoking. **(D)** Stratified by marital status.

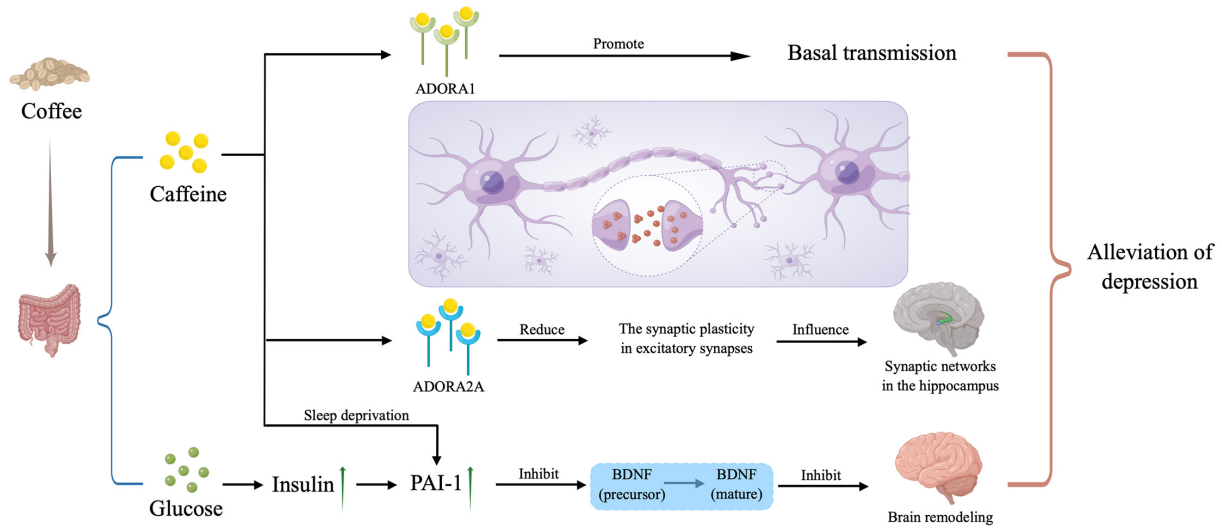

FIGURE 4
Potential mechanisms. Caffeine plays a role in the depression alleviation by combining with *ADORA1* and *ADORA2A* to influence our neural network in the brain, and inhibiting the mature of brain-derived neurotrophic factor (BDNF), and the latter way can also be triggered by large amounts of sugar taken in with caffeine when drinking coffee. PAI-1, plasminogen activator inhibitor; *ADORA1*, adenosine A1 receptor; *ADORA2A*, adenosine A2A receptor. The picture was drawn by Figdraw.

is prevented, and depression symptoms gets to relief. The mechanism above was shown in Figure 4.

In the present study, people who are female, younger, sleepier, have married but separated or haven't married, have not received higher education, have less moderate recreational activities, tend to smoke, and have less caffeine intake are more likely to be depressive. A previous report found that people with lower education showed a higher ratio of depression (17). The difference might be due to the different academic demands (18). Smoking is also associated with depression. Depressive patients tend to smoke as a self-meditation because nicotine will combine with nicotinic-cholinergic receptors (nAChRs) and then stimulates dopamine release in the nucleus accumbens, relieving depression symptoms (19). While education status is not easy to change, doctors could offer the patients who smoke to relieve depression alternatives to regulate reward so that they will not depend on cigarette smoking, for example, caffeine (20), as patients with more caffeine intake had less PHQ-9 score when caffeine consumption is lower than 90 mg. In fact, the chronic exposure to caffeine can afford the neuroprotection against a lot of noxious stimulation to the brain besides depression, thus substituting nicotine as self-administration in many aspects (19). (Food and beverage which would provide 90 mg of caffeine was included in Table 5. A dose of 90 mg is provided by a large cup of instant coffee). The co-administration between caffeine and depression deserves further discussion, on the one hand, caffeine can enhance the release of dopamine induced by nicotine (21); on the other hand, the function of nAChRs was under the control of A2A receptor (caffeine is the antagonist of A2A receptor) (22).

There are some limitations in the methods of the present study. First, this was a cross-sectional study based on a database, so only the association between caffeine intake and depression could be described while the casual connection cannot be accurately demonstrated. Also, only eight confounding factors were included, other potential confounding factors, such as trauma history, personal health history, income, and body mass index were not included. Only 3,263 samples were included in this study as only one cycle of the NHANES survey (2017–2018) was used and all samples with missing answers were excluded. The present study also excluded patients with too

TABLE 5 The consumption of food that contains 90 mg of caffeine (data provided by the USDA's Food and Nutrient Database for Dietary Studies).

Food and beverages	Consumption
Not reconstituted instant coffee	2.87 g
Reconstituted instant coffee	346.15 g
Espresso	42.45 g
Brewed coffee	225.00 g
Mocha coffee	272.73 g
Cappuccino	250.00 g
Green tea	750.00 g
Black tea	450.00 g
Black chocolate	160.71 g
Regular cola	1000.00 g
Diet cola	750.00 g
Energy drink (Red Bull)	310.34 g

much caffeine intake (more than 500 mg) as too much caffeine can cause other health problems. Moreover, this study did not conduct clinical evaluations of depression, but merely used the data provided by NHANES using PHQ-9 depression scale to evaluate the depression level of patients, which would not be so accurate. Meditation wasn't considered in the collection of caffeine intake, though caffeine is used in some medicine (23). Besides, the present study relied on a questionnaire rather than using the available biological measures to estimate the intake of coffee. In fact, although questionnaires have previously been shown to correlate with caffeine intake, the correlation is rather poor (circa 0.45) and it is observed that relations based on questionnaires are not always in line with data relying on biological estimates. Meanwhile, NHANES didn't establish the amount of caffeine in drinking water according to different areas. The sugary content in the beverage wasn't assessed, though previous study had found that hyperinsulinemia may contribute to the pathogenesis of depression (24). Another limitation of this study is that caffeine is only one of the xanthines acting as an antagonist of adenosine receptors, and other xanthines like theobromine (more abundant in cocoa/chocolate) is often more tightly related with alterations of brain function than caffeine (25) and theobromine is near equi-effective with caffeine to modulate the adenosine modulation system in the brain (26). Finally, most of the samples were non-Hispanic white.

In conclusion, the present study found that depression was negatively associated with caffeine intake (less than 90 mg). With the prevalence of depression reaching 22% worldwide, further research is needed to investigate the causal connection between caffeine and depression, and the different β value in different subgroups, to figure out whether and how caffeine could be applied to the treatment of depression. Also, in future studies the co-administration of glucose should be evaluated to elucidate its influence also on the affective state and depressive symptomatology.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary material**.

Ethics statement

The studies involving human participants were reviewed and approved by the National Center for Health Statistics (NCHS) Research Ethics Review Board (ERB). The patients/participants provided their written informed consent to participate in this study.

Author contributions

JB: writing—original draft, formal analysis, writing—review and editing, data curation, and project administration. PL: writing—original draft, formal analysis, writing—review and editing, conceptualization, and software. YG: writing—original draft, formal analysis, writing—review and editing, and data curation. YZ, MS, and JH: writing—original draft, formal analysis, and writing—review and editing. All authors contributed to the article and approved the submitted version.

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We appreciate the figdraw (https://www.figdraw.com) providing the tools to draw the **Figure 4**.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2022.1037579/full#supplementary-material

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Icariin reduces cognitive dysfunction induced by surgical trauma in aged rats by inhibiting hippocampal neuroinflammation

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Postoperative cognitive dysfunction (POCD) is a common postsurgical complication in elderly individuals, significantly impacting the quality of life of patients; however, there is currently no effective clinical treatment for POCD. Recent studies have shown that Icariin (ICA) has antiaging effects and improves cognitive function, but its effect in POCD has not been studied. In this study, we investigated the influence of ICA on cognitive function and the TLR4/NF-κB signaling pathway in a POCD rat model. We found that ICA reduced surgery-induced memory impairment, decreased hippocampal inflammatory responses, ameliorated neuronal injury in the hippocampus and inhibited microglial activation. In addition, we also observed that ICA inhibited activation of the TLR4/NF-κB signaling pathway. In summary, our research suggest that ICA can ameliorate surgery-induced memory impairment and that the improvements resulting from administration of ICA may be associated with inhibition of hippocampal neuroinflammation. Our research findings also provide insight into potential therapeutic targets and methods for POCD.

KEYWORDS

postoperative cognitive dysfunction, Icariin, neuroinflammation, surgery, cognitive impairment, TLR4/NF-κB

Introduction

Postoperative cognitive dysfunction (POCD) is a prevalent complication in elderly individuals following surgery, manifesting mainly as memory impairment, decreased information processing ability and reduced attention, and is also associated with various negative consequences, such as mood and personality alterations (Lin et al., 2020). According to the International Study of Postoperative Cognitive Dysfunction (ISPOCD), the incidence of POCD in elderly patients (aged over 60) was roughly 25.8% within seven days after surgery and approximately 10% within three months after surgery (Moller et al., 1998). Clinical evidence indicates that POCD may lead to long-term cognitive deficits, seriously decrease the quality of life of patients, prolong hospitalization after surgery, increase medical expenses and impose a substantial burden on both patients and society (Steinmetz et al., 2009). However, the pathogenic mechanism underlying POCD remains poorly understood, and there is no promising therapeutic agent for POCD.

The neuroinflammation caused by surgery plays a vital role in the development of POCD (Safavynia and Goldstein, 2018). Surgical trauma facilitates the proinflammatory state by triggering the release of excessive inflammatory mediators, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), from the peripheral immune system (Hirsch et al., 2016). These inflammatory cytokines can be transported to the brain through the disrupted blood–brain barrier (BBB), ultimately leading to neuroinflammation (Liu and Yin, 2018). Microglia are innate immune cells in the brain with immune regulation and phagocytosis functions. The increased levels of proinflammatory cytokines in the brain can hyperactivate microglia, inducing further release of proinflammatory cytokines, which causes the inflammatory cascade reaction and leads to a vicious cycle of neuroinflammation (Perry, 2004; Teeling and Perry, 2009). In addition, hyperactivated microglia elicit neurotoxic effects, leading to neuronal damage and influencing the function of neurons associated with learning and memory, ultimately causing cognitive impairment (Garden and Moller, 2006).

Icariin (ICA) is a flavonoid compound extracted from the leaves and stems of Herba Epimedii (Figure 1A), a traditional Chinese medicine plant, which has a broad spectrum of therapeutic effects, such as anti-osteoporosis, anti-aging, anti-inflammatory, and antioxidant effects (Wang S. et al., 2021). In the mouse model of diabetic nephropathy induced by streptozotocin (STZ), ICA inhibited the TLR4 signaling pathway, reduced the renal inflammatory response, and played a renoprotective role (Qi et al., 2021). In addition, ICA can improve learning and memory in animals during normal aging. Accumulating studies have provided robust evidence that ICA is a potential therapeutic agent for nervous system diseases (Li et al., 2021). In an Alzheimer's disease (AD) mouse model, ICA administration significantly enhanced learning and memory abilities in APP/PS1 transgenic mice during the Y maze task (Jin et al., 2014). Additionally, ICA decreased the amyloid beta (Aβ) load and amyloid plaque accumulation in the hippocampus of APP transgenic mice by lowering the expression of APP and BACE-1 (Zhang et al., 2014). Liu et al. (2019) found that ICA ameliorated neuroinflammation in the hippocampus by inhibiting the HMGB1/RAGE signaling pathway and played a neuroprotective role by activating the TLR4/NF-κB signaling pathway, resulting in reduced depressive behaviors in mice. Our preliminary experimental results showed that ICA can improve cognitive impairment induced by surgery in rats (Supplementary material); however, it remains unknown whether ICA can decrease neuroinflammation induced by surgery and thereby alleviate postoperative cognitive dysfunction. Consequently, this study aimed to assess the influence of ICA on POCD and further explore the role of the TLR4/NF-κB signaling pathway in the neuroprotective effect of ICA.

Materials and methods

Animals and grouping

Twenty-month-old male Sprague–Dawley rats, weighing between 600 and 650 g, were utilized in the experiments. These animals were procured from the Laboratory Animal Center of Gannan Medical University (Ganzhou, China). The rats were housed in a room with controlled humidity and temperature, following a 12-hour light/dark cycle, and provided with unrestricted access to food and water. The experiments were approved by the Ethics Committee of Gannan Medical University and adhered to the guidelines for the Care and Use of Laboratory Animals by the National Institutes of Health (NIH Publications No. 80-23). All experiments were performed such that they minimized both the number of animals used and the suffering of those animals.

To investigate the effect of ICA on the cognitive function of POCD rats, a total of 36 rats were randomly assigned to one of three groups: the control group, surgery group and surgery+ICA group (Figure 1B). The rats in the surgery+ICA group were gavaged with ICA (60 mg/kg, ICA was suspended in saline containing 1% DMSO) (Solarbio, Beijing, China) once a day for 7 consecutive days after surgery, while the rats in the other groups were gavaged with an equivalent volume of normal saline. The dosage of ICA was determined based on previous literature (Jiang et al., 2019).

Intramedullary fixation of tibia fracture

The rat model of POCD was established by intramedullary fixation of a tibial fracture, following the methods previously reported (Netto et al., 2018). Rats were anesthetized with 2.1% isoflurane, and the knee joint of the left hind limb was then depilated and locally sterilized. A longitudinal incision was made from the knee to the middle third of the tibia, the subcutaneous tissue was dissected and the muscle was removed from the patellar tendon and the tibial periosteum. A hole was drilled in the tibial tuberosity, and then a sterile Kirschner wire (diameter, 1 mm) was inserted into the intramedullary canal to the distal third of the tibia. Subsequently, osteotomy was performed at the junction of the middle and distal third of the tibia, and the incision was then sutured with 4/0 nylon after washing with sterile physiological saline. After surgery, rats were placed on a thermally insulating electric blanket until they regained consciousness. Penicillin was used to prevent infection, and lidocaine gel was applied for analgesia. The rats in the control group had their skin cut under isoflurane anesthesia and then sewn back on without causing a tibial fracture, and received the same postoperative anti-infective and analgesic treatment as the rats in the other groups.

Morris water maze test

The hippocampal-dependent memory and cognitive abilities were assessed using the Morris water maze, as detailed in a previous study (Feng et al., 2017). In brief, rats were placed in a circular swimming pool filled with warm water in which an escape platform was located 2 cm below the water surface in one quadrant's center. In the acquisition phase, rats were randomly released individually into the water facing the pool wall in each of the four quadrants four times per day for 7 continuous days before surgery. The animals were allowed 90 s per trial to find the platform (10 cm diameter) that was placed below the water surface and were allowed to rest

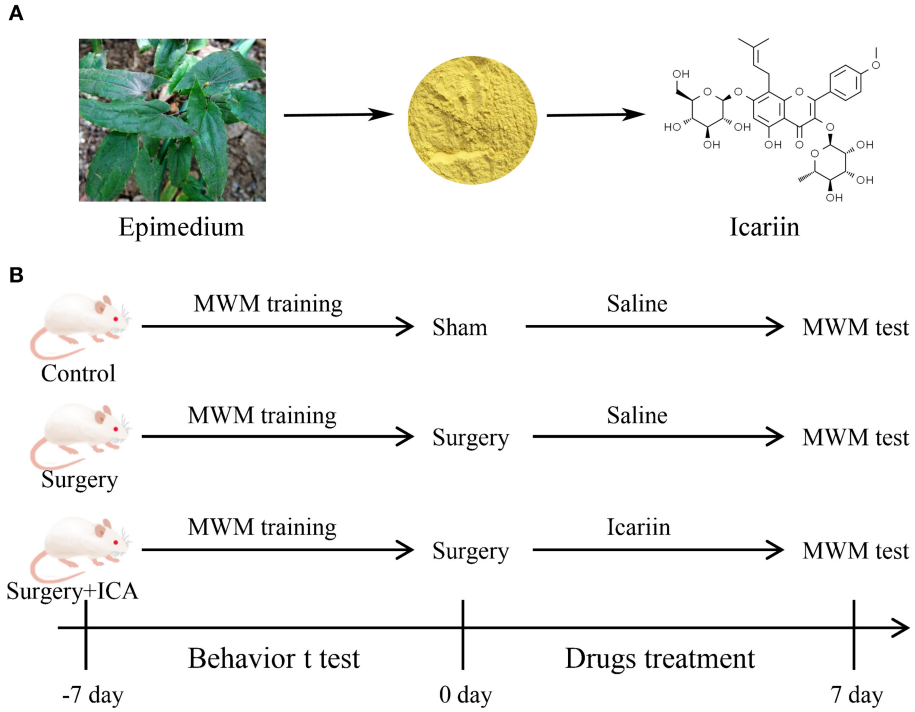

FIGURE 1
(A) Natural sources and chemical structure of Icariin (ICA). **(B)** Diagram of the experimental design. Thirty-six rats were randomly divided into the control, surgery and surgery + ICA groups. All rats were trained on the water maze test for 7 continuous days before the surgery. After modeling, each group was given ICA or saline solution once a day for 7 consecutive days. Subsequently, the water maze test was performed to examine spatial memory.

on it for 15 s. If a rat failed to find the platform within the allotted time (90 sec), an experimenter guided the animal to the platform. The time taken to climb onto the platform from entering the water was documented as the escape latency (maximum 90 sec). One week post POCD surgery, rats were subjected to a spatial probe test during which the platform was retracted from the pool. The number of platform crossings, the total time spent in the target quadrant, the time to first platform crossing and the swimming speed were automatically analyzed from video recordings using EthoVision® XT software (Noldus, Wageningen, Netherlands).

Hematoxylin and eosin (HE) staining and Nissl staining

After the Morris water maze test, the brains of the rats in each group were removed, and the brain tissue containing the hippocampus was then fixed with 4% paraformaldehyde at 4°C overnight. Then, the tissue samples were gradually dehydrated in an alcohol gradient and submerged in xylene. We embedded the samples in paraffin and then sectioned them at a thickness of 5 μM. After deparaffinization and rehydration at room temperature, the slices were stained with HE (Solarbio, Beijing, China) and Nissl staining solution (Solarbio, Beijing, China) in accordance with the manufacturer's instructions. The number of Nissl-positive cells in the hippocampal region was determined by a pathologist blinded to the grouping using a light microscope (Olympus, Tokyo, Japan) at 200× magnification. For each sample, three sections were taken, and three random fields were observed in each section. The total cell count was then divided by the number of observed fields to quantify the number of Nissl-positive cells in each sample.

Enzyme-linked immunosorbent assay (ELISA)

Blood samples and hippocampal samples were obtained after the Morris water maze test. The concentrations of TNF-α, IL-1β, and IL-6 in the serum and hippocampus were determined using enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, Wuhan, China) according to the manufacturer's protocol. The optical density (OD) value of each well was measured by an ELISA plate reader (Thermo Scientific, Waltham, MA, USA) at 450 nm. The experiments were repeated three times to allow calculation of mean values.

Western blot

Hippocampal tissues were homogenized and extracted by utilizing RIPA lysis buffer (Applygen Technology, Beijing, China), which contained protease inhibitor (TransGen Biotechnology, Beijing, China) and phosphatase inhibitor

(TransGen Biotechnology, Beijing, China). The total protein concentration in the supernatant was measured with a BCA protein assay kit (Solarbio, Beijing, China). Equal amounts of protein (50 μg/lane) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE), and subsequently transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA, USA). Next, the membranes were blocked with 5% BSA solution at room temperature for 2 hours and then incubated with primary antibodies specific for the following proteins overnight at 4°C: TLR4 (1:750, Proteintech Cat# 19811-1-AP), p-NF-κB p65 (1:1,250, Affinity Biosciences Cat# AF2006), NF-κB p65 (1:2,500, Proteintech Cat# 10745-1-AP) and GAPDH (1:20,000, Proteintech Cat# 10494-1-AP). After five washes with TBST, the membranes were incubated with the corresponding HRP-conjugated secondary antibodies (1:2,000, Proteintech Cat# SA00001-2) at room temperature for 1 hour. Finally, the membranes were visualized using ECL kits (Epizyme Biotechnology, Shanghai, China), and images were acquired by a chemiluminescent gel imaging system (ChemiDoc XRS+, Bio-Rad, PA, USA). Densitometric analysis of the protein bands was performed with Image Lab software.

Immunohistochemistry (IHC)

Rat brain samples were preserved using 4% paraformaldehyde, encased in paraffin, and sectioned into 5 μm-thick slices. After paraffin dewaxing and hydration, the slices were incubated with 3% H_2O_2 at room temperature for 10-15 min to inhibit endogenous peroxidase activity in the tissues. Then, the slices were subjected to antigen repair with 0.01 M sodium citrate using the thermal repair method in a microwave. Goat serum (10%) was used to block the nonspecific binding sites at 37°C for 30 min. The slices were incubated with the primary antibody against IBA-1 (1:500, Proteintech Cat# 10904-1-AP) at 4°C overnight and then incubated with goat anti-rabbit HRP-linked secondary antibody (1:1,000, Proteintech Cat# SA00001-2) at 37°C for 60 min. Then, the slices were coated with DAB buffer (Zhong Shan Golden Bridge Biotechnology, Beijing, China), and hematoxylin was used to counterstain nuclei. Finally, the hippocampal region was observed under a bright field fluorescence microscope (Carl Zeiss, Oberkochen, Germany) at 200× magnification. An experimenter who was unaware of the sample identities counted the IBA-1-positive cells using ImageJ software. The number of IBA-1-positive cells from three sections (three visual fields per section) were averaged for each animal and converted to cells/field.

Statistical methods

All the data are shown as the mean ± standard deviation (SD). GraphPad Prism 8.0 software was used for statistical analysis. One-way analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparisons test were used to evaluate the significance of differences. $P < 0.05$ indicated a statistically significant difference.

Results

ICA improves cognitive impairment of POCD rats

To test whether ICA improves cognitive function in POCD rats, the Morris water maze test was conducted. In the acquisition phase, all rats could find the platform after training and showed improvement in spatial learning and memory over time. There was no significant difference in the escape latency or swimming speed in the acquisition phase among the groups (Figures 2A–C), indicating that the rats in each group had the same baseline learning and memory ability. The spatial probe test (Figure 3E) was conducted on the 7th day after surgery, and the swimming speed of the rats did not differ significantly among the groups (Figure 3A), suggesting that fracture surgery did not affect the motor function of the rats. In comparison to the control group, both the time spent in the target quadrant and the number of platform crossings of rats in the surgery group were notably reduced (Figures 3C, D), and the time to first platform crossing of rats in the surgery group was significantly increased (Figure 3B), indicating that intramedullary fixation of tibia fracture resulted in cognitive dysfunction in rats, thus successfully establishing a POCD rat model. The effect of surgery was reversed by ICA treatment (Figures 3B–D), suggesting that ICA treatment improved memory capabilities in aged POCD rats. Taken together, these findings propose that ICA can alleviate cognitive impairment induced by surgery.

ICA alleviates neuroinflammation in hippocampus of POCD rats

Neuroinflammation associated with surgical trauma is the key pathogenic factor in POCD. Surgical stress leads to an increase in the levels of peripheral inflammatory factors, which subsequently leads to an increase in the level of inflammation in the hippocampus. Thus, we assessed the levels of inflammatory factors in the peripheral blood and hippocampus one week postsurgery. The serum concentrations of TNF-α rose in the surgery group (Figure 4A), while the serum levels of IL-1β and IL-6 were not significantly different among the groups (Figures 4B, C), indicating that peripheral inflammation returned to normal levels 7 days after surgery, and ICA had no significant impact on the normal peripheral inflammation level (Figures 4A–C). However, on the 7th day after surgery, the level of inflammation in the hippocampus remained high in the surgery group, while ICA treatment considerably reduced the levels of TNF-α, IL-1β, and IL-6 in the hippocampus of rats (Figures 4D–F). These data indicate that ICA can reduce neuroinflammation in the hippocampus.

ICA ameliorates neuronal damage in hippocampus of POCD rats

The HE staining results revealed that the neurons in the CA1, CA3 and DG regions in the control group were arranged in an orderly pattern, evenly stained, and regular in shape,

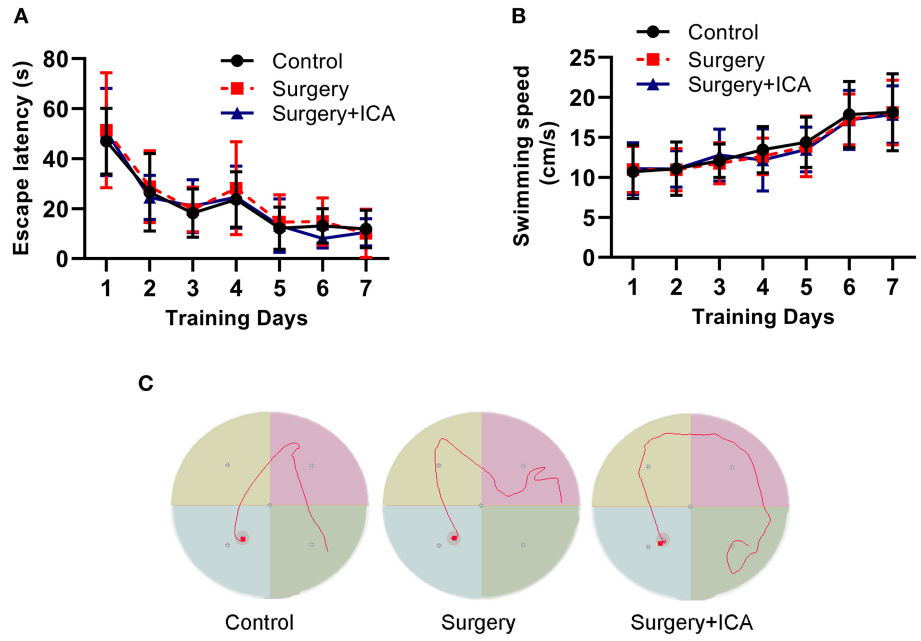

FIGURE 2
Performance of rats in the training phase of the water maze test. **(A, B)** Rats were trained to find the platform for 7 continuous days preoperatively, and there was no significant difference in the escape latency and swimming speed among the groups. **(C)** Results of motion trials of rats after 7 days of training in the Morris water maze test. $n = 12$ per group.

whereas those in the surgery group were scattered, irregular, and unevenly stained (Figure 5). After ICA treatment, the neurons were regularly shaped, and the staining was uniform, indicating that the pathological damage to neurons was reversed by ICA (Figure 5). To evaluate neuronal survival in the CA1, CA3 and DG regions of the hippocampus, Nissl staining of brain sections was performed (Figure 6A). Nissl staining showed that the density of neurons in these hippocampal regions was normal in the control group, while the number of Nissl-positive cells obviously decreased in the surgery group (Figures 6B–D). However, ICA treatment significantly attenuated this effect (Figures 6B–D).

ICA inhibits the activation of microglia of POCD rats

Microglia have a crucial function in mediating neuroinflammation and neuronal damage in the hippocampus. Therefore, we labeled microglia with IBA-1 7 days postsurgery. As shown in Figure 7A, the number of microglia labeled with IBA-1 in the hippocampus of rats from the surgery group significantly increased. In addition, the number of endpoints and branch length of microglia in the surgery group decreased (Supplementary Figures 1B, C), indicating that microglia were activated in rats in the surgery group. After ICA treatment, the number of IBA-1-tagged microglia decreased (Figures 7B–D), the number of endpoints and branch length of microglia increased (Supplementary Figures 1B, C), indicating that the activation of microglia was inhibited (Figures 7B–D). These data suggest that ICA treatment can inhibit the activation of microglia and thus protect neurons in the hippocampus.

ICA inhibits the activation of TLR4/NF-κB signaling pathway

The TLR4/NF-κB signaling pathway is closely related to neuroinflammation in central nervous system diseases. We evaluated the expression of proteins associated with the TLR4/NF-κB signaling pathway (Figure 8A). In comparison to the control group, the protein levels of TLR4 and phosphorylated NF-κB in rats in the surgery group increased (Figures 8B–D), suggesting that surgical stimulation activated the TLR4/NF-κB signaling pathway, consistent with previous studies (Shi et al., 2020). In contrast, ICA treatment decreased the protein levels of TLR4 and phosphorylated NF-κB (Figures 8B–D), indicating that the activation of the TLR4/NF-κB signaling pathway was likely inhibited by ICA in POCD rats, which may ultimately inhibit microglia.

Discussion

In this research, we investigated the effect of ICA on the cognitive function of POCD rats and the potential mechanism underlying this effect. Our research showed that ICA reduced the level of cognitive impairment induced by surgery, which was manifested by the increase in the number of platform crossings, the time spent in the target quadrant and in the decrease in the time to first platform crossing after ICA treatment in the water maze test. ICA also lowered the level

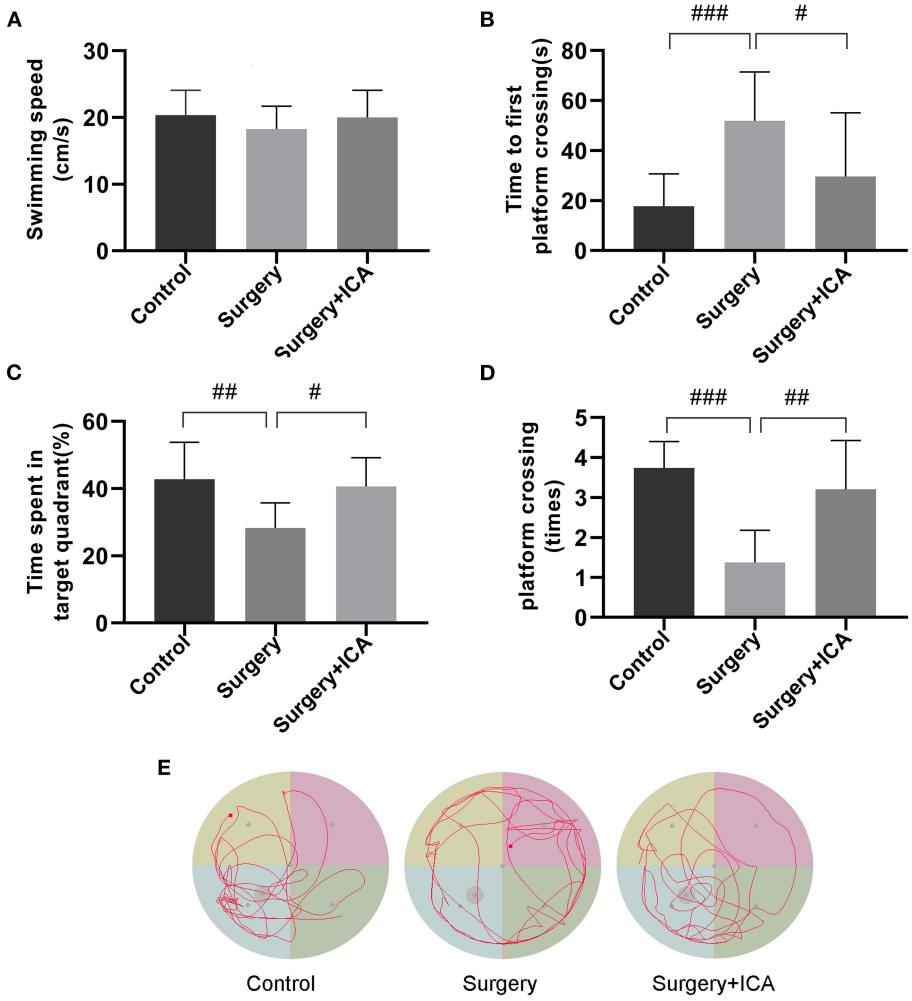

FIGURE 3
ICA improves cognitive impairment in POCD rats. **(A–D)** In the spatial probe test, swimming speed, time to first platform crossing, the time spent in the target quadrant and the number of platform crossings were used to assess motor function and memory ability by analyzing the motion trajectory. **(E)** The motion trajectory of rats in each group. The data are expressed as the means ± standard deviations; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. $n = 12$ per group.

of inflammatory factors in the hippocampus. HE staining, Nissl staining and immunohistochemistry showed that ICA ameliorated neuronal damage in the hippocampus and inhibited the activation of microglia. More importantly, we also found that ICA inhibited the activation of the TLR4/NF-κB signaling pathway in the hippocampus.

Currently, surgical trauma is believed to be the main cause of POCD (Saxena and Maze, 2018); thus, surgical trauma was used to establish a POCD model in this study. In addition, it was found that POCD was more common in patients who underwent clinical fracture surgery (Uzoigwe et al., 2020). To better simulate the clinical characteristics, we used intramedullary fixation of tibial fractures to induce POCD in this experiment. The Morris water maze is a classical method for evaluating the cognitive function of rodents. In this study, we conducted a navigation experiment with the Morris water maze test before surgery. Rats were tested four times a day for seven consecutive days to ensure that all rats in each group could find the platform and had the same spatial memory ability in the water maze test. On the 7th day after surgery, a spatial probe test was conducted on rats in each group. The findings revealed that the number of platform crossings and the time spent in the target quadrant were decreased and the time to first platform crossing was increased after surgical trauma stimulation, indicating that surgical stress caused cognitive dysfunction in rats and that the POCD model was successfully established. However, after ICA was given to POCD rats, the number of platform crossings and the time spent in the target quadrant increased, and the time to first platform crossing decreased in the water maze test, indicating that ICA can improve cognitive function in POCD rats.

Previous reports have indicated a close relationship between neuroinflammation and cognitive dysfunction caused by surgery (Subramaniyan and Terrando, 2019; Wang J. et al., 2021). To investigate whether the improvement in cognitive function after administration of ICA in aged POCD rats was related to the inhibition of neuroinflammation, we measured the levels of proinflammatory cytokines such as TNF-α, IL-1β and IL-6 in the

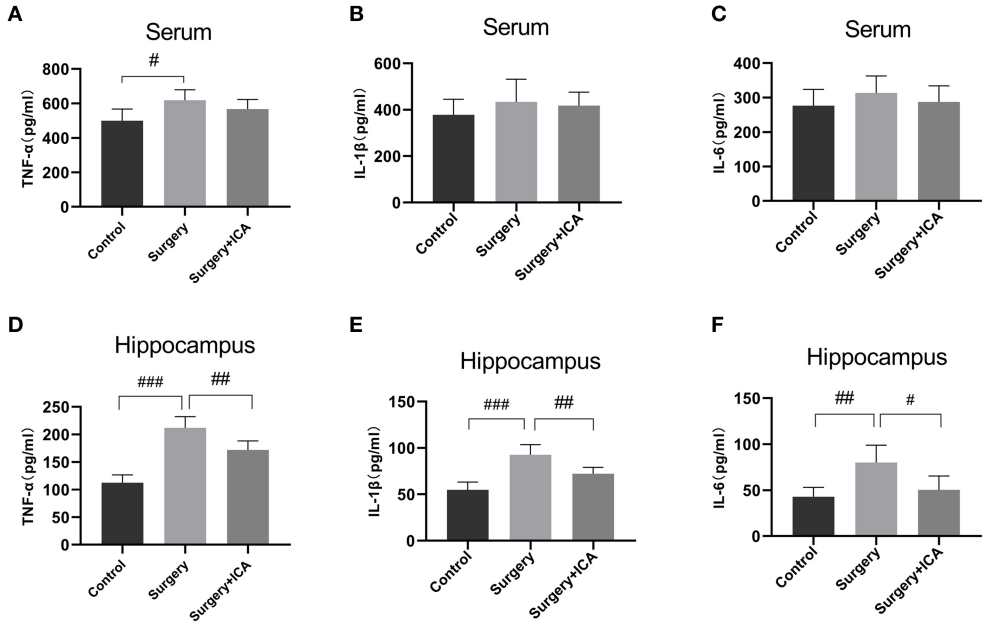

FIGURE 4
ICA alleviates neuroinflammation in the hippocampus of POCD rats. **(A–C)** The levels of serum inflammatory cytokines TNF-α, IL-1β and IL-6 among the groups were analyzed using an ELISA. **(D–F)** The levels of neuroinflammation were assessed by detecting the expression of TNF-α, IL-1β and IL-6 in the hippocampus. Data are expressed as the means ± standard deviation; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. $n = 6$ per group.

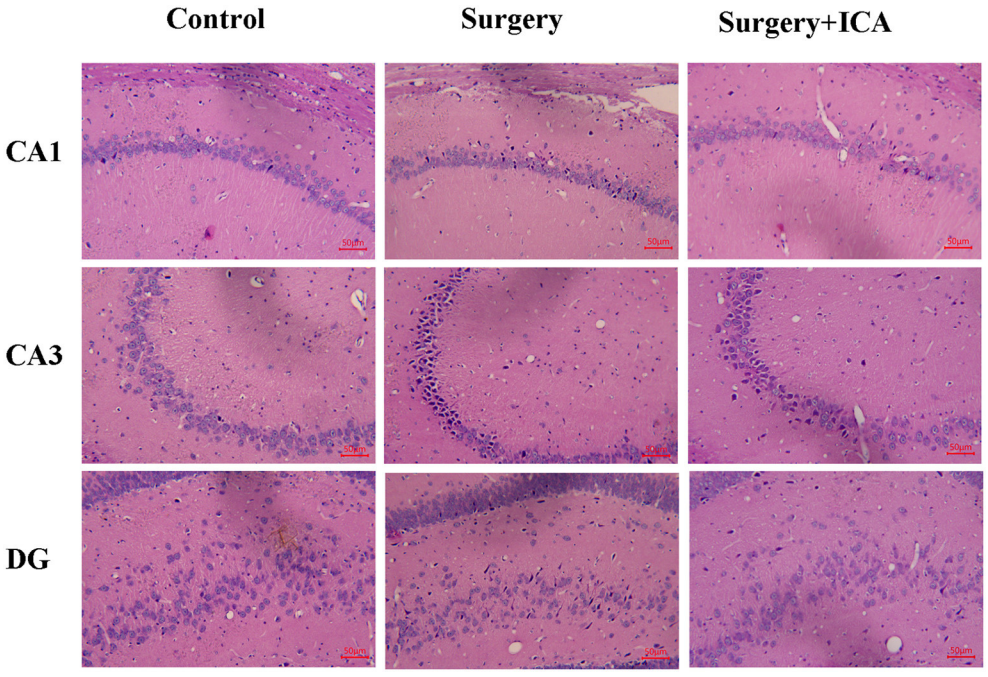

FIGURE 5
ICA ameliorates neuronal damage in the hippocampus of POCD rats. After HE staining, the morphological changes in the CA1, CA3 and DG regions of the rat hippocampus were observed under an optical microscope (200×).

peripheral blood and hippocampus. It is believed that surgical stress leads to increases in the levels of peripheral inflammatory factors, which can enter the central nervous system through the damaged BBB, leading to increased neuroinflammation (Saxena et al., 2019). Our study showed that the levels of peripheral inflammatory factors were basically restored to the baseline levels 7 days after surgery, while hippocampal inflammation remained high. ICA treatment did not reduce the baseline level of peripheral inflammation but did

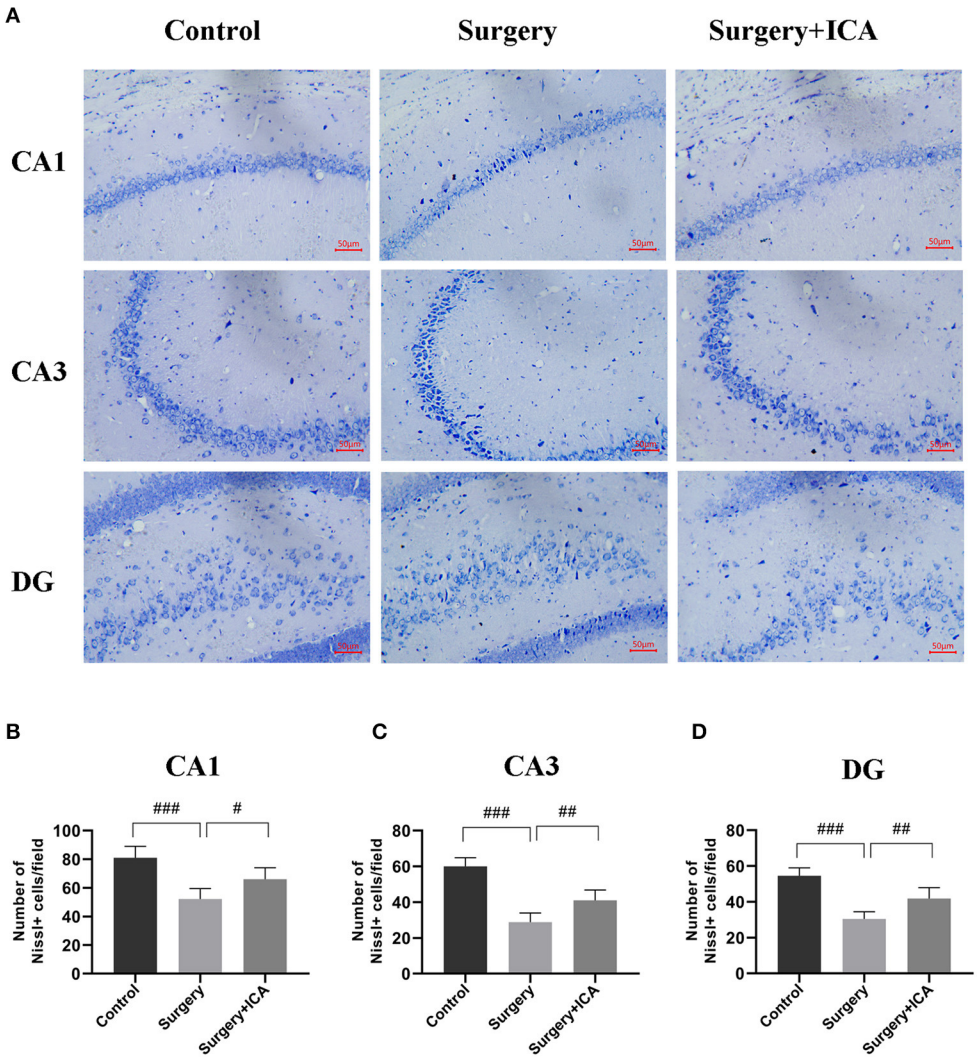

FIGURE 6
ICA ameliorates neuronal damage in the hippocampus of POCD rats. **(A)** Nissl staining was applied to detect the histopathological changes in hippocampal neurons in CA1, CA3 and DG (scale bar = 50 μm). **(B–D)** The number of Nissl-positive cells in CA1, CA3 and DG were quantified. The data are expressed as the means ± standard deviations; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. $n = 6$ per group.

reduce the high level of neuroinflammation in the hippocampus, suggesting that ICA inhibited inflammation in the hippocampus. Notably, ICA can also reduce oxidative stress to accelerate fracture healing and promote postoperative recovery (Gurbuz et al., 2019), which may be another reason that ICA ameliorated cognitive impairment induced by surgery.

Accumulating evidence has demonstrated that activation of microglia can aggravate neuroinflammation (Li et al., 2020). Normally, microglia are in an inactive state. However, microglia can be activated during inflammation and BBB destruction. On the one hand, activated microglia can secrete numerous inflammatory factors, such as TNF-α, IL-1β and IL-6, and exacerbate neuroinflammation; on the other hand, activated microglia are neurotoxic and can cause damage to neurons (Chen et al., 2019; Wang Z. et al., 2021). In this study, we examined the morphology and number of neurons in the CA1, CA3 and DG regions of the hippocampus and the activation of microglia. Our results showed that surgery induced the activation of microglia and caused damage to neurons, and ICA reversed this change, consistent with the reduction in hippocampal neuroinflammation.

In addition, we observed that ICA inhibited the TLR4/NF-κB signaling pathway. In the central nervous system, TLR4 is expressed by microglia, astrocytes and neurons (Islam et al., 2022). Activation of TLR4 promotes NF-κB nuclear translocation, which subsequently regulates the mRNA transcription of various proinflammatory mediators, including TNF-α, IL-1β and IL-6, aggravating central nervous inflammation (Zhou et al., 2020). In addition, the TLR4/NF-κB signaling cascade promotes microglial polarization toward the M1 phenotype, which has potent phagocytic properties and is proinflammatory (Ebrahim Soltani et al., 2022). Therefore, the TLR4/NF-κB signaling pathway is crucial in microglia activation and neuroinflammation. Our research showed that intramedullary fixation of tibial fracture activated the TLR4/NF-κB signaling pathway, aligning with prior

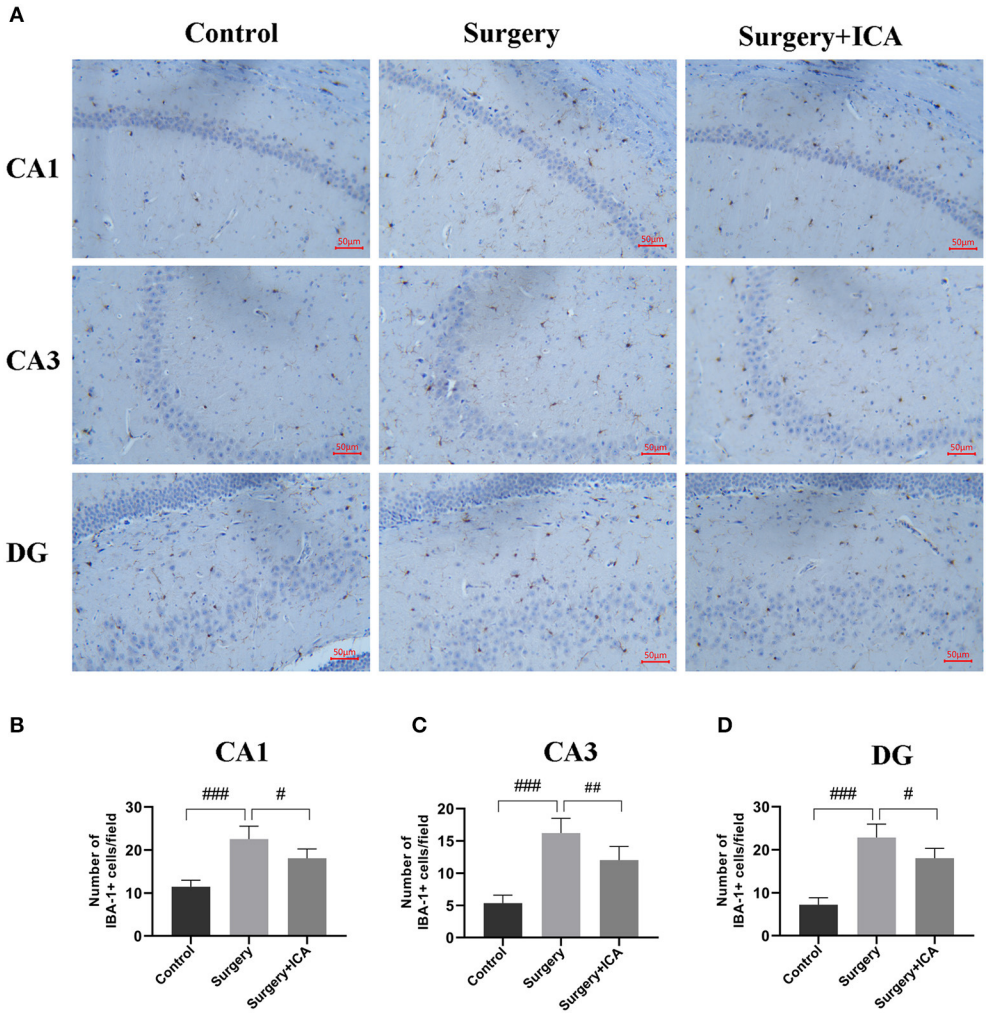

FIGURE 7
ICA inhibits the activation of microglia in POCD rats. **(A)** The activation of microglia in the CA1, CA3 and DG regions of the hippocampus was evaluated by immunohistochemistry (scale bar = 50 μm) in each group. **(B–D)** The number of IBA-1-positive cells in CA1, CA3 and DG was counted using ImageJ software. The data are expressed as the means ± standard deviations; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. $n = 6$ per group.

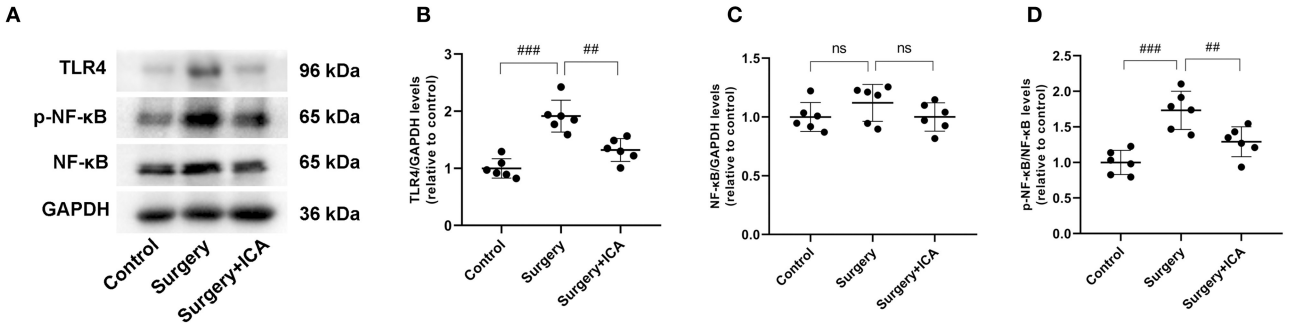

FIGURE 8
ICA inhibits the activation of the TLR4/NF-κB signaling pathway. **(A)** The protein expression levels of TLR4, NF-κB p65, and p-NF-κB p65 were analyzed using Western blotting. **(B)** The ratio between the optical density value of TLR4 versus GAPDH in the dentate gyrus. **(C)** The ratio between the optical density value of NF-κB versus GAPDH in the dentate gyrus. **(D)** The ratio between the optical density value of p-NF-κB versus the total NF-κB in the dentate gyrus. The data are expressed as the means ± standard deviations; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. $n = 6$ per group.

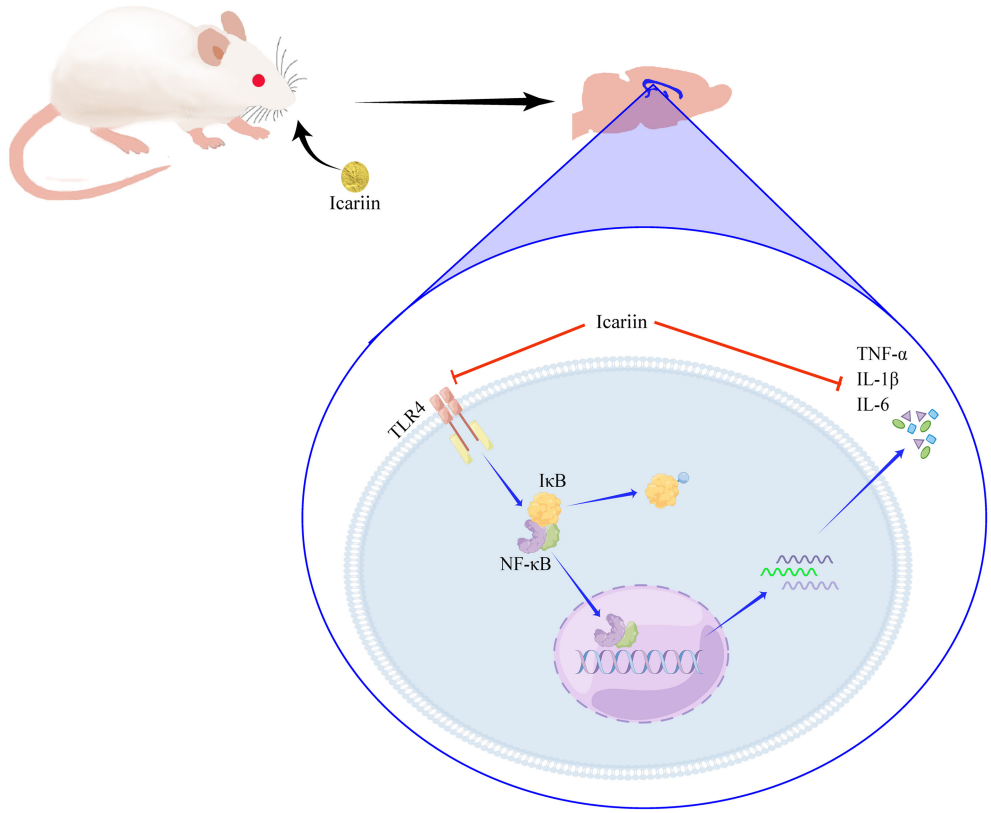

FIGURE 9
ICA attenuated neuroinflammation by inhibiting the TLR4/NF-κB signaling pathway.

research findings (Wang et al., 2013; Lu et al., 2015). After treatment with ICA, the TLR4/NF-κB signaling pathway was inhibited. Therefore, it is speculated that ICA may inhibit the release of proinflammatory factors and ameliorate POCD by inhibiting the TLR4/NF-κB signaling pathway. However, a previous study showed that ICA played a neuroprotective role and reduced depressive behavior in mice by activating the TLR4/NF-κB signaling pathway, which contradicts our findings (Liu et al., 2019). These contradictory results may be due to the TLR4/NF-κB pathway playing different roles in different stages of neuroinflammation. In the early stage of neuroinflammation, the TLR4/NF-κB signaling pathway can promote inflammation, while in the late stage of neuroinflammation, the TLR4/NF-κB signaling pathway plays a neuroprotective role and promotes the regeneration of neurons.

This study had several limitations. First, this study solely explored the expression of the TLR4/NF-κB signaling pathway in the rat hippocampus, without assessing its presence in neurons or glial cells. As reports have indicated that the TLR4/NF-κB signaling pathway exists in both neurons and neuroglia, future research should explore the expression of the TLR4/NF-κB signaling pathway in neurons and glial cells of the rat hippocampus by using double immunofluorescence staining. Second, our assessment of inflammation caused by surgery was limited to the hippocampus. However, surgery may have different effects on inflammation in different regions of the brain, such as the striatum, prefrontal cortex, and amygdale (Hovens et al., 2015). Future research should focus on the potential impact of surgery on other brain regions. Third, the present investigation primarily centered on early postoperative cognitive performance, and further research is needed to observe long-term cognitive function. Finally, in this study, we only used male rats, and future research should further investigate the effects of Icariin on postoperative cognitive dysfunction and neuroinflammation in female rats.

Conclusion

In conclusion, our study showed that ICA can decrease cognitive dysfunction by ameliorating neuroinflammation in aged POCD rats. Moreover, the underlying mechanisms may involve the suppression of the TLR4/NF-κB signaling pathway (Figure 9). ICA may be a promising drug for the treatment of postoperative cognitive dysfunction in elderly patients.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Ethics Committee of Gannan Medical University.

Author contributions

JY and MZ contributed to the conception and designed the research study. LW and GP wrote the manuscript, did the HE and Nissl staining, and did the Western blots. JY revised the manuscript. MZ, LC, and BW did the animal caring and surgery. YZ and JZ performed the MWM. BW and YZ did the Elisa assay. JZ and MG did the immunohistochemistry. JY, LW, and GP did the data collection and conducted the statistical analysis. All authors participated in manuscript revision, proofreading, and approved the submitted version criteria.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnbeh.2023.1162009/full#supplementary-material

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Coffee consumption decreases the connectivity of the posterior Default Mode Network (DMN) at rest

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Habitual coffee consumers justify their life choices by arguing that they become more alert and increase motor and cognitive performance and efficiency; however, these subjective impressions still do not have a neurobiological correlation. Using functional connectivity approaches to study resting-state fMRI data in a group of habitual coffee drinkers, we herein show that coffee consumption decreased connectivity of the posterior default mode network (DMN) and between the somatosensory/motor networks and the prefrontal cortex, while the connectivity in nodes of the higher visual and the right executive control network (RECN) is increased after drinking coffee; data also show that caffeine intake only replicated the impact of coffee on the posterior DMN, thus disentangling the neurochemical effects of caffeine from the experience of having a coffee.

KEYWORDS

coffee, resting-state, connectomics, default mode network, executive control network

1. Introduction

There is a common expectation, namely among habitual coffee drinkers, that coffee increases alertness and psychomotor functioning. For these reasons, many individuals keep drinking coffee to counteract fatigue, stay alert, increase cognitive performance, and increase work efficiency (Smith, 2002). Coffee beverages are constituted of numerous compounds known to affect human behavior, among which are caffeine and chlorogenic acids (Sadiq Butt et al., 2011). From the neurobiological perspective, both caffeine and chlorogenic acids have well-documented psychoactive actions, whereas caffeine is mostly an antagonist of the main adenosine receptors in the brain—A_1 and A_{2A} receptors, leading to the disinhibition of excitatory neurotransmitter release and enhancement of dopamine transmission via D2 receptors (Fredholm et al., 2005) to sharpen brain metabolism and bolster memory performance (Paiva et al., 2022); chlorogenic acids can directly impact neuronal performance through mechanisms that still need to be understood (Lorist and Tops, 2003; Fernandes et al., 2021).

While the neurochemical action of these compounds seems to be reasonably understood, the psychological effect of coffee/caffeine, although largely genuine, remains a matter of debate and should be considered in the context of its use. In fact, some studies show that caffeine has effects on cognitive performance and mood if taken by non-habitual coffee drinkers, but that these effects may decline due to the development of tolerance (James and Rogers, 2005). While some studies seem to point to an effect of caffeine in decreasing lethargy/fatigue and increasing vigor (Judelson et al., 2013), others suggest that the cognitive and emotional effects only occur after 8 h of abstinence (Heatherley et al., 2005), and others even point to the fact that a significant component of the psychological effect of coffee/caffeine should be attributable to the reversal of adverse withdrawal effects associated with short periods of abstinence from the intake (James and Rogers, 2005) or even to the suggestion of having had coffee beverages (Liguori and Hughes, 1997; Dawkins et al., 2011).

Only limited information is available about the impact of coffee intake on the whole-brain network activity, for which the use of novel imaging techniques has proven to be of relevance. Functional magnetic resonance imaging (fMRI) allows us to study, in a non-invasive way, the function of the human brain during the execution of specific tasks or at rest (Soares et al., 2016). In the context of coffee consumption, some previous studies show that there is the activation of different cortical and subcortical areas during a visuomotor task (Park et al., 2014), or upon the hedonic evaluation of caffeine and saccharin (Gramling et al., 2019), or of the frontopolar and cingulate cortex during a two-back verbal working memory task (Koppelstaetter et al., 2008; Haller et al., 2017), or in a modified Sternberg task (Klaassen et al., 2013). Moreover, recent studies suggest an impact of coffee on brain functional connectivity at rest prompting a functional reorganization toward more efficient network properties with implications in emotionality, alertness, and action readiness (Kim et al., 2021; Magalhães et al., 2021).

Despite these results, there is still an open question on what drives habitual coffee drinkers to keep drinking coffee. Herein, we aim to explore the resting-state fMRI data in a group of habitual coffee drinkers before and after acute coffee consumption, using network functional connectivity approaches. We hypothesize that coffee consumption will lead to higher integration of networks linked to the prefrontal cortex associated with executive memory and with brain health throughout the lifespan, such as the posterior default mode network (DMN) (Buckner et al., 2009; Leech et al., 2011).

2. Materials and methods

2.1. Ethics statement

The present study was conducted in accordance with the principles expressed in the Declaration of Helsinki (59th amendment) and was approved by the ethics committee of Hospital de Braga. All participants gave written informed consent upon the explanation of the aims of the study and were allowed to withdraw from it and stop their participation in the experiments at any time during the study.

2.2. Subject recruitment and assessment

All subjects were recruited from the general healthy Portuguese population. Exclusion criteria included the presence of neurological or psychiatric disorders, habitual consumption of mind-altering substances (such as cannabinoid substances), and the inability to undergo an MRI scanning session. The inclusion criteria in order to be considered a coffee drinker was to drink a minimum of one cup of coffee per day. Then, volunteer subjects who agreed to take part in the study were asked to abstain from consuming caffeinated beverages or food for at least 3 h before participating in the study. In total, 47 subjects were recruited who fulfilled the inclusion criteria. Subjects' sociodemographic data were collected during an interview and then they performed an MRI/fMRI scanning session.

2.3. MRI brain imaging

MRI scans were conducted using a Siemens Verio 3T (Siemens, Erlangen, Germany) located in Hospital de Braga (Braga, Portugal) using a 32-channel head antenna. The scanning session included as an anatomical acquisition one sagittal magnetization-prepared rapid acquisition with gradient echo (MPRAGE, TR/TE = 2,420/4.12 ms, FA = 9°, 1 mm^3 isometric voxel size, and Field-of-View = 176 × 256 × 256 mm^3). The resting state fMRI (rs-fMRI) acquisition used a multi-band echo planar imaging sequence, CMRR EPI 2D [R2016A, Center for Magnetic Resonance Research, University of Minnesota, Minnesota, USA (Feinberg et al., 2010; Moeller et al., 2010; Xu et al., 2013)] sensitive to fluctuations in the blood oxygenation-dependent level contrast (two sets of parameters were used: TR/TE = 1,190/34 ms, FA = 62°, 2 mm^3 isometric voxel size, and 76 axial slices over a matrix of 256 × 256 mm^2; and TR/TE =1,000/27 ms, FA = 62°, 2 mm^3 isometric voxel size, and 64 axial slices over a matrix of 200 × 200 mm^2). Each rs-fMRI acquisition had a duration of 7 and a half min, during which subjects were instructed to remain with their eyes closed, relaxed, and let their minds wander freely. Each subject was scanned twice using such acquisitions: a baseline acquisition at the start of the scanning session, immediately followed by coffee intake (containing 85 mg of caffeine over 100 ml of water, no added sugar), and 30 min after coffee intake, a second fMRI acquisition was performed.

2.4. Preprocessing of MRI data

MRI results included in this article come from preprocessing performed using fMRIPrep 1.4.1 (Esteban et al., 2019). A full description of the preprocessing pipeline can be found in the Supplementary material.

2.5. Resting-state analysis

For all resting-state analyses, a dummy variable was included as a covariate to control for the potential impact of the two different resting-state sequences used. Moreover, sex, age, and

education level were also included as covariates. Then, a general-linear model (GLM) was performed in order to remove the effect of the confounding variables from both resting-state acquisitions, and the residuals were used as a signal of interest for the analysis.

2.5.1. Independent component analysis

Resting-state network (RSN) maps were analyzed voxel-wise through a probabilistic independent component analysis (ICA) as implemented in multivariate exploratory linear optimized decomposition into independent components (MELODIC), distributed with FSL (Beckmann and Smith, 2004). Probabilistic ICA is a fully data-driven approach that enables the isolation of components based on the temporal correlation of the corresponding areas while maximizing the spatial independence between components. Then, dual-regression analysis was performed to estimate the subject-specific components that correspond to the group-wise RSNs. Because the probabilistic ICA approach may identify noisy components corresponding to non-biological signals, such as movement artifacts, the independent components were selected after the visual inspection of their spatial distribution (Horowitz-Kraus et al., 2015). Specifically, components that were mainly present in regions that do not generate the blood oxygen level-dependent (BOLD) signal (white matter, ventricles, or outside the brain) were excluded from the analysis.

Then, the RSNs functional connectivity (FC) was compared between both resting-state sequences (after the removal of confounding effects), using paired t-tests within a non-parametric permutation procedure implemented in the randomize tool from FSL (Winkler et al., 2014). Threshold-free cluster enhancement (TFCE) was used to detect widespread significant differences and control the family-wise error rate (FWE-R) at $\alpha = 0.05$. For each contrast, 5,000 permutations were performed.

2.5.2. Functional connectomics analysis

For the functional connectomics analysis, data were first extracted for each subject from a 284 regions atlas that combined 200 cortical regions from the Schaefer parcellation (doi: 10.1093/cercor/bhx179) with 84 subcortical functional regions from the Shen atlas (Shen et al., 2013), thus combining two different functional atlases for full brain coverage. For each subject pair of acquisitions, and to transform the data into matrices of FC, a *Pearson's* correlation between time series was calculated, followed by the Fisher's Z transformation to normalize its distribution.

To overcome the issue of multiple comparisons induced by the large number of connections in the network, we applied the network-based statistics (NBS) approach (Zalesky et al., 2010), as we have previously described (Magalhães et al., 2021). This approach provides a correction equivalent to the FWE-R by estimating the probability of identifying, in a random permutation of the tested data, networks to a larger extent than the ones identified in the hypothesis tested. This is done in two phases. The first step tests the statistical hypothesis at each point of the matrix, which is then filtered by a user-chosen statistical edge threshold. Significant network components are identified as sets of threshold-surviving connections between nodes, such that each node in the network can be reached from any other, through significant connections. The component size is calculated as the number of significant connections. While the connection threshold is not directly determinant of the network significance, it determines the possible extent of the network, with lower significance edge thresholds revealing larger and more widespread networks and higher thresholds resulting in smaller and more focused effects. As such, following the toolbox authors' recommendation, we explored a range of edge thresholds between 0.005 and 0.00005. Then, step 2 creates random permutations of the data set and applies the same methodology as step 1 to each permutation, determining the size of network components found. Finally, the calculated distribution of components' size is used to estimate the probability of finding random components with a size greater than the one found in our hypothesis. A total of 5,000 permutations were used, together with a FWE corrected network significance of 0.05. To test the acute impact of caffeine intake, we used a paired t-test comparison between the pre- and post-coffee intake acquisitions.

2.6. Impact of caffeine on the results

To help understand if the results from the previously described analysis extend beyond the impact of caffeine consumption, we conducted a complementary analysis using a second population of habitual coffee drinkers ($N = 36$). Instead of a coffee drink, subjects from this group were given the same amount of hot water with the same amount of caffeine diluted in it. Following each of the previous analyses, we extracted the data from the networks of interest for the ICA analysis and from the significant network component for NBS and tested for the same effect within the caffeine drinkers. This analysis was conducted using paired sample t-tests.

3. Results

For the analysis of coffee consumption, we have studied 47 individuals (31 women), with an average age of 30 years (SD 7.9). To discriminate the effect of caffeine in the results, we studied an additional group of 36 individuals (27 women), with 32.1 (SD 11.1) years of age.

3.1. Independent-components analysis

A total of 30 components were obtained from the probabilistic ICA, and 12 of these components were found to be representative of the most typical RSNs (see Supplementary Figure 1). We found significant FWE-R TFCE corrected pre-post coffee intake differences in several networks (see Figure 1).

In the higher visual network, the connectivity with the left middle occipital gyrus (MOG) was increased after drinking coffee (MNI coordinates = −26, −82, 34; 3 voxels; peak t-value = 4.54),

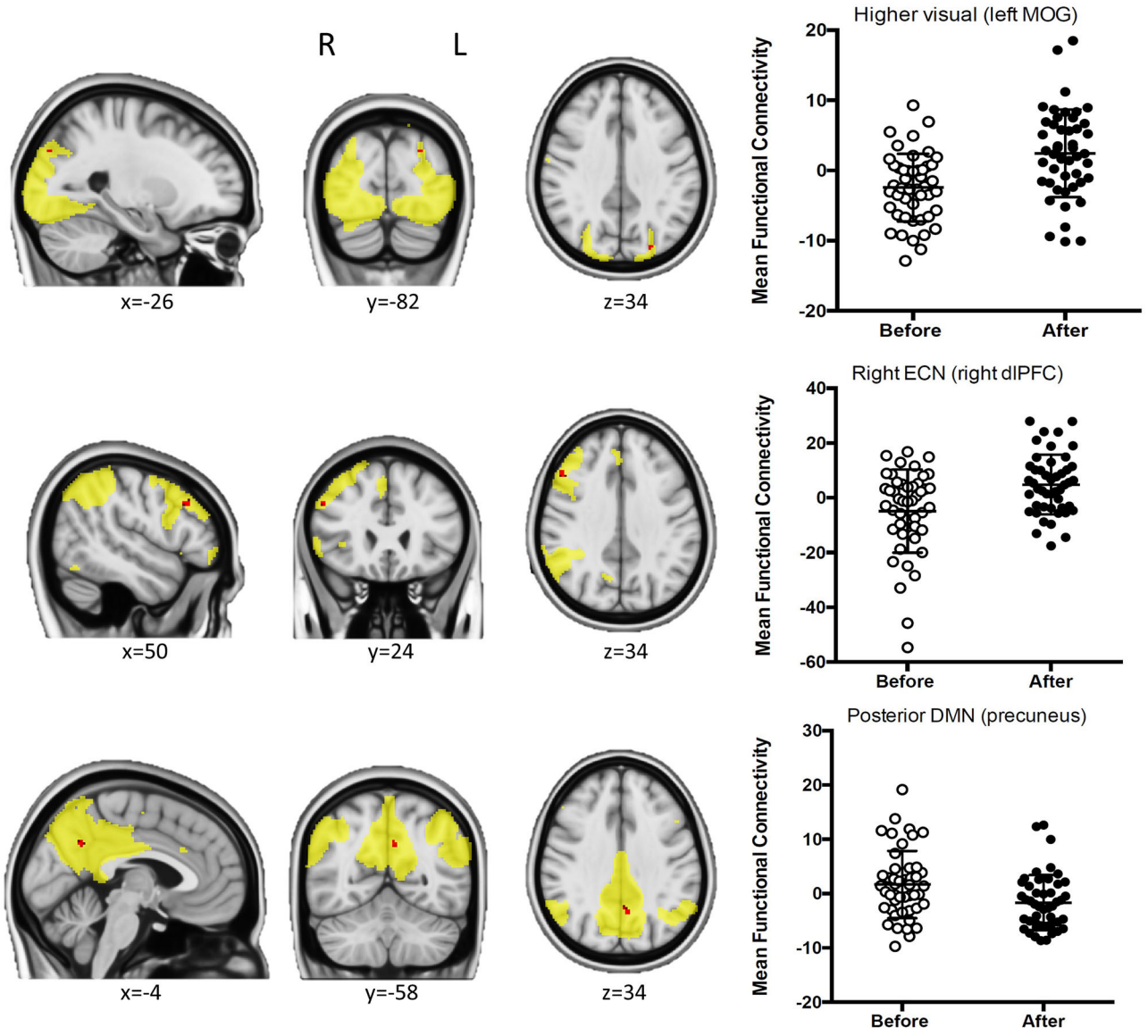

FIGURE 1
Results from ICA. For those clusters showing significant pre-post coffee intake connectivity differences, sagittal, coronal, and axial views **(left)** of the clusters, and box plots **(right)** representing the mean connectivity before and after are shown. The FWE-R TFCE corrected clusters are presented in red and overlaid on their corresponding networks in yellow.

as well as the connectivity of the right dorsolateral prefrontal cortex (dlPFC) within the right executive control network (RECN; MNI coordinates = 50, 24, and 34; 13 voxels; peak *t*-value = 4.81).

On the other hand, for the posterior DMN, the connectivity in a cluster within the left precuneus decreased after coffee intake (MNI coordinates = −4, −58, 34; 14 voxels; peak *t*-value = 4.63).

When running paired *t*-tests in the caffeine group for the ICA networks showing significant differences in this analysis (higher visual, RECN, and posterior DMN), we found no significant modulation of the higher visual and the RECN networks, while for the posterior DMN, we found a significant effect and in the same direction than in the group that drank coffee (decreased connectivity after caffeine intake; MNI coordinates = −8, −84, 44; 29 voxels; peak *t*-value = 5.35).

3.2. Connectomics analysis

The NBS analysis revealed a network of decreased functional connectivity that was modulated by coffee consumption. Components with different extents of this network were found to be significant for thresholds higher than 0.001 (statistics for all tested thresholds are presented in Table 1). To facilitate visualization, we present here only the network obtained for a threshold of p = 5e-05 [t(threshold) = 4.26, network p = 0.022, hedge's g = 0.71, eight nodes and eight edges; Figure 2]. This network was mostly composed of nodes from the somatosensory/motor and default mode networks and from the prefrontal cortex. Specifically, we highlight regions overlapping the precentral gyrus (nodes #118 and #16), the superior and medial frontal gyrus (#81, #88, #160, and

TABLE 1 Results of the functional connectomics analysis using NBS.

Threshold (p, t)	p (network)	g	N nodes	N edges	FC before	FC after
0.005, 2.69	0.061	–	–	–	–	–
0.001, 3.27	0.036	0.60	86	143	0.087	−0.088
0.0005, 3.52	0.030	0.63	60	81	0.092	−0.091
0.0001, 4.04	0.039	0.70	9	10	0.109	−0.109
0.00005, 4.26	0.022	0.71	8	8	0.112	−0.112

For each of the five thresholds used, we report here the p significance of the networks (for $p < 0.1$), Hedge's g, as a measure representative of the effect size, as well as the network size represented as the number of nodes, the number of edges in the network, and the mean network FC for each moment.

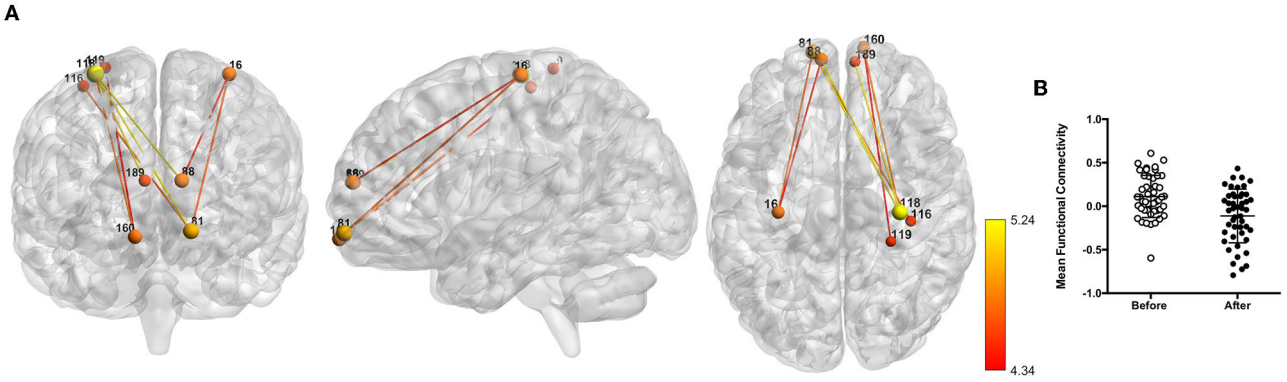

FIGURE 2
Network of reduced functional connectivity (FC) after coffee consumption for a threshold of $p < 5e\text{-}05$. **(A)** Coronal, sagittal, and axial view of the networks, with connections color-coded from red to yellow according to the statistical strength of the effect. **(B)** Box plots representing the before and after coffee consumption average FC.

#189), and the middle frontal gyrus (#88). Furthermore, at lower threshold levels, effects can also be found in other brain regions (Supplementary Figure 2).

Testing for significant differences in the caffeine group within the networks obtained in this analysis, we found a trend for a difference in this network ($p = 0.087$).

4. Discussion

The current study, by comparing resting-state FC before and after coffee consumption in habitual coffee drinkers, reveals that the connectivity of the posterior DMN is decreased after drinking coffee, while the connectivity in nodes of the higher visual and the right executive control network (RECN) is increased after drinking coffee. Importantly, the decrease in the posterior DMN connectivity is replicated by caffeine intake, whereas the alterations observed in the higher visual and the RECN are not. These observations add to our knowledge of the motivation to drink coffee and disentangle the brain connectivity effects that are attributable to caffeine (posterior DMN), from those that are triggered by other dimensions of coffee intake.

One of the major findings of this study relates to the impact of coffee (and caffeine) on the connectivity of the posterior DMN, namely in the left precuneus. The components of the DMN are known as a cognitive and physiological neurobiological framework necessary for the brain to respond to external stimuli (Buckner et al., 2008). In particular, the precuneus is a dynamic area of the brain involved in self-consciousness, memory, and visuospatial imagery (Fletcher et al., 1995; Lou et al., 2004; Cavanna and Trimble, 2006; Andrews-Hanna et al., 2010; Boly et al., 2012), functions commonly reported to be altered after coffee intake. Similarly, coffee and caffeine are well-known to induce wakefulness, and the finding herein reported is noticeable given that the precuneus network is connected to subcortical areas of the reticular activating system implicated in arousal (Tomasi and Volkow, 2011). Additionally, it is worth noting that the precuneus has emerged as a central node of the DMN and perhaps the most connected hub in the cortex (Cavanna, 2007; Buckner et al., 2008; Tomasi and Volkow, 2011; Utevsky et al., 2014; Pereira-Pedro and Bruner, 2016) and, therefore, the observation of decreased connectivity after coffee and caffeine intake at rest in this node points to a higher preparedness to switch from resting to task-context processing after coffee intake (Childs and De Wit, 2006). Curiously, and in accordance, the precuneus has been implicated in the guidance of motor responses but also in higher achievement motivation, namely in the left hemisphere (Childs and De Wit, 2006).

Another relevant finding of this study was the coffee-associated decreased functional connectivity between the somatosensory/motor networks and the prefrontal cortex; more specifically, there was decreased functional connectivity between the precentral gyrus and the superior and medial middle frontal gyrus after coffee intake. Although not statistically significant, there

was a relevant trend toward a similar observation after caffeine intake. This result reveals that caffeine consumption in habitual coffee drinkers prepares them for action. Briefly, the initiation and execution of movements comprise a prefrontal area involved in movement planning, a frontal premotor area involved in movement coordination, the primary motor cortex (i.e., precentral gyrus), from which the final movement command emerges, and the primary sensory cortex (i.e., postcentral gyrus), which allows adaptation through sensory feedback. It has been suggested that the connectivity strength between motor regions and the prefrontal cortex seems to be more associated with cognitive control because the extent to which these interactions occur at rest might be an indication of motor performance during subsequent task performance (Langan et al., 2010; Seidler et al., 2015); this would explain the reported gains in psychomotor efficiency experienced after the intake of caffeinated coffee (Smith, 2002).

Interestingly, increased functional connectivity was observed after coffee, but not caffeine, intake in the higher visual and RECN networks. The middle occipital lobe is implicated in visual processing, and the executive control network (a frontoparietal network that includes among others the dorsolateral prefrontal cortex) is engaged in high-level cognitive functions such as working memory, cognitive control, and goal-directed behavior (Seeley et al., 2007; Menon, 2011; Niendam et al., 2012). Our observation that coffee, but not caffeine, impacts the connectivity in these networks is interesting as higher functional connectivity in executive control and visual networks may underlie cognitive control and visual imagery but also because that is attributable to other components of coffee intake besides caffeine. The latter points to an imaginary sensation (in this case visual) that may trigger pleasure as observed for other stimuli like music (Zatorrea and Salimpoor, 2013) that do not seem to rely on the established neurochemical effects of caffeine in adenosine receptors but rather on the sensory experience of having coffee, which is determined by a complex interaction of multiple compounds present in coffee beverages (Poole and Tordoff, 2017; Lang et al., 2020).

One important issue that should be discussed whenever studying the effect of caffeine in brain networks relates to the design and interpretation of resting-state BOLD fMRI studies, as caffeine is a cerebral vasoconstrictor that may cause an increase in the concentration of deoxyhemoglobin and thus a decrease in the BOLD baseline resting signal (Mulderink et al., 2002). Indeed, it was shown that caffeine reduced the measures of resting-state BOLD connectivity in the motor cortex in parallel with decreased baseline cerebral blood flow and spectral energy in the low-frequency BOLD fluctuations (Rack-Gomer et al., 2009), and another study confirmed not only a reduction in whole-brain global perfusion (Haller et al., 2013) after caffeine but also a caffeine-enhanced task-related activation in a local and distributed network that was most pronounced in the bilateral striatum and to a lesser degree in the right middle and inferior frontal gyrus, bilateral insula, left superior and inferior parietal lobule, as well as in the cerebellum bilaterally. In that same study, it was shown that functional connectivity was significantly enhanced in caffeine vs. placebo in a distributed and task-relevant network including the prefrontal cortex, the supplementary motor area, the ventral premotor cortex, and the parietal cortex, as well as the occipital cortex (visual stimuli) and basal ganglia; importantly, the activation strength of the task-relevant-network component correlated with response accuracy for caffeine yet not for placebo, indicating a selective cognitive effect of caffeine (Haller et al., 2013). Another layer of complexity when analyzing the effects of caffeine in resting-state brain networks relates to the individual heterogeneity in the response and in the nature of the effect. In fact, some studies show that functional connectivity decreases due to caffeine vary among subjects, and a correlation analysis between the changes in functional connectivity and regional blood flow suggested that the effect of caffeine on BOLD functional connectivity was predominantly neural (motor/visual cortices) and partly vascular (DMN). However, it was shown that, after caffeine ingestion, the DMN involved more attentional networks, and more extrastriatal areas were integrated into the functional connectivity of the visual cortex, which may be associated with the known pharmacological effect of caffeine in elevating alertness (Wu et al., 2014). In further support of the cognitive effect of caffeine, it is important to also highlight the findings that the thalamus is involved in mediating the interaction of attention and arousal in an attentional task under different levels of arousal (sleep deprivation or caffeine administration) (Portas et al., 1998) that caffeine stabilizes the extent of neuronal activation in repetitive word stem completion, counteracting the general task practice effect (Bendlin et al., 2007), and the modulatory effect of caffeine on brain regions (medial frontopolar and anterior cingulate cortex) that have been associated with attentional and executive functions (Koppelstaetter et al., 2008).

Finally, it is important to highlight some methodological limitations of the present study. One of the limitations is the absence of a non-drinker control sample (to rule out the withdrawal effect) or an alternative group consuming decaf coffee (to rule out the placebo effect of coffee intake); this fact should be taken into account in future studies on this topic. However, as a methodological strength, we have included a group that took caffeine (instead of coffee) to discriminate the effects that should be attributed to caffeine and not to expectations of having coffee, which is of relevance as there are studies showing that both caffeine and expectation of having consumed caffeine improved attention and psychomotor speed (Dawkins et al., 2011). Another limitation of the current study relates to the fact that we have only studied resting-state brain connectivity, and further studies should also address the impact of coffee/caffeine in task-related events, which are certainly more insightful in the establishment of psycho-cognitive effects of coffee intake and in the discrimination of differences in the patterns of responses to caffeine by habitual consumers and habitual non-consumers. The latter may go some way to explaining why some individuals become caffeine consumers, in light of evidence showing that caffeine tended to benefit consumers' mood more while improving performance more in the non-consumers (Haskell et al., 2005).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Hospital de Braga. The patients/participants provided their written informed consent to participate in this study.

Author contributions

NS, RM, PM, and AC contributed to the conception and design of the manuscript. RV, TC, LA, and MS organized the database. MP-P and RM performed the statistical analysis and wrote the first draft of the manuscript. NS and RC wrote sections of the manuscript. All authors contributed to the manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnbeh.2023.1176382/full#supplementary-material

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Role of environmental enrichment on social interaction, anxiety, locomotion, and memory in Wistar rats under chronic methylphenidate intake

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Introduction: Chronic use of various compounds can have long-lasting effects on animal behavior, and some of these effects can be influenced by the environment. Many environmental enrichment protocols have the potential to induce behavioral changes.

Aim: The aim of the present study was to investigate how environmental enrichment can mitigate the effects of chronic methylphenidate consumption on the behavior of Wistar rats.

Methods: The animals were housed for 20 days under either an environmental enrichment protocol (which included tubes of different shapes) or standard housing conditions. After seven days, half of the rats received 13 days of oral administration of methylphenidate (2 mg/kg). After seven days, the rats underwent behavioral tests, including the elevated plus maze (anxiety), open field (locomotion), object-in-place recognition test (spatial memory), and a test for social interaction (social behavior).

Results: The results showed that the enriched environmental condition reversed the enhanced time in the open arms of the elevated plus maze induced by methylphenidate ($F_{[1,43]} = 4.275$, $p = 0.045$). Methylphenidate also enhanced exploratory rearing in the open field ($F_{[1,43]} = 4.663$, $p = 0.036$) and the time spent in the open area of the open field ($H[3] = 8.786$, $p = 0.032$). The enriched environment mitigated the inhibition of social interaction with peers induced by methylphenidate ($H[3] = 16.755$, $p < 0.001$) as well as the preference for single exploratory behavior ($H[3] = 9.041$, $p = 0.029$).

Discussion: These findings suggest that environmental enrichment can counteract some of the effects of methylphenidate. These results are relevant for the clinical treatment of the long-lasting secondary effects associated with methylphenidate pharmacological treatment.

KEYWORDS

methylphenidate, psychostimulants, social interaction, anxiety, enriched environment, ADHD, rat

Introduction

"Methylphenidate (MPH) is the most commonly prescribed pharmacological treatment for attention deficit hyperactivity disorder (ADHD) and belongs to a class of psychostimulant drugs (Jaeschke et al., 2021). Psychostimulant drugs have the potential to impact behavior, cognition, and emotional functions in both animals and humans (Anghelescu and Ahlers, 2020). When administered to humans, psychostimulants can lead to various behavioral effects, including euphoria and alterations in cognitive or physical performance; the specific effects depend on several factors such as the type of psychostimulant (e.g., cocaine, amphetamine, and methylphenidate), route of administration, and the individual's condition at the time of drug intake (Koob et al., 2020). While MPH can significantly improve the functioning of brain structures affected by ADHD, it may have detrimental effects on other areas (Coon et al., 2014). Furthermore, under certain conditions, the use of MPH can increase the risk of substance abuse (Linssen et al., 2014). These considerations highlight the need to investigate potential adverse effects associated with unnecessary consumption of psychostimulant drugs, including MPH as the most prescribed medication for ADHD.

Additionally, there is a growing interest in examining other factors that may mitigate the consequences of chronic MPH use, such as environmental enrichment, which is recognized as a protective factor against substance abuse (Rodríguez-Ortega and Cubero, 2018) and as a useful tool in reversing the emotional and behavioral effects induced by early life stressful situations (Corredor et al., 2022).

It has been demonstrated that low doses of psychostimulants such as MPH can improve attention and memory processes in both humans and rats (Spencer et al., 2015). According to the same authors, low doses are the ones used clinically to treat attention deficits in humans. For the pharmacological treatment of ADHD in humans, a low dose of MPH is effective in improving attention and memory by addressing prefrontal cortex hypoactivity (Cheng et al., 2014). However, high doses of the same drug have been shown to lead to aggressive behaviors and other behavioral and cognitive impairments (Smith and Farah, 2011). Environmental enrichment has also demonstrated the ability to mitigate the effects of stress on memory and cognitive functions, thereby enhancing their development (Macartney et al., 2022).

MPH consumption can not only affect cognitive processes but also interfere with emotional processing, such as anxiety. It has been reported that MPH can reduce anxiety levels, unlike other stimulants such as methamphetamines (Boyette-Davis et al., 2018). This potential anxiolytic effect is supported by reports on the enhanced time spent in the open arms of the elevated plus maze after MPH administration (Martin et al., 2018). However, it is important to consider that the anxiolytic effect of methylphenidate can vary depending on the baseline anxiety levels of each animal prior to consumption (Segev et al., 2016). In contrast, there is also evidence that in some cases, methylphenidate consumption can increase anxiety in animals, and this effect can be mitigated by physical activity or forced exercise in the animals (Motaghinejad et al., 2015). The evidence regarding the consumption of psychostimulants and their impact on anxiety is extensive and, at times, contradictory. Therefore, it is important to continue studying this topic. There is an interest in investigating whether other environmental variables, such as environmental enrichment, can mitigate the effect of chronic MPH consumption on anxiety.

Social interaction is another area of significant behavioral impact of psychostimulants. The social environment of an animal, including humans, directly influences its development (Grigoryan et al., 2022). Consequently, in recent years, the social behavior of animals has been extensively studied (Hackenberg et al., 2021), with many of these studies being conducted on rats. In the social behavior of rats, the mere proximity between individuals is a good predictor of the intention to interact. It is known that social behavior can be inhibited by MPH consumption (Vanderschuren et al., 2008). Conversely, other variables, such as environmental enrichment, have shown to promote the development of social behavior in rats by facilitating social responses between individuals (Neal et al., 2018). Consequently, there is an increasing interest in studying the effect of environmental enrichment on human social behavior and its therapeutic potential in treating neurodevelopmental problems (Ball et al., 2019; Corredor et al., 2022).

Furthermore, several studies have demonstrated that psychostimulants, while suppressing social behavior in animals, can also modify locomotion and increase rearing behavior (Šlamberová et al., 2015). Specifically, it has been shown that MPH consumption can increase locomotion in animals both in open-field tests and in their normal home-cage activity (Martin et al., 2018). These changes in locomotion can be explained by observed alterations in the animals' dopaminergic system resulting from chronic methylphenidate treatment and the environmental conditions (standard vs. enriched) in which they are raised (Gill et al., 2013). Environmental enrichment has been shown to influence animals' sensitization to modify their locomotion in response to methylphenidate (Malone et al., 2022). Given the potential of this variable, it is relevant to continue studying whether an enriched environment can reverse the effects of a psychostimulant on animal locomotion.

The present study aims to analyze the role of environmental enrichment on chronic, low-dose consumption of the psychostimulant methylphenidate in relation to anxiety, memory, social interaction, and locomotion in Wistar rats. To achieve this, animals receiving chronic treatment with MPH were housed in either a standard or enriched environment, and behavioral tests for anxiety, locomotion, exploration, and social interaction were conducted.

Method

Animals

A total of forty-seven male Wistar rats were utilized for this experiment. The rats were derived from a Wistar strain from Charles River Laboratories and were grouped in sets of four individuals and placed in polycarbonate cages (16.5 cm × 50 cm × 35 cm). The cages were maintained in a controlled environment featuring a 12-h light/12-h dark cycle (lights on at 06:00), ad libitum availability of food (Zeigler 104) and water, a consistent room temperature of 22 ± 2°C, and humidity maintained at 57 ± 10%. Due to the fact that the interest of this work is not the comparison of data between males and females, females were not included in the experiments in order to avoid possible hormonal interferences in the effects of methylphenidate and exposure to enriched environments. In fact, there are reports that show complex effects of the enriched environment on females, possibly due to hormonal factors (Corredor et al., 2022). Random assignment was conducted to allocate

the rats into two different housing conditions: standard housing conditions (St) and an enriched environment (Ee). Each housing condition group was further divided into two subgroups: one receiving methylphenidate administration (MPH) and the other receiving vehicle administration (Vh). The rats were housed in their respective conditions from postnatal day 25 (PND25) to PND46. Throughout the study, the rats had unrestricted access to food and water.

For the social interaction experiments, an additional twenty-four animals from the same facility were utilized as "external peers."

All experimental procedures were carried out in compliance with the Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the Universidad de Los Andes.

Drugs

Methylphenidate (Ritalin) was administered orally in a dose of 2 mg/Kg in syrup presentation. The animals in the control groups received only vehicle. To administer the drug to the animals, the tablet was crushed daily, and a 2 mg/kg dose was measured and diluted in 0.05 mL of a water and honey syrup. Subsequently, each rat's weight was recorded, and the dose was calculated accordingly. Once the required amount was determined, it was drawn into a syringe and orally administered to each rat. This method proved effortless, as the rats readily consumed the entire dosage. The animals that were part of the group that did not receive the drug also received the honey and water solution using the same procedure. Several studies that have allowed a washout period of 3 to 15 days from the last dose of Ritalin have demonstrated that the behavioral effects of the drug persist beyond this period (Askenasy et al., 2007).

Procedure

At postnatal day 25 (PND25), the subjects were placed in their designated housing condition. The enriched condition consisted of circular platforms and squared or circular PVC pipe parts, which were alternated every other day to prevent habituation, following the environmental enrichment protocols established in the Laboratory of Neuroscience and Behavior of the University of Los Andes. This involves using three distinct PVC pipe shapes to modify cage structures, offering novel surfaces and hiding spots for interaction with new items. To prevent habituation, objects are rotated every 3 days within the housing box. This form of enrichment is considered passive, as it does not require direct animal interaction. The standard condition animals were housed without any additional elements inside their cages.

After a period of 7 days (PND32), the animals were randomly assigned to one of the two treatment groups: methylphenidate or vehicle. The administration of methylphenidate or the vehicle began at this point and continued once a day for a duration of 13 days, until PND45. The drug was administered orally in syrup form, chosen for its pleasant taste and to minimize any stress associated with alternative administration methods. Following the completion of the drug administration, a six-day withdrawal period was provided to the animals to avoid immediate withdrawal effects.

Subsequently, all animals underwent behavioral tests as outlined in Figure 1, which describes the procedure.

Behavioral testing

Elevated plus maze

On postnatal day 53 (PND53), all the animals underwent the Elevated Plus Maze (EPM) test. The maze comprises two open

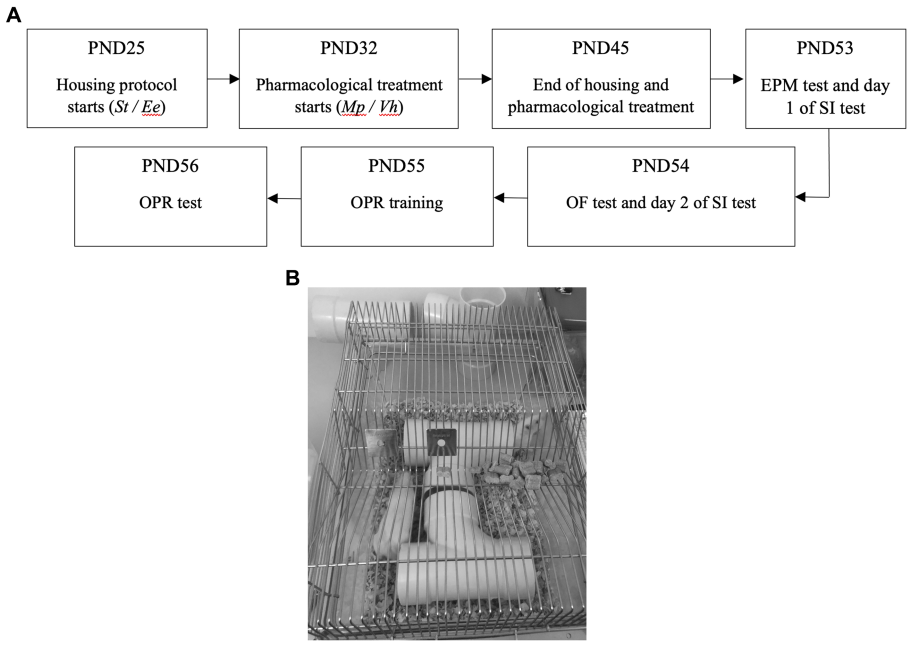

FIGURE 1
(A) Outline of the experimental timeline. PND: Post-natal day; St: Standard; Ee: Enriched environment; Mp: Pethylphenidate; Vh: Vehicle; EPM: Elevated plus maze; SI: Social interaction; OF; Open field; OPR: Object in place recognition; **(B)** Typical enriched environment.

platforms intersected at right angles by two enclosed platforms with 40 cm high walls, forming open and closed arms. Each arm measures 50 cm in length and 10 cm in width. A 1 cm high plexiglass rim prevents rat falls. Constructed from wood coated with black melamine, the maze is elevated 50 cm above the floor, positioned in a secluded test room illuminated by white LEDs (60 Lux at the center). Rats, unexposed to the maze previously, were individually tested, starting in the center, and exploring for 5 min. After testing, rats returned to their cages, and the maze was cleaned using 10% alcohol and disposable towels. Digital recordings of the experiment were analyzed, tracking both arm entries and time spent. An arm entry was defined as placing all four paws on the arm's surface. Each subject was placed in the center of the maze and allowed to freely explore for a duration of 5 minutes. The time spent and the number of entries into both the open and closed arms were recorded. Additionally, other behaviors such as grooming, rearing, head dipping, and stretching were also observed and recorded during the test.

Social interaction

Immediately after the Elevated Plus Maze (EPM) test, the animals entered the social interaction test. This test evaluates social interaction with an unfamiliar animal in a round open field and is used to assess social skills. Even low doses of psychostimulants have been reported to affect certain social behaviors (Meaney et al., 1981). Each experimental animal was placed in a cage with an unknown animal (external peer) and allowed to freely interact for 10 minutes. During this period, the frequency and latency of various social behaviors were recorded. These behaviors include: (1) Pinning, where one animal lies on its back with another animal on top; (2) Pouncing, which involves sniffing or rubbing the nose against the neck of another animal; (3) Contact, which includes grooming between peers and other forms of social exploration (Achterberg et al., 2015); (4) Exploration, representing moments of individual exploration; (5) Passing, when one animal passes over the other; and (6) Following, where one animal chases the other. These behaviors were selected to be observed with the intention of assessing the "natural" sociability of the animals, which is understood as non-violent interactions driven by the interest animals show in engaging with their peers (Vanderschuren et al., 2016). Some of these behaviors, including playful actions that are a part of the animals' natural sociability and may be influenced by the environmental conditions in which the animals are situated, as well as other non-playful behaviors driven solely by an interest in interacting (Achterberg et al., 2014), were chosen for observation. After the social interaction test, the animals were returned to their home cages. The test was repeated 24-h later.

Open field

After a 24-h interval, the open field test was conducted. The animals were placed in a square plexiglass arena measuring 80 × 80 × 50 cm. Each subject was gently placed in the center of the open field and allowed to freely explore for a duration of 5 minutes. During this time, the amount of time spent in the center of the arena versus near the periphery, as well as the total distance covered, were recorded. After the completion of the five-minute exploration period, the animals were returned to their respective home cages.

Object in place recognition

Twenty-four hours after the open field test, the object in place recognition test was conducted to assess memory. This test is commonly used to evaluate learning and memory abilities.

The habituation phase took place on the first day, during which the animal was introduced to an open field measuring 80 × 80 × 50 cm and allowed to freely explore for 5 minutes. The purpose of this phase was to familiarize the animal with the test environment.

The following day, the animal was placed back into the same arena, this time with two identical objects positioned in specific locations near the center of the field. The animal was given time to explore the objects and their surrounding areas.

After a 24-h interval, the training phase commenced. This phase consisted of five trials, with the two identical objects being placed in the same positions throughout all trials. Visual cues were present in the field to provide spatial references.

24-h later, the testing phase began. During this phase, the location of one of the objects was changed while the other remained in its original position. The behavior of the animal, specifically the time spent circling the new location versus the old one, was recorded. It is important to note that the objects used in all tests were identical to ensure that the animal's preference was based solely on the location rather than any physical characteristics of the objects.

The duration of the exploration behavior (direction of the animal's snout and/or contact of the forepaws with the object) for each object was calculated. The following calculation was used:

$$\text{EI} = \text{Exploration novel object}\,(\text{O1}) - \text{Exploration familiar object}\,(\text{O2}) \,/\, \text{Total exploration time in the trial}$$

Data analysis

The collected data were analyzed using a two-way analysis of variance (ANOVA). Group mean differences were compared using the Student Newman–Keuls *post hoc* test, whenever necessary. If the data did not pass the normality test (Shapiro–Wilk) or exhibited unequal variances, non-parametric analysis (Kruskal-Wallis Analysis of Variance on Ranks) was conducted. In all instances, the alpha level was set at 0.05.

Results

In Tables 1, 2, a summary of the statistical findings for the two-way ANOVA and the Kruskal-Wallis test (ANOVA on ranks) is provided when normality and variance tests failed.

Emotional response

Regarding the time spent in the open arms of the elevated plus maze (see Figure 2), the ANOVA revealed significant differences ($F_{[1,43]} = 4.275$, $p = 0.045$) in the interaction between the factors

TABLE 1 Statistical results of parametric analysis (Two Way ANOVA) for the performance in all the behavioral tests.

Behavior	Normality test (Shapiro–Wilk)	Equal variance test	Treatment (tt) (Vh / Mp)		Environment (hsg) (St / EE)		Interaction (tt × hsg)	
			$F_{[1,43]}$	p	$F_{[1,43]}$	p	$F_{[1,43]}$	p
Elevated plus-maze								
Entries into the closed arms	0.586	0.533	6.545	0.014*	2.975	0.092	0.345	0.560
Time spent in closed arms	0.050	0.796	2.780	0.103	0.131	0.720	2.453	0.125
Entries into the open arms	0.491	0.707	2.840	0.099	1.577	0.216	3.269	0.078
Time spent in open arms	0.055	0.779	3.398	0.072	0.000	0.993	4.275	0.045*
Percentage of entries into the open arms	0.743	0.512	0.061	0.806	3.280	0.077	3.252	0.078
Crossings by the central square	0.149	0.873	6.830	0.012*	0.470	0.496	0.929	0.341
Time spent in the central square	0.477	0.390	0.246	0.622	0.719	0.401	0.030	0.864
Total distance run in the maze	0.087	0.629	6.187	0.017*	10.06	0.003*	0.239	0.627
Average speed while exploring the maze	0.084	0.619	6.467	0.015*	1.044	0.002*	0.250	0.619
Object recognition test								
Time spent exploring the new object	0.930	0.675	0.018	0.895	5.498	0.024*	0.186	0.886
Social interaction test								
Time of interaction with peers	0.625	0.066	0.213	0.647	0.713	0.403	0.309	0.581

Alpha value set in 0.05. Please refer to the text for *post hoc* comparisons. Vh, vehicle; Mp, methylphenidate; St, standard housing; EE, enriched environment; tt, treatment; hsg, housing.
*Significant statistical difference.

TABLE 2 Statistical results of non-parametric analysis (Kruskal-Wallis ANOVA on ranks) for the performance in all the behavioral tests.

Behavior	Normality test (Shapiro–Wilk)	Equal variance test	H[3]	p
Elevated plus maze				
Total time of inactivity (different than freezing)	<0.050	<0.050	16.451	<0.001*
Open field				
Total time exploring the central area	<0.050	0.718	8.786	0.032*
Average of speed while exploring the periphery	<0.050	0.163	13.714	0.003*
Average of speed while exploring the central area	<0.050	0.194	2.302	0.512
Time spent self-grooming	<0.050	0.348	4.079	0.253
Time spent rearing	0.094	<0.050	9.625	0.022*
Object recognition test				
Time spent exploring the familiar object	<0.050	0.987	5.217	0.157
Index of exploration of the new object (time exploring new object / time exploring familiar object)	<0.050	0.480	6.146	0.105
Social interaction test				
Time pouncing	<0.050	<0.050	0.456	0.928
Rejection by pressing peers time	<0.050	<0.050	0.850	0.837
Time spent following peers (playing)	<0.050	<0.050	16.755	<0.001*
Time spent in single exploration	<0.050	0.641	9.041	0.029*

Alpha value set in 0.05. Please refer to the text for *post hoc* comparisons. *Significant statistical difference.

(treatment × housing condition). *Post hoc* analysis of group means differences using the Student Newman–Keuls test showed that, for animals housed in the standard condition, those treated with methylphenidate spent a significantly longer time in the open arms compared to those receiving the vehicle. However, this effect was not observed in animals housed in the enriched environment condition.

Exploration and locomotion

The Kruskall-Wallis test found significant differences in rearing in the open field ($H[3] = 9.625$, $p = 0.022$, see Figure 3A). *Post hoc* comparison of group means using the Dunn's method revealed that, in general, animals receiving

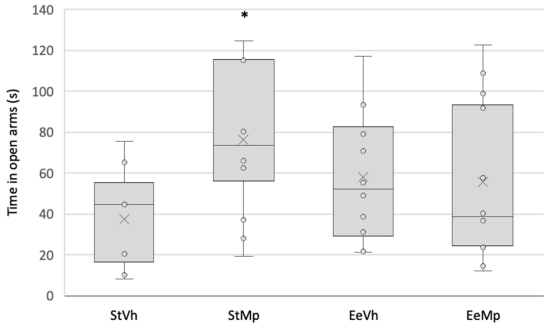

FIGURE 2
Time spent in the open arms of the elevated plus maze for all groups. Median represented by the inner line in the box and Average represented by the inner "x". *: different from the pharmacological control in the same housing condition.

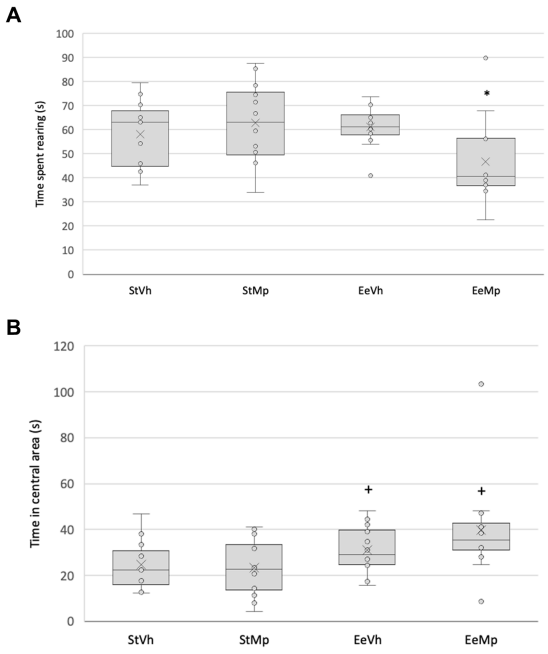

FIGURE 3
Time spent in explorative rearing behavior **(A)** and exploring the central area **(B)** of the open field for all groups. Median represented by the inner line in the box and Average represented by the inner "x". +: different from the control environment housing condition with the same treatment.

methylphenidate and housed under the enriched condition explored less than those housed in the standard environment condition.

The Kruskal-Wallis test also found significant differences in exploration time in the central area ($H[3] = 8.786$, $p = 0.032$). *Post hoc* comparison of group means using the Dunn's method revealed that, in general, animals housed in the enriched environment explored the central area more than those in the standard environment (see Figure 3B).

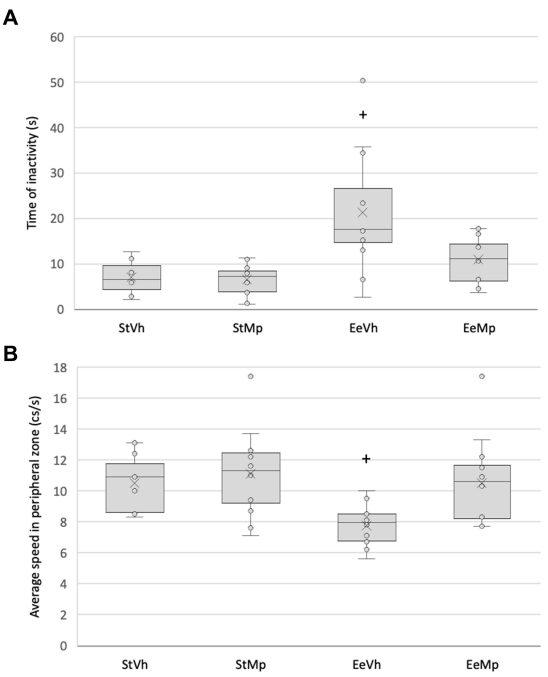

FIGURE 4
(A) total time of inactivity for all the subjects in the elevated plus maze and **(B)** average speed during the exploration of the periphery of the elevated plus maze. Median represented by the inner line in the box and Average represented by the inner "x". +: different from the control environment housing condition with the same treatment.

Behavioral regulation

The Kruskal-Wallis test showed significant differences for the inactivity time of the animals in the elevated plus maze ($H[3] = 16.451$, $p < 0.001$). *Post hoc* comparison of group means using the Dunn's method revealed that in the enriched environment condition, animals that received methylphenidate were less inactive than those that received the vehicle (see Figure 4A).

Regarding the exploration speed of the periphery in the open field, the Kruskal-Wallis test found significant differences ($H[3] = 13.714$, $p = 0.039$). *Post hoc* comparison of group means using the Dunn's method showed that for animals that received the vehicle, those in the enriched environmental condition exhibited a decreased exploration speed compared to those in the standard condition (see Figure 4B).

Social behavior

In the social interaction test, the Kruskal-Wallis test revealed significant differences ($H[3] = 16.755$, $p < 0.001$) in the time spent following another individual. The *post hoc* comparison of means using the Dunn's method showed that animals housed in the standard environment condition and receiving methylphenidate had a lower amount of time spent chasing their companions (see Figure 5A).

The Kruskal-Wallis test found significant differences ($H[3] = 9.041$, $p = 0.029$) in the amount of time spent in single

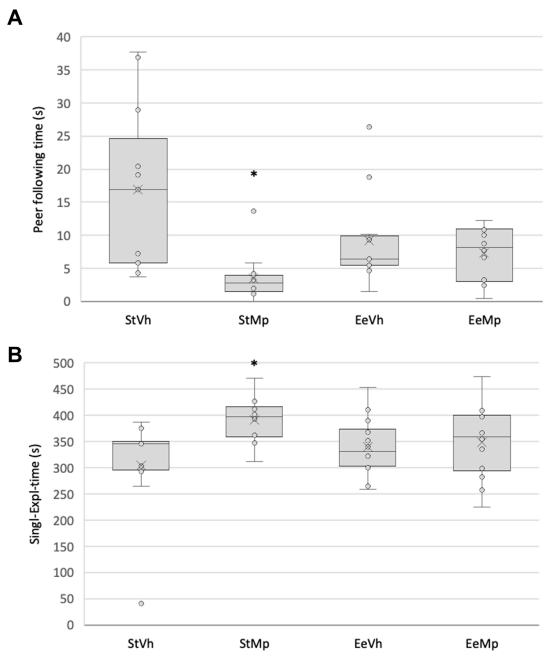

FIGURE 5
Total time spent **(A)** following another individual and **(B)** single exploration in the social interaction situation. Median represented by the inner line in the box and Average represented by the inner "x". *: different from the vehicle in the same environment housing condition.

exploration. The *post hoc* comparison of group means using the Dunn's method revealed that animals that received methylphenidate and were housed in the standard environment had longer durations of single exploration. This effect was not observed in animals that received methylphenidate and were housed in the enriched environment (see Figure 5B).

Memory

No significant differences were found between the groups in the exploration time of the novel object in the object recognition test.

Discussion

This study aimed to determine whether environmental enrichment can reverse or mitigate some of the effects of MPH on anxiety, locomotion, social interaction, and memory in Wistar rats. For this purpose, animals housed under standard or enriched conditions received chronic low-dose treatment of MPH or vehicle. The objective of our study was to investigate the long-term impact of chronic use of methylphenidate during early adolescence. To avoid interference from both the effect of methylphenidate and its withdrawal on the behaviors studied, it was decided to wait 7 days after the last administration, a period after which it can be ensured that there will be no interference from these two processes (Askenasy et al., 2007). Similarly, we sought to have a period of time not very different between the last administration of methylphenidate and the behavioral tests performed. For this reason, the behavioral testing was carried out with a little time of rest between them. In order to optimize the behavioral protocol used, the habituation session to the object recognition test was carried out in an open field arena so this session was used for both purposes, habituation to the place where the object recognition test was to be done and open field test.

There is no consensus in the literature regarding the potential interference of the order between different tests (Blokland et al., 2012), especially when working in mice (McIlwain et al., 2001; Võikar et al., 2004), nor on the best time that should be left between each of them. Blokland et al. (2012) reports that there may be an interference effect of the application of tests such as the Zero-Maze and the open field on performance in the forced swim test. Their results are understandable because, since the forced swim test is a test focused on the evaluation of depression, its performance can be interfered with by a stress effect after the application of other tests. In our case, neither the object recognition test nor the social interaction test have been reported as forms of measuring depression, nor as possible tests for measuring emotional factors, so an interaction effect between them with the elevated plus maze and the open field was not expected.

On the other hand, some authors propose that a period of 3 days between the completion of one test and the start of another is adequate (Sosa et al., 2019), when dealing with tests that involve large motivational loads for animals. In our case, none of the tests used causes emotional effects or induces emotional conditioning, so interaction between them is not plausible.

In particular, and due to the high sensitivity of the elevated plus maze for the measurement of anxiety (Hogg, 1996; Morato and Brandão, 1996), it is always chosen to perform it first and before any other. Similarly, and perhaps based on the initial work of Kostowski et al. (1989), it is common practice in many laboratories to sequentially evaluate behavioral tests that do not involve the use of painful or emotional learning stimuli (Kostowski et al., 1989), a situation that also reduces stress due to manipulation and ensures that there are not too many variations in the physiological conditions under which the behavioral evaluation is performed (Corredor et al., 2022).

Finally, it is worth mentioning that since control groups were used for each of the factors (environment and pharmacological treatment) and since all animals received exactly the same treatment and had exactly the same experimental times, any interference that any of these behavioral tests could have had on the subsequent ones would have also been present in the control groups, and therefore it can be ensured that this hypothetical effect would be present in all conditions.

To assess anxiety, we analyzed the time spent in the open arms of the EPM. Some studies have reported a moderate anxiolytic-like effect of environmental enrichment (Corredor et al., 2022). However, paradoxically, our results showed that the increased exploration of the open arms observed in animals housed under standard conditions and treated with MPH was reversed by the enriched environment. This suggests a paradoxical anxiogenic

effect of the enriched environment. However, it is possible that the increased exploration of the open arms in animals treated with MPH does not indicate an anxiolytic effect but rather a lack of behavioral regulation, indicative of impulsive-like behavior. If this is the case, the paradoxical effect of the enriched environment could be better understood as a reversal of the impulsive behavior induced by MPH. The lack of effect of MPH on the time spent exploring the central area of the open field supports the notion that it does not reflect a genuine decrease in anxiety. While time spent in open arms is often interpreted as a reduction in anxiety, it's important to continue exploring other potential explanations that could account for this phenomenon. For instance, within the context of our study, when linking environmental enrichment with the consumption of a psychostimulant drug wherein the environment mitigates the drug's effect, it's plausible that the mitigated impact pertains to the stimulant effect on animal locomotion. This mitigation could lead to an increase in impulsive movements, which are typically suppressed by anxiety. We also consider the possibility that the drug, by inducing impulsivity, diminishes the animal's risk assessment facilitated by anxiety. Consequently, this could explain why the animal spends more time in the open arms of the elevated plus maze. As highlighted in previous studies, it is crucial to consider that the impact of MPH on anxiety is influenced, to some extent, by the specific anxiety-inducing stimuli (Crawford et al., 2013). Therefore, conducting a more comprehensive investigation into the mechanisms underlying the heightened stimulation of the open arms induced by MPH would be valuable.

Moreover, it could be suggested that the increase in time spent in the open arms is associated with locomotion and reactivity to novelty, as proposed by certain authors (Dow-Edwards et al., 2008). However, this does not appear to be the case in our study. In fact, our results demonstrated no difference in total distance covered in the elevated plus maze, nor in the average exploration speed in the open field, suggesting the absence of any MPH-induced effects on locomotion. Nevertheless, further exploration of impulsive behavior in the elevated plus maze is needed.

Rearing behavior, which involves the animal raising its front legs and relying solely on its hind legs, is commonly used as a measure of exploration (Sturman et al., 2018). Our results indicate that housing animals treated with MPH in an enriched environment reduces the tendency to engage in rearing behavior. This unexpected effect could suggest that MPH-treated animals are more susceptible to the effects of environmental enrichment. However, it is crucial to delve into how the enriched environment might be mitigating the drug's impact on the animal's exploratory behaviors. In the event that methylphenidate consumption enhances exploratory behavior such as rearing, this effect could also be attributed to impulsive motion, and conceivably, the enriched environment could be counteracting the impulsivity induced by the drug. Nonetheless, there is a need to continue investigating various motor and emotional variables that could drive an animal to explore an environment, in order to better comprehend the circumstances under which this could be beneficial or not for the animal. In our study, we contend that irrespective of the reasons behind the animal's engaging in the behavior, the environment is ameliorating the drug's effects, thus bolstering the notion that the environment can function as a protective factor against substance consumption effects. Further research is necessary to explore exploratory behaviors like rearing and other variables that may be linked to these behavioral changes. Lastly, since this experiment used rats without any attentional disorder, it raises the question of how MPH would affect exploratory rearing in rats with attentional impairments.

Certain authors have demonstrated that MPH and atomoxetine can inhibit social gambling behavior by altering the noradrenergic system (Achterberg et al., 2015). While this study assesses behaviors other than gambling, its objective is to evaluate the impact of MPH on the fundamental social interaction of animals.

Social behavior was assessed using a test that enabled free interaction while also providing the option to maintain distance from others. By observing specific social interactions, behaviors such as physical contact between animals, interest in proximity to others, the intention to initiate social interaction, and preference for individual exploration can be measured. One particular behavior, known as "following behavior," involves rodents initiating social interaction by approaching and sniffing the anogenital area of another animal (Meaney et al., 1981).

Our findings demonstrated that chronic MPH significantly inhibits the initiation of social behavior. Additionally, we observed that MPH administration increased the time spent in solitary exploration. However, when animals treated with MPH were housed in an enriched environment, the duration of social behavior (specifically, "following behavior") was comparable to that of animals receiving the vehicle. The same is true for single exploration.

From these results, it is evident that the enriched environment has the ability to reverse the decreased interest in initiating social contacts. It is noteworthy that when comparing the time spent following other peers between animals housed under the two different conditions, the enriched condition resulted in less variability compared to the standard housing condition. In future studies, it would be intriguing to explore neurobiological changes that can shed light on the mechanisms through which the enriched environment manages to counteract the drug's impact on the social interaction of animals. Given that this study solely observed behavior, it allows us to speculate that once again, the enriched environment serves as a protective factor against the impact of addictive drug consumption on the social interaction of the animals, through a modulating effect on anxiety. In this context, if methylphenidate is consumed within an enriched environment, it could potentially reverse the decrease in social interaction induced by the drug. However, the implications of this would vary depending on the circumstances, as it hinges on whether harnessing the drug's effect is required or advantageous for the animal within a specific context. It is crucial to highlight that social behavior serves as an adaptive function in mammals, and it is directly linked to survival and reproduction in adulthood (Achterberg et al., 2015). Furthermore, social experiences during adolescence play a fundamental role in the social, cognitive, and emotional development of rats (Hodges et al., 2018).

Additionally, it is worth noting that in humans, the inhibition of social behaviors may be one of the factors contributing to the efficacy of MPH as a treatment for ADHD, as it reduces attention to environmental stimuli (Vanderschuren et al., 1997). Therefore, the

observed effect of the enriched environment on social interaction needs to be carefully considered, as it could have unintended consequences or be seen as an undesired effect.

In relation to locomotion, our findings revealed that environmental enrichment has a regulatory effect on behavior. Specifically, rats housed in the enriched condition exhibited increased time of inactivity (distinct from freezing) in the elevated plus maze. This inactivity could be interpreted as a strategy to regulate impulsive behavior, allowing the animals to better consolidate acquired information. This interpretation aligns with the observed effect of MPH on open arm exploration. If this is the case, our results suggest that the enriched environment was unable to counteract the impulsive behavior induced by MPH. In fact, rats receiving MPH and housed in the enriched condition did not exhibit an enhanced duration of inactivity. It is also plausible that the mechanism through which inactivity regulates impulsivity is related to reduced levels of anxiety (Holubová-Kroupová and Šlamberová, 2021).

The pseudo-anxiolytic-like effect observed with MPH may potentially indicate a compensatory mechanism in response to chronic drug consumption (Crawford et al., 2013). In this scenario, it could imply a general increase in motor activity without a specific exploratory aim.

In relation to memory, although there were no significant differences in the interaction between the pharmacological treatment and housing condition, there are noteworthy findings to highlight. Firstly, environmental enrichment as a standalone factor appeared to enhance memory by increasing the exploration time of the novel object. This finding aligns with previous studies suggesting that an enriched environment can improve memory by facilitating cognitive changes (Leger et al., 2015).

Secondly, although it is striking that unlike most other studies, MPH did not induce any memory changes in our experiment. In other studies, even at low doses, MPH has been shown to enhance memory (Carmack et al., 2014). Therefore, it is essential to consider additional environmental variables that may interfere with the results and influence the effects of MPH on memory.

We propose the possibility that the lack of MPH effect on memory in our study could be attributed to the specific protocol used for the object recognition task. Unlike most other studies, our protocol focuses on measuring declarative memory rather than spatial memory, as it involves changing the object itself rather than its location. It is important to mention that this protocol has been standardized and utilized by the Laboratory of Neuroscience and Behavior at the University of Los Andes (documentation available at the university's library repository).

Another aspect to take into consideration is the interval that elapsed between the completion of drug administration and the commencement of the experiments. Variables such as the number of days of drug administration and the withdrawal period need to be accounted for in future research to determine the implementation of environmental enrichment protocols and behavioral observation. Further studies are necessary to attain more distinct conclusions regarding the impact of methylphenidate on memory.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by IACUC – Universidad de los Andes. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Cannabidiol and brain function: current knowledge and future perspectives

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Cannabidiol (CBD) is a naturally occurring non-psychoactive cannabinoid found in *Cannabis sativa*, commonly known as cannabis or hemp. Although currently available CBD products do not meet the safety standards of most food safety authorities to be approved as a dietary supplement or food additive, CBD has been gaining widespread attention in recent years due to its various potential health benefits. While primarily known for its therapeutic effects in managing epileptic seizures, psychosis, anxiety, (neuropathic) pain, and inflammation, CBD's influence on brain function has also piqued the interest of researchers and individuals seeking to enhance cognitive performance. The primary objective of this review is to gather, synthesize, and consolidate scientifically proven evidence on the impact of CBD on brain function and its therapeutic significance in treating neurological and mental disorders. First, basic background information on CBD, including its biomolecular properties and mechanisms of action is presented. Next, evidence for CBD effects in the human brain is provided followed by a discussion on the potential implications of CBD as a neurotherapeutic agent. The potential effectiveness of CBD in reducing chronic pain is considered but also in reducing the symptoms of various brain disorders such as epilepsy, Alzheimer's, Huntington's and Parkinson's disease. Additionally, the implications of using CBD to manage psychiatric conditions such as psychosis, anxiety and fear, depression, and substance use disorders are explored. An overview of the beneficial effects of CBD on aspects of human behavior, such as sleep, motor control, cognition and memory, is then provided. As CBD products remain largely unregulated, it is crucial to address the ethical concerns associated with their use, including product quality, consistency, and safety. Therefore, this review discusses the need for responsible research and regulation of CBD to ensure its safety and efficacy as a therapeutic agent for brain disorders or to stimulate behavioral and cognitive abilities of healthy individuals.

KEYWORDS

CBD, cannabis, hemp, endocannabinoid system, receptor, brain activity, neurological disease, mental disorder

1 Introduction

1.1 Overview and statistics

Cannabidiol (CBD) is one of the over 140 types of cannabinoids found in *Cannabis sativa*. It is non-psychoactive and influences signaling pathways in the brain in a different way than tetrahydrocannabinol (THC), the key and most potent psychoactive compound of cannabis. THC is responsible for the "high" that is brought by the consumption of cannabis and is also responsible for dependence (Urits et al., 2021; Legare et al., 2022), whereas CBD is not intoxicating and does not induce abuse or dependence potential (Chesney et al., 2020; Urits et al., 2021). Furthermore, CBD is characterized by high lipophilicity and is therefore quickly distributed in the brain, adipose tissue, and other organs upon intake. However, it has low solubility and absorption in water when given in capsules, which causes variable pharmacokinetics. Bioavailability via inhalation averages 31%, while oral bioavailability is about 6% in humans (Chayasirisobhon, 2020).

The use of products derived from CBD is steadily increasing, especially in nations that have flexible regulations on cannabis-based products. However, even in countries where CBD products lack regulatory approval, they are still being sold and used to self-medicate against various conditions (Britch et al., 2021). CBD is mainly used to improve wellbeing and relieve symptoms of several diseases. Statistics on the prevalence of CBD as a therapeutic product globally are not widely available. The usage of CBD-based products as therapeutic agent is high in the United States and Canada, with 26.1% and 16.2%, respectively, whereas it is comparatively less popular in Europe, with a prevalence between 10.9% and 14% (Casanova et al., 2022; Goodman et al., 2022).

The results of an online survey to determine patterns of use, dose, and self-perceived effects of CBD showed that most users were aged between 25 and 54 years (72.2%) (Moltke and Hindocha, 2021). The most cited reasons for CBD use in this sample were anxiety (42.6%), sleep problems (42.5%), stress (37%), and general health and wellbeing (37%). Older people were more likely to use CBD for pain relief than any other reason, and CBD use was higher in males than in females. Respondents lauded the effectiveness of CBD and did not report adverse effects.

1.2 Importance of understanding the effects of CBD on brain function

There is increasing interest in the application of CBD as a therapeutic agent. Indeed, CBD has putative anticonvulsant, antipsychotic, anxiolytic, anti-inflammatory and anti-craving properties, which are critical in healthcare (Batalla et al., 2020). Since CBD has anticonvulsant properties, Epidiolex, the only FDA-approved prescription medicine based on CBD, is used worldwide to treat seizures associated with Lennox-Gastaut syndrome, Dravet syndrome and tuberous sclerosis complex. However, the low availability and affordability of antiseizure medications, especially in low-income countries, are the major barriers to epilepsy treatment (Leitinger et al., 2020). CBD may also be used to manage symptoms of anxiety and other mental disorders by regulating brain activity and connectivity patterns (O'Neill et al., 2021). Most conventional antipsychotics and antidepressants are linked to low response rates, adverse effects, limited tolerance, and adherence (Kruizinga et al., 2021). Mass production and distribution of CBD-based drugs might provide a solution for these challenges and could significantly increase the accessibility and adherence to psychiatric treatments while reducing adverse effects. However, knowledge gaps on the effects of CBD on brain function but also on the safety of CBD consumption first need to be addressed and a new regulatory pathway for CBD needs to be defined.

Understanding the effects of CBD on brain function has several advantages. First, it is important to establish the effectiveness of CBD-based products as an intervention for neurological and mental disorders, not to mention possible other applications in pain relief, sleep promotion, stress reduction, and cognitive enhancement. Second, investigating the effects of CBD on the brain is an opportunity to assess the risk to the brain posed by CBD, whether long-term or not. Finally, understanding whether CBD positively influences brain function has an implication for policymakers. As it stands, many nations are yet to legalize cannabis-based products, even for therapeutic purposes. Scientific evidence is crucial to drive policy change. If CBD demonstrates to be a viable intervention for any or all the named health issues, its use may improve the quality of life for millions of people globally. It is, therefore, important to review CBD's effects on the brain using various scientific sources and methods.

1.3 Scope of the review

This review aims to gather, synthesize, analyze, and consolidate scientifically proven evidence on the effects of CBD on brain function and its significance in the treatment of brain disorders. To achieve this aim, some background information on CBD is provided, including its biomolecular properties and mechanisms of action. Next, the results emerging from human brain imaging and neurophysiological studies are discussed. Thereafter, the potential implications of CBD as a neurotherapeutic agent are addressed: the clinical applications of CBD in treating neurological and mental disorders are considered, as well as the applications of CBD to enhance aspects of human behavior. Finally, an overview of future research on CBD and its ethical implications is provided.

2 CBD and the endocannabinoid system

2.1 The endocannabinoid system (ECS) and its role in regulating brain function

The endocannabinoid system (ECS) controls most bodily functions, including sleep, temperature, pain reception, inflammatory and immune responses, learning and memory, processing emotions, and eating, making it the subject of most drug development research. The ECS has emerged as a mediator of short-term and long-term synaptic plasticity. It comprises at least two G protein-coupled receptors (GPCR), the accompanying

endogenous ligands such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and enzymes needed for synthesis and degradation (Lu and Mackie, 2016). The two major GPCRs are cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2). The former is considered the most abundant GPCR in the central nervous system and is expressed widely across the prefrontal cortex, the hippocampus, and the basal ganglia (Freund et al., 2003; Harkany et al., 2008). CB2, on the other hand, is more prevalent immune cells.

The term "extended endocannabinoid system" is used to refer to a broader network of signaling molecules and receptors that interact with the ECS (Cristino et al., 2020). This is involved in the regulation of various physiological processes, including pain, inflammation, metabolism, and cardiovascular function. This expanded system includes other lipid signaling molecules such as N-acylethanolamines (NAEs) different than AEA that can activate cannabinoid receptors, as well as other receptors and enzymes that are involved in the regulation of various physiological processes. For example, NAEs such as palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) can activate CB1 and CB2, as well as other receptors such as peroxisome proliferator-activated receptor alpha (PPARα) and transient receptor potential vanilloid channel 1 (TRPV1), which are involved in the regulation of pain and inflammation (Kasatkina et al., 2021). Similarly, 2-AG can activate both CB1 and CB2, as well as other receptors such as G protein-coupled receptor 55 (GPR55), which is involved in the regulation of blood pressure and inflammation (Sugiura and Waku, 2000; Balenga et al., 2011).

The fact that CB1 receptors form homodimers and heterodimers with other GPCRs, including CB2 receptors and dopamine, opioid, serotonin, and orexin receptors, and that several heterodimers have been described as specific modulators of hippocampal function further supports the location-dependent modulation of cannabinoid signaling (Batalla et al., 2020; Cristino et al., 2020). Activations of the heterodimers lead to effects that oppose the effects of isolated CB1 receptor activation. This phenomenon, together with different G proteins that can bind CB1, explain the diverse nature of the CB1 action depending on the neighboring protein interaction and location.

The ECS controls the activity-based forms of plasticity by inducing long-term changes in the γ -aminobutyric acid (GABA) release (Winters and Vaughan, 2021). Hippocampal excitation can induce the production of 2-AG, which then acts on the presynaptic GABAergic synapsis, thereby modulating excitability by regulating the inhibitory tone of hippocampal circuits (Abate et al., 2021). Further, synaptic plasticity in the amygdala is critical in the acquisition, storage, and extinction of aversive memories, and the ECS has emerged as a crucial mediator of such neuroplasticity-related phenomena (Viveros et al., 2007). It was proposed that endocannabinoids facilitate the extinction of aversive memories through their selective inhibitory effects on local inhibitory networks in the amygdala. This provides evidence for the functional role of endocannabinoid release-based synaptic plasticity (Marsicano et al., 2002; Azad et al., 2004). In addition to the amygdala, the hypothalamus has been suggested as a potential site for cannabinoid-induced neural plasticity. In this area, cannabinoid-dependent synaptic plasticity is believed to play a role in regulating the stress-response system (Di et al., 2003; Riedel and Davies, 2005).

2.2 CBD-related signal transduction

Although the molecular pathways and mechanisms through which CBD acts have not been fully established yet (Batalla et al., 2020), it is suggested that CBD can directly interact with different receptor-dependent and independent mechanisms (Schouten et al., 2022), thereby targeting multiple pathways and mechanisms of action which contribute to different therapeutic applications (Britch et al., 2021; Legare et al., 2022) (see Figure 1). CBD engages in the activation of channels (e.g., TRPV1), transcription factors (e.g., PPARγ), and different GPCRs, such as CB1, CB2, GPR55, serotonin 1A receptor (5-HT1A), and adenosine A2a receptor.

TRPV1 regulates the transduction of (noxious) chemical and physical stimuli such as acid, capsaicin (hot pepper), allyl isothiocyanate (wasabi), and heat, but also of endocannabinoids. Upon activation, desensitization of the receptor might occur, which relieves the symptoms of nociceptive behavior. CBD acts as a TRPV1 agonist and can thus induce receptor desensitization resulting in analgesic effects, as was observed in animal models of neuropathic and inflammatory pain (Costa et al., 2004). Less is known about the effect of CBD on TRP1A (a sensor of cold, pain, and itch) and TRPV2 (a high-threshold thermosensor), though it was found that CBD also activates and desensitizes these receptors, which was reported to play a role in neuronal inflammation (Wang et al., 2019) and development (Santoni and Amantini, 2019). In contrast, CBD is an antagonist of the cold and menthol receptor (TRPM8), which is also expressed in sensory neurons, but the functional role of CBD on TRPM8 remains to be further elucidated. It was recently also shown that CBD inhibits voltage-gated sodium channels (Huang et al., 2023) and activates the voltage-gated potassium channel KCNQ2 (Ma et al., 2023), which both play a major role in regulating neuronal excitability.

As mentioned before, CBD does not only exert its action via the activation of channels but also via transcription factors such as PPARγ which is engaged in metabolic and immune functions. As an agonist of PPARγ, CBD was shown to exert anti-inflammatory (Hou et al., 2012) and neuroprotective (Esposito et al., 2011) effects.

Due to its low affinity for CB1 and CB2 (Bisogno et al., 2001), CBD mainly mediates CB1 and CB2-related signaling via indirect ways. CBD inhibits the enzymatic activity of fatty acid amide hydrolase which is responsible for the degradation of AEA. Since AEA also acts as an agonist of the cannabinoid receptors, CBD and its related increase in AEA abundance might therefore indirectly stimulate CB1 and CB2 signaling. However, CBD might also attenuate binding of CB1 agonists, thereby antagonizing CB1 signaling (Laprairie et al., 2015). In the CNS, CBD-related effects on mainly CB1 and CB2 are involved in the regulation of neuropathic and nociceptive pain but also anxiety. Additionally, CB2 is highly expressed in immune cells, and agonism of CB2 induces anti-inflammatory effects. CBD is also an antagonist of GPR55, via which it might exert anti-inflammatory effects (Lin et al., 2011). Furthermore, CBD may act via 5-HT1A which plays a protective role in oxidative stress and more specifically lipid

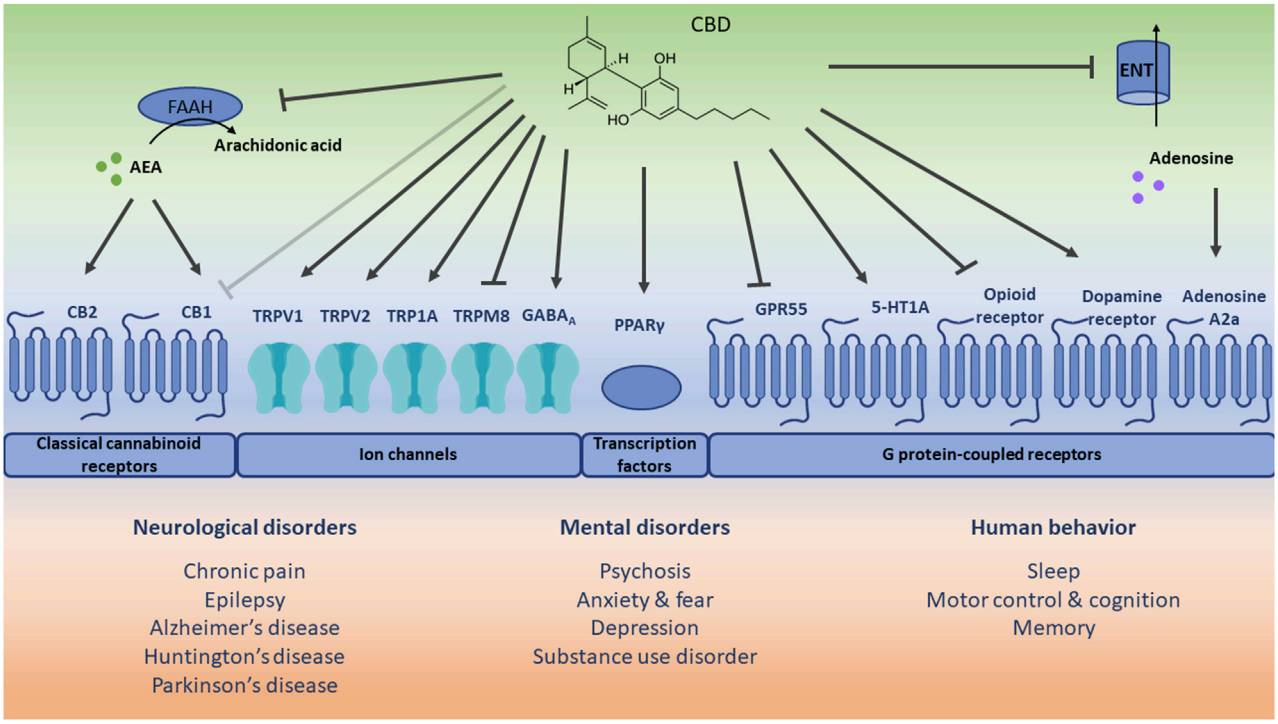

FIGURE 1
Schematic representation of potential receptor-dependent and independent mechanisms via which cannabidiol (CBD) may play a role in the treatment of therapeutic applications. The direct (ant)agonistic actions of CBD are depicted with arrows and lines toward the respective receptors. Additionally, the indirect receptor mechanisms of CBD, i.e., via increased anandamide (AEA) concentration [by inhibiting its hydrolysis to arachidonic acid via fatty acid amide hydrolase (FAAH)], and increased extracellular adenosine concentration [by inhibiting its uptake via equilibrative nucleoside transporter (ENT)] are included. The light grey inhibitory arrow indicates an antagonistic effect of CBD towards cannabinoid receptor 1 (CB1). However, it should be noted that CBD displays a low affinity for CB1 and a minimal direct activity at CB1. CB2: cannabinoid receptor 2; TRPV1: transient receptor potential vanilloid channel 1; TRPV2: transient receptor potential vanilloid channel 2; TRPA1: transient receptor potential ankyrin channel 1; TRPM8: transient receptor potential melastin channel 8; $GABA_A$: γ-aminobutyric acid type A receptor; PPARγ: peroxisome proliferator-activated receptor gamma; GPR55: G protein-coupled receptor 55; 5-HT1A: serotonin 1A receptor.

peroxidation. In an animal model of hypoxic-ischemic brain injury, CBD treatment partially prevented oxidative stress, inflammation and excitotoxicity via 5-HT1A (Pazos et al., 2013). Finally, CBD also affects the adenosine A2a receptor via an indirect mechanism, i.e., CBD inhibits the equilibrative nucleoside transporter, which controls extracellular adenosine availability for other cell receptors. The accumulation of extracellular adenosine levels eventually stimulates the adenosine A2a receptor via which CBD might exert anti-inflammatory (Carrier et al., 2006) and neuroprotective (Castillo et al., 2010) effects.

There is also evidence that CBD affects the binding capacity of ligands to receptors of the opioid and dopaminergic system. CBD decreases the binding capacity of agonists to μ- and δ-opioid receptors, thereby downregulating opioid receptor signaling (Kathmann et al., 2006). This might hold clues for CBD treatment as a strategy for addictions (Hurd et al., 2015), such as alcohol misuse (Viudez-Martínez et al., 2018a). CBD also acts as a partial agonist of dopamine D2 receptors, which might have antipsychotic effects (Seeman, 2016). Finally, via facilitated binding of agonists to GABA type A ($GABA_A$) receptors, CBD can be relevant for anticonvulsant and anxiolytic effects (Bakas et al., 2017). Although promising, it should be noted that these relationships and therapeutic applications require further investigation.

3 Evidence for CBD effects in the human brain

Human neuroscience techniques such as magnetic resonance imaging (MRI), positron emission tomography (PET), single-photon emission computed tomography (SPECT), and electroencephalography (EEG) can be used to shed light and guide the use of CBD. It has been established that depending on the region and dose, CBD can elicit different reactions (Millar et al., 2018). Brain imaging helps to understand which dose of CBD elicits what kind of reaction in a specific brain region (Weinstein et al., 2016). Nonetheless, these brain regions are interconnected, and the effects of CBD on one region can have implications for the functioning of others (Lorenzetti et al., 2023). It is worth noting that research in this field is still ongoing, and there is much to learn about the interaction between CBD and the brain.

3.1 Magnetic resonance imaging

Several functional MRI (fMRI) studies investigated the effects of CBD on brain function in healthy individuals (Supplementary Table S1). Overall, CBD was found to reduce resting-state activity and

connectivity across several brain regions, potentially indicating an anxiolytic (anxiety-reducing) effect. In a cross-over study using fMRI, sixteen healthy volunteers were given 600 mg of oral CBD (Grimm et al., 2018). The group that was given CBD significantly increased the fronto-striatal connectivity compared to the placebo group. THC, on the other hand, did not show a significant change in connectivity. A direct comparison between THC and CBD revealed that CBD increased connectivity relative to THC between the right putamen and the frontal pole and paracingulate gyrus. In response to inhibition, CBD was found to attenuate brain activity in the left posterior insula, left superior temporal gyrus, and left transverse temporal gyrus. The left medial prefrontal cortex was attenuated by CBD during the presentation of salient stimuli relative to non-salient stimuli (Borgwardt et al., 2008). In addition, CBD increased the connectivity between the left caudate nucleus, the left inferior frontal gyrus, and the left dorsal striatum. In contrast, it decreased connectivity between the left dorsal striatum, the left anterior cingulate, and the left thalamus.

Other studies reported that CBD may have a modulatory effect on brain regions associated with anxiety and stress, such as the amygdala and prefrontal cortex. For instance, a study documented that, during emotional processing, CBD attenuated bilateral activity in the posterior lobe of the cerebellum (Batalla et al., 2020). In intense fear, CBD attenuated activity in the amygdala, anterior parahippocampal gyrus, anterior and posterior cingulate gyri, and the right posterior lobe of the cerebellum (Batalla et al., 2020), and disrupted the connectivity between the left amygdala and the left anterior cingulate cortex when processing fear. Also, CBD increased activity in the right occipital lobe, lingual gyrus, cerebellum, and cuneus during visual stimulation (Winton-Brown et al., 2011).

An fMRI study compared brain activation during reward, loss, and neutral anticipation in medication-naïve individuals with high risk for psychosis (Wilson et al., 2019). The group given CBD showed intermediate activation in the left insula and parietal operculum, left superior frontal gyrus, and left frontal operculum. In patients with established psychosis, CBD treatment showed bilateral activation in the inferior frontal and left middle frontal gyrus (Fusar-Poli, 2012). In patients with anxiety disorder, fMRI studies reported the activation of the right posterior cingulate gyrus (Li et al., 2018), while activity was decreased in the left parahippocampal gyrus and hippocampus (Cha et al., 2016). Finally, an MR spectroscopy study showed that CBD decreased GABA in patients with autism but not in the healthy control groups (Pretzsch et al., 2019).

3.2 Nuclear medicine imaging

PET and SPECT studies have been less common in investigating the effects of CBD compared to MRI studies. Some of these studies have explored the binding of CBD to specific receptors in the brain, such as the serotonin 5-HT1A receptor, which is associated with mood regulation.

A study used PET to investigate the effects of CBD on brain function in people at high risk of psychosis (Bhattacharyya et al., 2018). The administration of 600 mg of CBD was found to be associated with changes in brain function in the medial temporal, midbrain, and striatal regions, which are areas that are involved in the development of psychosis. These changes were associated with improvements in cognitive performance and reduced symptoms of psychosis. The findings corroborate the idea that CBD may have potential as a treatment for people at high risk of developing psychosis. A case report study provided further evidence for the positive effects of CBD in psychosis (Koethe et al., 2023), suggesting that these may be due to enhanced cerebral glucose utilization.

Other studies on the neural effects of CBD were conducted using SPECT. Specifically, in two SPECT studies healthy individuals (Crippa et al., 2004) and treatment-naïve patients with social anxiety disorder (Crippa et al., 2011) were administered with 400 mg of CBD or placebo, in a double-blind procedure. CBD significantly decreased subjective anxiety and increased mental sedation, while placebo did not induce significant changes in both studies. In healthy individuals, lower activity in CBD than placebo was revealed in the left amygdala and the left posterior cingulate gyrus. In patients with social anxiety disorder, CBD administration reduced activity in the left parahippocampal gyrus, hippocampus, and inferior temporal gyrus, relative to placebo. Taken together, these results suggest that CBD can reduce anxiety, and that this is related to its effects on activity in limbic and paralimbic brain areas.

3.3 Electroencephalography

Evidence for the effects of CBD on EEG activity in healthy individuals is currently lacking. EEG recordings were mainly used to document the effects of CBD on patients with seizures. For instance, a case study on a nine-year-old with Lennox-Gastaut Syndrome, a severe and rare form of epilepsy, documented that CBD produced an unprecedented dramatic normalization of the baseline EEG activity (Prakash, 2020). More generally, a meta-analysis of 104 EEG studies from 52 patients with pediatric-onset refractory epilepsy reported that 74% of the patients had a reduction in interictal epileptiform discharges (IEDs), 46% in ictal findings, and 17% experienced a change in sleep architecture after CBD treatment (Herlopian et al., 2022). Another study assessed the longitudinal impact of CBD on EEG measures in subjects with treatment-resistant epilepsy (Grayson et al., 2021). It showed that CBD has positive effects on interictal epileptiform discharge frequency but no effects on other clinical EEG measures; the effects of CBD did not appear to be dependent on dose. Another longitudinal EEG study in patients with refractory epilepsy (Armstrong et al., 2022) revealed that there are subtle changes in certain neural metrics even at baseline that may not be perceived during qualitative analysis and that could be used in the future as a biomarker to predict a patient's clinical response to CBD administration.

4 Implications for the potential of CBD as a therapeutic agent

The following sections review clinical and pre-clinical data concerning the therapeutic potential of CBD in a variety of neurological and mental disorders as well as its effects on aspects of human behavior (Supplementary Table S2). However, in some disorders clinical trials are scarce and conclusions are predominantly based on preclinical studies, highlighting the need for human intervention trials.

4.1 Neurological disorders

4.1.1 Chronic pain

Pain lasting longer than 3 months can be classified as chronic pain, and involves neuropathic, nociceptive, musculoskeletal, inflammatory, psychogenic, and mechanical pain (Cohen et al., 2021).

Preclinical studies show great potential for CBD as an analgesic agent (Costa et al., 2007; Belardo et al., 2019; De Gregorio et al., 2019; Jesus et al., 2019; Malvestio et al., 2021). The analgesic properties of CBD are suggested to be induced by the agonistic actions of CBD at TRPV1, a mediator in pain signaling (Henson et al., 2022). Once TRPV1 channels are activated, they desensitize and enter a refractory period during which sensory neurons are not responsive to further stimulation. Via this mechanism, symptoms of nociceptive behaviour are relieved. Indeed, rodent models of neuropathic and inflammatory pain showed that the antihyperalgesic actions of CBD were reversed by TRPV1 antagonists (Costa et al., 2004; Costa et al., 2007; De Gregorio et al., 2019). Additionally, CBD's analgesic actions may be mediated via the activation of the serotonergic system through 5-HT1A receptors. In male diabetic rats, mechanical allodynia was attenuated by treatment with CBD (0.3 or 3 mg/kg). This effect was completely prevented by the pre-treatment with the 5-HT1A antagonist WAY 100135 (Jesus et al., 2019). Furthermore, in rats subjected to a nerve injury model that induces neuropathic pain, 5-HT firing activity decreased, resulting in mechanical allodynia. Treatment with CBD (5 mg/kg/d) normalized 5-HT activity and reduced mechanical allodynia. However, after antagonism of 5-HT1A with WAY 100635 (2 mg/kg/d for 7 days) this effect was partially prevented (De Gregorio et al., 2019). These findings are in accordance with a similar study in rats subjected to a nerve injury model, which also showed that treatment with CBD (30 nmol microinjected into the prelimbic division of the medial prefrontal cortex) attenuated mechanical allodynia in a 5-HT1A-dependent manner (Malvestio et al., 2021). Interestingly, it was found that the analgesic effect of CBD was also blocked by the CB1 receptor antagonist, providing an alternative mechanism of action (Malvestio et al., 2021).

Although these preclinical studies repeatedly show the analgesic properties of CBD, evidence in human clinical trials remains scarce. A survey investigating patients' perspectives and attitudes about CBD for the treatment of pain symptoms showed that 62% of the participants used a CBD product. Furthermore, the majority of patients (59%) who ever used CBD products perceived reductions in pain and in 67.6% of these patients, the use of CBD allowed them to reduce their pain medications (Schilling et al., 2021). Another explorative study evaluated the effect of CBD treatment on self-reported quality of life and showed that patients with (non-)cancer chronic pain symptoms reported reductions in pain scores upon CBD treatment, while patients with neurological symptoms did not perceive any improvements (Gulbransen et al., 2020). The lack of a control group in this study makes it difficult to identify any causal relationship for CBD treatment and reduced pain scores. Additionally, there was a large loss of participants (36.3%) during the study. Furthermore, the applied doses ranged from 40 to 300 mg/d and were reported inconsistently and incompletely by patients, which further added complexity to the interpretation of these results.

In a study involving seven kidney transplant patients with chronic pain, CBD treatment (100–300 mg/d for 3 weeks) resulted in total pain reduction in two patients and a partial reduction in four patients, while one patient did not perceive any change in symptoms at all (Cuñetti et al., 2018). Again, the lack of a control group and the low sample size make it impossible to draw any conclusions from this study, as acknowledged by the authors. On the other hand, a placebo-controlled randomized trial involving 29 patients with symptomatic peripheral neuropathy provided evidence that transdermal application of CBD oil (250 mg/3 fL. Oz for 4 weeks) can yield a significant reduction in intense pain, sharp pain, cold and itchy sensations compared to placebo (Xu et al., 2020).

To date, clinical studies on the effect of CBD in patients with chronic pain remain inconclusive. The majority of clinical studies for the treatment of chronic pain typically utilized a mixture of THC and CBD [reviewed in (Henson et al., 2022; Argueta et al., 2020)] and studies evaluating the isolated effect of CBD are rather scarce and unconvincing. As such, there is a high need for placebo-controlled clinical studies evaluating the effect of isolated CBD supplements to clarify the role and the underlying mechanisms of CBD in chronic pain management.

4.1.2 Epilepsy

Epilepsy is a neurological disease characterized by cerebral hyperactivity or synchronous neuronal activity (Fisher et al., 2005). A large proportion of epileptic patients suffer from drug-resistant seizures that further increase the rate of cognitive impairment as well as psychiatric and physical disability.

CBD has been shown to exert anti-convulsant effects in various animal models of epileptic seizures (Jones et al., 2012; Klein et al., 2017; Costa et al., 2022). It is hypothesized that CBD may act preferentially to reduce seizure spread (Jones et al., 2012), but the exact mechanism underlying the anti-convulsant effects of CBD has not yet been elucidated. Given its low affinity for CB1 and CB2, CBD most likely modulates neuronal hyperexcitability via cannabinoid receptor-independent pathways. Proposed mechanisms include: 1) regulation of Ca^{2+} homeostasis in neurons under a normal or a highly excitable state (Ryan et al., 2009); 2) agonistic properties at 5-HT1A receptors eliciting membrane hyperpolarising responses (Theodore, 2003; Merlet et al., 2004); 3) enhancing endogenous adenosine levels in the CNS thereby increasing inhibitory adenosinergic tone which aids in seizure suppression (Boison, 2006; Carrier et al., 2006); and 4) inducing neuroprotective and anti-inflammatory actions via modulation of PPARγ (Costa et al., 2022). It is critical to understand these mechanisms of action to improve CBD's efficacy and implementation as a therapeutic agent in epilepsy.

Also in human clinical trials, the potential of CBD as a therapeutic agent for drug-resistant seizures has recently been investigated. Its effectiveness in childhood epilepsy including, Lennox–Gastaut syndrome and Dravet syndrome has been studied extensively and summarized in recent systematic reviews (da Silva Rodrigues et al., 2023; Silvinato et al., 2022). It was concluded that treatment with CBD successfully reduced the frequency of seizures by 33%, supporting the use of CBD in these patient populations. These positive results have led to the approval of Epidiolex as a prescription medicine for seizures associated with Lennox–Gastaut syndrome and Dravet syndrome.

4.1.3 Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disease that is characterized by parenchymal deposition of amyloid-β in plaques and intraneuronal accumulation of hyperphosphorylated tau protein, inducing chronic inflammation and oxidative damage (DeTure and Dickson, 2019). Several studies showed a potential role for CBD in the inhibition of AD progression [reviewed in (Xiong and Lim, 2021)].

Treatment with CBD was reported to reduce amyloid-β production, tau phosphorylation, and neuroinflammation *in vitro* (Scuderi et al., 2014; Libro et al., 2016). These effects were also observed in *in vivo* studies where CBD treatment suppressed the neuroinflammatory response induced by amyloid-β deposition in mice (Esposito et al., 2007) and rats (Esposito et al., 2011), and stimulated hippocampal neurogenesis (Esposito et al., 2011) thereby delaying disease progression. CBD also improved cognitive performance, which is typically affected in AD. More specifically, long-term treatment with CBD (20 mg/kg/d for 8 months) prevented the development of social recognition deficit in a transgenic mouse model of AD (Cheng et al., 2014a). Additionally, sub-chronic treatment with CBD (20 mg/kg daily for 1 week, 3x/week for the following 2 weeks) prevented the cognitive impairment in mice injected with amyloid-β as shown by their performance in the Morris water maze test (Martín-Moreno et al., 2011). These positive effects on cognitive functioning were associated with a reduction in neuroinflammation (Martín-Moreno et al., 2011; Cheng et al., 2014a).

The mechanisms underlying the neuroprotective effect of CBD against AD progression have not yet been fully elucidated. There is emerging evidence that the effect is mediated via PPARγ activation and subsequent inhibition of Nuclear Factor-Kappa B (Esposito et al., 2011; Scuderi et al., 2014). Nevertheless, mediation via alternative receptors has also been proposed, including the activation of TRPV1 and PI3K/Akt pathway (Libro et al., 2016). Future studies that determine the underlying mechanisms are warranted.

4.1.4 Huntington's disease

Huntington's disease (HD) is an inherited disorder characterized by neuronal lesions in brain areas that help to control voluntary (intentional) movement, such as the cerebral cortex and the striatum (Rikani et al., 2014). As a result, HD patients develop uncontrollable movements (chorea) and abnormal body postures.

The neuroprotective properties of CBD make it a potential candidate in the treatment of HD. Indeed, in rats where striatal lesions were induced by 3-nitropropionic acid (3NP, an inhibitor of mitochondrial complex II), CBD reversed 3NP-induced reductions in GABA contents and reduced 3NP-induced striatal atrophy. The neuroprotective effects of CBD acted preferentially on striatal neurons that project to the substantia nigra and resulted from antioxidant and cannabinoid receptor-independent mechanisms (Sagredo et al., 2007).

To the best of our knowledge, there is only one clinical trial available that investigated the effects of CBD administration in patients suffering from HD. In this study, treatment with CBD (10 mg/kg/d for 6 weeks) in neuroleptic-free patients with HD did not show any effects on chorea severity or other therapeutic outcomes (Consroe et al., 1991). Other clinical trials investigating the effects of CBD on HD symptoms included THC-containing medicines, such as nabilone or Sativex (Curtis et al., 2009; López-Sendón Moreno et al., 2016). Besides the fact that these studies showed contradicting results, they did not allow us to determine the role of isolated CBD supplements in the treatment of HD.

Overall, evidence for the potential of CBD as a therapeutic agent to treat HD is lacking and future studies should aim to elucidate the isolated effects of CBD on HD symptoms.

4.1.5 Parkinson's disease

Parkinson's disease (PD) is another neurodegenerative disease that is characterized by reduced dopamine levels as a result of dopaminergic neuron degeneration. Although the pathophysiology of PD is complicated, evidence suggests that α-synuclein (α-syn) aggregates play a significant role in the dopaminergic neurodegenerative process, leading to impairments of cellular function and oxidative stress (Pupyshev et al., 2018). The symptoms of PD include motor as well as non-motor signs such as depression, anxiety, apathy, sleep disorders, and psychosis (Poewe, 2008; Sveinbjornsdottir, 2016).

Currently, the dopamine replacement agent, I-DOPA, is often prescribed to treat PD. However, chronic use of I-DOPA is associated with (non-)motor complications including tardive dyskinesia (TD), a hyperkinetic movement disorder that is described by involuntary and repetitive movements (Hauser et al., 2022). Interestingly, CBD was able to reduce symptoms of orofacial dyskinesia induced by anti-dopaminergic drugs in mice (Sonego et al., 2018; Sonego et al., 2021) showing its potential as an add-on therapy in PD.

Furthermore, studies show that CBD ameliorates motor and cognitive impairments in animal models of PD and TD (da Cruz Guedes et al., 2023; Peres et al., 2016). More specifically, pretreatment with CBD (0.5 mg/kg) attenuated the increase in cataleptic behavior, oral movements and memory deficits, but not locomotor activity induced by reserpine administration in rats (Peres et al., 2016). In another study where *C. Elegans* were exposed to reserpine, CBD exposure recovered reserpine-induced alterations in locomotion rate/food-sensing behavior, attenuated morphologic alterations and dopaminergic neuron degeneration, and reduced human α-syn protein accumulation (da Cruz Guedes et al., 2023). These studies highlight the potential of CBD as a neuroprotector against PD.

However, clinical trials on the effects of CBD on PD symptoms show inconsistent results. Treatment with CBD for 10–15 days (titrated from 5 to 20–25 mg/kg/d) improved total and motor Movement Disorder Society Unified Parkinson Disease Rating Scale (UPDRS) scores in 10 PD patients (Leehey et al., 2020). These results were confirmed in an open-label pilot study where six patients received a flexible dose of CBD for 4 weeks (starting at 150 mg/d with weekly increases of ~150 mg/d, depending on the clinical response). When CBD was added to their treatments, patients showed improvements in total UPDRS score (Zuardi et al., 2009). In contrast, a placebo-controlled double-blind RCT showed no changes in UPDRS score in participants who were treated with CBD (75 or 300 mg/d), although the scores on overall wellbeing and quality of life significantly improved (Chagas et al., 2014a).

As mentioned above, symptoms of PD also include non-motor signs such as anxiety, sleep disorders, and psychosis. Acute CBD supplementation (300 mg) was shown to reduce perceived feelings of anxiety and tremor amplitude in PD patients who underwent the simulated public speaking test (de Faria et al., 2020). Furthermore, psychotic symptoms were reduced in PD patients receiving a flexible dose of CBD (starting at 150 mg/d) for 4 weeks (Zuardi et al., 2009). Since the increased psychotic symptoms in PD patients are associated with the use of dopaminergic drugs, the authors hypothesized that the reduction in psychotic symptoms upon CBD treatment resulted from attenuated dopaminergic activity in brain areas related to psychotic symptoms. Regarding the effect of CBD on sleep disorders in PD, some discrepancies are present in the existing results. A case study of 4 PD patients suffering from rapid eye movement (REM) sleep behavior disorder (RBD) showed a reduction in the frequency of RBD events, including nightmares and active behavior during dreaming in all 4 patients after treatment with CBD (75 or 300 mg/d for 6 weeks) (Chagas et al., 2014b). However, these results were not confirmed in a placebo-controlled randomized trial in PD patients with Restless Legs Syndrome and RBD. In this study, treatment with CBD (doses gradually increasing from 75 to 300 mg for 12 weeks) did not result in improvements in subjective, nor in objective sleep quality measured by polysomnography, as compared to placebo (de Almeida et al., 2023).

Because of the different nature of these study designs (i.e., case studies and randomized controlled trials), the small sample sizes, and the different dosing schedules, it is difficult to draw solid conclusions on the effect of CBD on motor and non-motor symptoms in PD. Additional placebo-controlled trials are required to properly understand the role of CBD in this neurodegenerative disease.

4.2 Mental disorders

4.2.1 Psychosis

Conventional treatment of psychosis is through drugs that are highly selective dopamine D2 receptor antagonists, such as haloperidol and amisulpride. Although effective, these treatments are often accompanied by severe side effects including deficits in motor control (Strange, 2001). Interestingly, animal models used for screening antipsychotic drugs, show that CBD treatment inhibited catalepsy or hyperlocomotion induced by apomorphine or ketamine (Zuardi et al., 1991; Moreira and Guimarães, 2005). These preclinical data indicate that CBD acts via different mechanisms, exhibiting a profile of atypical antipsychotic drugs. Therefore, CBD may be proposed as an alternative to classical drugs. The antipsychotic potential of CBD was demonstrated in schizophrenia (Zuardi et al., 1995; Leweke et al., 2012; McGuire et al., 2018) and PD (Zuardi et al., 2009).

While still speculative, current literature suggests that CBD may reduce psychotic symptoms via the inhibition of FAAH and subsequent increases in AEA levels. Indeed, in non-medicated acute schizophrenic patients, AEA cerebrospinal fluid concentrations showed a negative correlation with psychotic symptoms (Giuffrida et al., 2004). Interestingly, CBD (800 mg/d) treatment in acute schizophrenic patients increased AEA levels which was associated with reductions in psychotic symptoms (Leweke et al., 2012). Whereas these data present a convincing mechanism, alternative mechanisms of action such as the attenuation of increased glial reactivity (Gomes et al., 2015) or via parvalbumin-positive GABA neurons (Campos et al., 2017) cannot be excluded and further research is warranted.

A first individual case report of a female schizophrenia patient in which haloperidol treatment was terminated because of severe side effects, showed that treatment with CBD (1500 mg/d for 26 days) resulted in similar improvements in psychiatric symptoms without any side effects (Zuardi et al., 1995). Additionally, a clinical trial comparing amisulpride and CBD (800 mg/d for 4 weeks) treatment in acute schizophrenic patients showed that CBD was equally effective in treating clinical effects with fewer side effects compared to amisulpride (Leweke et al., 2012). Further evidence for the antipsychotic effects of CBD were provided in a trial where schizophrenia patients added CBD (1000 mg/d for 6 weeks) to their usual medication and perceived improvements in psychotic symptoms, as was supported by the treating clinicians' impressions of improvement and illness severity (McGuire et al., 2018).

While these results are promising, it seems that higher doses up to 800 or 1000 mg/d are required to effectively treat psychotic symptoms, since chronic (6 weeks) or acute treatment with lower CBD doses (600 mg/d) did not improve symptoms in psychotic patients (Boggs et al., 2018) or cognitive impairments in schizophrenic patients (Hallak et al., 2010), respectively. Though, even these higher doses do not always seem to be effective, as it was shown that treatment with a high CBD dose of 1280 mg/d in three treatment-resistant schizophrenic patients only mildly improved symptoms of psychosis in one of the three patients after 35 days (Zuardi et al., 2006). It should be noted that the lack of effect in this case study may be explained by the severity of the disease state and does not necessarily exclude its effectiveness in non-resistant schizophrenic patients. Nevertheless, the application of CBD in psychotic patients requires further investigation regarding the optimal dosing schedule.

4.2.2 Anxiety and fear

CBD treatment successfully induced anxiolytic-like effects in several animal models, including the elevated plus-maze (Guimarães et al., 1990; Resstel et al., 2009; Gomes et al., 2011; Hsiao et al., 2012), Y-maze (Mori et al., 2021), Vogel conflict test (Moreira et al., 2006; Gomes et al., 2011) and social interaction test (Almeida et al., 2013). Nevertheless, the mechanisms underlying the anxiolytic effects of CBD are not fully elucidated yet.

As previously mentioned, SPECT studies in healthy individuals and social anxiety disorder patients showed that CBD reduced anxiety and fear via its action on limbic and paralimbic brain areas (Crippa et al., 2004; Crippa et al., 2011). Furthermore, several animal models showed that the anxiolytic effects of CBD were blocked when combined with a 5-HT1A antagonist (Resstel et al., 2009; Gomes et al., 2011), suggesting mediation via this receptor. Indeed, 5-HT1A receptors are widely present in brain areas related to stress and anxiety and agonism of this receptor has been associated with anxiolytic responses (Rioja et al., 2004) whereas 5-HT1A-knockout mice showed anxiogenic-like behavior (Tsetsenis et al., 2007). In rats, pretreatment with a CB1 antagonist counteracted the suppressive effect of CBD on panic-like behavior induced by $GABA_A$ receptor blockade (da Silva et al., 2015). Since CBD administration increases AEA levels, indirect agonism of CB1 might be an alternative mediating pathway

through which CBD reduces anxiety levels. In addition, the anxiolytic effects of CBD in ischemic mice were prevented by antagonism at CB1 and 5-HT1A, as well as at CB2 and PPARγ (Mori et al., 2021), showing the complex signaling role of CBD.

Also, in human clinical trials, the anxiolytic effects of CBD have been determined. For example, acute administration with 300 mg CBD reduced anxiety induced by the simulated public speaking test (SPST) in healthy individuals (Zuardi et al., 1993; Zuardi et al., 2017; Linares et al., 2019) and PD patients (de Faria et al., 2020). Furthermore, patients suffering from social anxiety disorder showed higher levels of anxiety during the SPST compared to healthy individuals, but the administration of 600 mg CBD completely abolished these differences (Bergamaschi et al., 2011). Contrarily, administration of 600 mg CBD in healthy individuals without experimentally induced anxiety did not show any anxiolytic effect, suggesting that CBD is only able to reduce anxiety levels in case of stress or fearful situations (Martin-Santos et al., 2012). Furthermore, the dose is of relevance as it was shown that CBD presents an inverted U-shape in which intermediate doses seem to be effective, whereas low and high doses are not (Zuardi et al., 2017; Linares et al., 2019). Besides reducing anxiety levels, CBD also affected fear memory expression (Lee et al., 2017) via acute reduction in fear expression (Jurkus et al., 2016), enhancement of memory extinction (Bitencourt et al., 2008; Das et al., 2013) and disruption of memory reconsolidation (Stern et al., 2012; Gazarini et al., 2014; Stern et al., 2015). These effects may be of relevance for the treatment of phobias and post-traumatic stress disorder (PTSD).

4.2.3 Depression

The antidepressant-like effects of CBD have been shown in several animal models including tail suspension (Schiavon et al., 2016), forced swimming (El-Alfy et al., 2010; Zanelati et al., 2010; Réus et al., 2011; Sales et al., 2019; Xu et al., 2019), the saccharine preference test (Shoval et al., 2016), and the novel object test (Shoval et al., 2016). It was repeatedly reported that CBD exerts its effect through 5-HT1A activation (Zanelati et al., 2010; Linge et al., 2016; Sartim et al., 2016) and increases in brain-derived neurotrophic factor (BDNF) levels in certain brain areas (Réus et al., 2011; Xu et al., 2019). Moreover, a recent study proposed that 5-HT1A activation by CBD, either directly or indirectly via elevated serotonin levels, may increase BDNF levels, which ultimately leads to mTOR activation and synaptogenesis (Sales et al., 2019). Nevertheless, not all animal studies replicate these findings as unaltered BDNF levels following CBD treatment have also been reported (Zanelati et al., 2010). These contradictory findings may be explained by the use of different animal species, different CBD doses, and different administration routes. In fact, the effectiveness of CBD seems highly dependent on the applied dose and studies suggest a U-shaped dose-response curve (Zanelati et al., 2010; Réus et al., 2011) as mentioned earlier. Additionally, doses that induce behavioral improvements do not always correspond to the doses that induce desired neurological adaptations (Réus et al., 2011; Schiavon et al., 2016). Future studies are warranted to properly understand the underlying mechanisms by which CBD improves symptoms of depression and to determine effective dose regimens.

Compared to the preclinical research, almost no studies investigated the effect of CBD on depression in humans. One case report showed that a patient suffering from neurofibromatosis type 1 perceived improvements in depressive symptoms when she switched from conventional antidepressants to CBD oil (Hegazy and Platnick, 2019). Given this scarcity, more clinical trials in humans are required to translate the preclinical antidepressant-like effects of CBD to therapeutic applications.

4.2.4 Substance use disorders

An increasing number of studies focusing on the anti-addictive properties of CBD is emerging. Animal studies show promising effects of CBD in the context of alcohol, opioids, and methamphetamine use. Accordingly, a limited number of human studies showed a positive impact of CBD on nicotine, cannabis, and opioid use (Paulus et al., 2022).

There is a wide range of possible mechanisms via which CBD may be involved in the regulation of drug use and addiction (Navarrete et al., 2021). CBD may interact with the dopaminergic system, which plays an important role in addictive disorders. The release of dopamine after drug consumption induces drug-rewarding effects via the activation of dopamine D2 receptors (Trifilieff et al., 2013). CBD potentially inhibits the increased dopaminergic signaling induced by drug intake. Indeed, animal studies report that CBD treatment inhibited the amphetamine (Renard et al., 2016) and cocaine (Galaj et al., 2020) induced increases in neuronal dopamine activity. Additionally, these effects were associated with reductions in self-administration of cocaine (Galaj et al., 2020). Furthermore, reduced intake of ethanol by CBD was associated with lower gene expression of the μ-opioid receptor and tyrosine hydroxylase (Viudez-Martínez et al., 2018a), which is the rate-limiting enzyme in the biosynthesis of dopamine. The μ-opioid receptor is known to modulate dopamine transmission via inhibition of GABAergic interneurons (Spanagel et al., 1992). As such these findings propose an interaction between CBD and the opioid system.

Furthermore, CBD might exert its actions in substance use disorders via the serotonergic system. As previously mentioned, the agonistic actions of CBD at 5-HT1A have been widely acknowledged and seem to be involved in the anxiolytic and anti-depressant effects of CBD. In addition, the activation of 5-HT1A by CBD may also play a role in its mediation of the drug-rewarding process. In fact, mice subjected to oral ethanol self-administration showed lower ethanol intake when treated with a combination of CBD (20 mg/kg/d for 2 weeks) and Naltrexone (0.7 mg/kg/d for 2 weeks) (Viudez-Martínez et al., 2018b). These effects were absent when the treatment was combined with a 5-HT1A antagonist. Accordingly, a study using a self-administering paradigm of low cocaine doses in rats reported that the reductions in cocaine intake induced by CBD (20 or 40 mg/kg) were blocked by pretreatment with the 5-HT1A antagonist (Galaj et al., 2020). Interestingly, CBD was not able to reduce cocaine intake when self-administered cocaine doses were high, suggesting a limited capacity for CBD to counteract the reward process linked to cocaine consumption.

CBD might also be involved in the process of drug memory expression (Ren et al., 2009), consolidation (de Carvalho and Takahashi, 2017), and extinction (Parker et al., 2004), which plays an important role in addiction. Indeed, even though CBD treatment (5 and 20 mg/kg) was unable to reduce self-administrated heroin intake in rats, it did inhibit cue-induced drug-seeking behavior, a measure of cue-heroin memory expression (Ren et al., 2009).

Additionally, several animal studies investigated the effect of CBD on drug memory consolidation and extinction using the drug-induced conditioned place preference paradigm, where increased time spent in the drug-paired place is associated with the rewarding properties of the drug. Using this paradigm, treatment with CBD impaired the preference for the place previously associated with amphetamine, morphine, or cocaine (Parker et al., 2004; de Carvalho and Takahashi, 2017). Whether CBD enhances extinction, impairs reconsolidation or influences both processes of drug memories is currently unclear and requires further investigation.

While the mechanisms mentioned above seem promising, most research in the field of the anti-addictive properties of CBD is based on animal models. The available evidence from clinical trials is scarce and human intervention trials are required to translate the preclinical findings towards therapeutic applications of CBD in substance use disorders.

4.3 Aspects of human behavior

4.3.1 Sleep

CBD has been suggested as therapeutic strategy to improve sleep. The exact mechanisms via which CBD affects sleep are not yet fully understood, but different hypotheses are based on preclinical and clinical studies. On a biological level, it can be hypothesized that FAAH inhibition via CBD increases AEA levels, which might directly affect CB1-mediated NREM stability (Pava et al., 2016). However, it remains to be confirmed whether this CBD-FAAH-CB1 axis effectively mediates sleep. Alternatively, CBD can also directly act upon $GABA_A$ receptors (Bakas et al., 2017), which play a beneficial role in sleep regulation (Gottesmann, 2002), e.g., reduced sleep latency and improved NREM sleep (Lancel, 1999). However, depending on the context (e.g., CBD levels and exposure time) and on the receptor subtype, CBD can act as a $GABA_A$ receptor agonist or antagonist. Therefore, the CBD-$GABA_A$ receptor axis also requires further investigation.

Despite biological mechanisms that might explain CBD-related improvements in sleep, it appears that CBD has no effect on sleep quality and quantity in subjects with an undisturbed and healthy sleep pattern (Linares et al., 2018). However, the calming effect of CBD on the CNS might reduce anxiety and promote relaxation (Shannon et al., 2019), via which CBD can indirectly improve sleep in people suffering from anxiety. In addition, some patient populations might also benefit from CBD use, since CBD increases sleeping time in insomnia patients (Carlini and Cunha, 1981) and improves REM sleep in Parkinson's disease patients (Chagas et al., 2014b). It should be noted that some reports showed that CBD increased the wakefulness during sleep in young adults (Nicholson et al., 2004), and even decreased the REM sleep in rats (Murillo-Rodríguez et al., 2006; Murillo-Rodríguez et al., 2008). This alerting effect of CBD requires a well-planned timing and dosing of CBD if being used as a strategy to improve sleep, but this effect also holds promise for the use of CBD as a strategy to improve excessive daytime sleepiness (Nicholson et al., 2004).

4.3.2 Motor control and cognition

Preclinical evidence indicates that acute and prolonged CBD use does not affect motor activity or spatial learning and recognition (Schleicher et al., 2019; Viudez-Martínez et al., 2019). However, in different (preclinical models of) diseases in which motor activity is compromised, CBD might improve some features of motor learning or attention.

Locomotion remained unaffected by CBD treatment in different preclinical models of hyper- and hypolocomotion, including Parkinson's disease (hypo) (Peres et al., 2016), fragile X syndrome (hyper) (Zieba et al., 2019), and an acute neuroinflammatory model (hypo) (Florensa-Zanuy et al., 2021). Likewise, in a genetically-induced preclinical Alzheimer's disease model, prolonged CBD treatment did not affect features of motor performance or sensorimotor gating (pole test, accelerod) (Coles et al., 2020). However, in a preclinical model of encephalopathy, CBD improved locomotion and cognition (Magen et al., 2009). An improvement in the memory deficit and object recognition memory was also observed in a Parkinson's (Peres et al., 2016) and Alzheimer's (Coles et al., 2020) disease model, respectively.

In schizophrenic patients, long-term but not acute CBD treatment (Hallak et al., 2010) improved the sustained attention assessed via the Continuous Performance Test (Leweke et al., 2021). Accordingly, CBD improved the attention lapse duration during a psychomotor vigilance test in healthy adults (Rudisill et al., 2023), whereas CBD had no effect on measures of attention during a vigilance test, and even exacerbated the impairing effect of THC in healthy rats (Moore et al., 2023). Altogether, CBD seems to have no consistent effect on motor performance in preclinical disease models. Some of the motor effects ascribed to CBD can be explained by its anxiolytic effect. It remains to be determined whether findings in preclinical models, often treated with supra-physiological doses of CBD, can be translated toward clinical populations.

4.3.3 Memory

Several preclinical studies show the potential of CBD to restore memory impairments in a large variety of animal models that induce neurological dysfunction (Magen et al., 2009; Cassol et al., 2010; Magen et al., 2010; Avraham et al., 2011; Barichello et al., 2012; Fagherazzi et al., 2012; Pazos et al., 2012; Wright et al., 2013; Cheng et al., 2014a; Cheng et al., 2014b; Schiavon et al., 2014; Campos et al., 2015). A suggested mechanism via which CBD may improve or restore memory impairments involves its antioxidant properties. Research demonstrated that oxidative damage of nuclear as well as mitochondrial DNA in brain cells can have adverse effects on memory processing and retention during ageing (Kandlur et al., 2020). Animal models demonstrated that, by diminishing the risk of oxidative stress, CBD has the potential to safeguard brain cells (da Silva et al., 2018), thereby promoting the preservation of memory function. This notion has been supported in a study where treatment with CBD (2.5, 5, or 10 mg/kg daily for 9 days) in rats submitted to sepsis prevented memory alterations. In this study, the positive effects of CBD on memory were associated with a decrease in oxidative damage in the brain (Cassol et al., 2010).

Alternatively, the anti-inflammatory actions of CBD may play a role in restoring memory deficits. Moreover, in a murine model of cerebral malaria, treatment with CBD (30 mg/kg/d for 3 days, in addition to the traditional anti-malaria drug Artesunate) inhibited the increase in proinflammatory cytokines (TNFα and IL-6) in the hippocampus and prefrontal cortex and subsequently restored malaria-induced memory deficits (Campos et al., 2015). These results were replicated in a model of hepatic encephalopathy

induced by bile-duct ligation in mice. Again, treatment with CBD (5 mg/kg/d for 4 weeks) restored impairments in cognition and locomotion and reduced the increase in hippocampal expression of the TNFα receptor 1 (Magen et al., 2009; Magen et al., 2010). These effects were mediated via 5-HT1A (Magen et al., 2010) and A2A adenosine (Magen et al., 2009) receptor activation, since treatment with their respective antagonists blocked the effects of CBD.

Interestingly, besides reducing neuroinflammation in these animal models, CBD treatment also restored hippocampal levels of BDNF (Magen et al., 2009; Magen et al., 2010; Campos et al., 2015). BDNF is a member of the neutrophin family and is highly involved in maintenance, growth, and survival of neurons. Given that previous studies show an association between increased BDNF levels and improved cognitive performance (Dincheva et al., 2012; Carlino et al., 2013), it seems plausible that CBD-induced increases in hippocampal BDNF explain, at least partly, the rescue of memory deficits in above mentioned models.

Lastly, it was hypothesized that CBD alters cerebral blood flow in brain regions that are involved in memory processing thereby influencing memory function. Moreover, in healthy human participants, acute supplementation with 600 mg CBD increased cerebral blood flow in the hippocampus (Bloomfield et al., 2020). Although higher resting hippocampal blood flow is associated with better memory performance (Heo et al., 2010), the current study was not able to show any effect of CBD supplementation on memory performance. The authors suggested that ceiling effects may have accounted for the lack of a relationship between hippocampal cerebral blood flow and memory task performance as the study population included healthy participants.

These results contrast with studies showing that CBD can restore or protect against episodic memory deficits induced by its psychotropic counterpart THC. More specifically, when healthy participants and cannabis users were administered THC (either 1.5 mg intravenously or 8 mg via inhalation) episodic memory and facial recognition performance were reduced. These effects were diminished when THC was combined with CBD (either 600 mg oral or 16 mg via inhalation) (Englund et al., 2013; Hindocha et al., 2015). However, these effects have not been confirmed in all studies and seem highly dependent on the dose, route of administration, and frequency of cannabis use of the study population (Morgan et al., 2018).

5 Future directions

5.1 Promising areas for future research

The research on CBD is constantly evolving, and there are several promising areas of research that need to be further explored (Figure 2). One of the primary areas that future studies need to focus on is establishing a narrow range for CBD dosage, specifically tailored to the targeted disorder, consumer weight, ethnicity, and gender (Batalla et al., 2020; Legare et al., 2022). As demonstrated in the above sections, the current lack of an effective CBD dosage range results in mixed results. By leveraging brain imaging technology, researchers can more accurately determine the appropriate dosage range for specific disorders.

Another promising area of research is the use of CBD in the treatment of neurodegenerative disorders. While there have been a few human studies, the sample sizes have been small, the applied doses showed a large variability and control groups were often lacking. As a result, more placebo-controlled clinical trials are needed to clarify the effectiveness of CBD in the treatment of chronic pain, Alzheimer's disease, Huntington's disease, and Parkinson's disease and to develop evidence-based guidelines for its use. Specifically for the management of chronic pain, there is a need to bridge the gap between what CBD users report in pain management and what is scientifically proven.

Finally, the effectiveness of CBD in treating mental disorders such as schizophrenia, anxiety and depression remains inconclusive. Also, the anti-addictive properties of CBD seem promising but human trials are scarce and future research should focus on the translation of the preclinical findings towards therapeutic applications of CBD.

5.2 Optimization of CBD's therapeutic potential

The therapeutic potential of CBD is vast, and there are several ways in which this potential can be improved. One approach involves determining the active functional groups in the structure of the compound and manipulating it to improve its solubility without altering its therapeutic potential. This is particularly important because CBD has low bioavailability when taken orally, with only 6% of the compound being absorbed (Chayasirisobhon, 2020). This leads to a significant amount of wastage, which can be costly when the drug is commercialized. Linking CBD to a water-soluble end or a lipid compound without destroying its therapeutic ability could significantly increase its bioavailability and make it a more viable treatment option. In fact, co-ingesting CBD supplements with a high-fat meal was reported to significantly improve its bioavailability (Taylor et al., 2018; Birnbaum et al., 2019). Another approach to improving the therapeutic potential of CBD involves exploring different methods of administration. For example, aerosolized CBD has been shown to have a bioavailability of 31%, making it a potentially effective method of delivery (Stott et al., 2013; Chayasirisobhon, 2020). Finally, genetic engineering can be used to create strains of the cannabis plant with high CBD content, which is key to ensuring a consistent supply of CBD for production (Luo et al., 2019). This approach has the potential to significantly increase the availability of CBD and make it a more accessible treatment option for a wide range of conditions.

5.3 Ethical and regulatory considerations

The research field surrounding CBD remains quite volatile, and one should take certain issues into account. While CBD has been proven not to induce dependence, researchers must ensure the high purity of CBD used in their studies and be vigilant for possible contamination with THC. Also, they should carefully monitor any potential side effects that may arise (Chesney et al., 2020). Overall CBD is well tolerated with a very safe adverse event profile. Some recurring mild adverse events include somnolence, decreased appetite, and gastrointestinal symptoms (Lattanzi et al., 2021). Additionally, CBD is metabolized in the liver and influences enzymatic systems, as such drug-drug interactions between CBD and other medications that

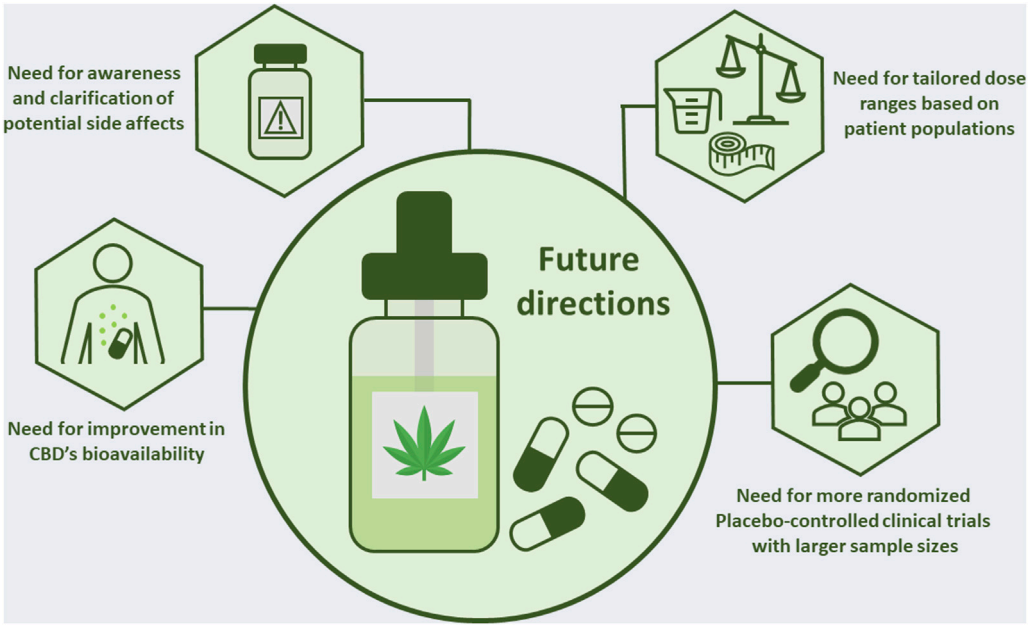

FIGURE 2
Future directions for research on CBD to confirm its use as a potential therapeutic agent.

are metabolized in the liver may occur. In fact, clinical studies involving patients with the Lennox–Gastaut syndrome (Devinsky et al., 2018) or Dravet syndrome (Lattanzi et al., 2021) showed elevated serum transaminases when CBD treatment was combined with antiepileptic drugs. Even though most cases resolve either spontaneously or after dose reduction, these risks should be carefully considered before CBD treatment is prescribed. This is particularly important as the therapeutic potential of CBD continues to be explored for various medical conditions. By taking these precautions and ensuring that research is conducted in a safe and ethical manner, we can continue to advance our understanding of CBD and its potential applications in medicine, while also prioritizing the safety and wellbeing of study participants.

6 Conclusion

With significant research, CBD has the potential to meet the healthcare needs of different groups of neurological and psychiatric patients. Current studies indicate that CBD interacts with several GPCRs, such as CB1, CB2, GPR55, 5-HT1A, adenosine A2a, opioid and dopamine receptors, but it can also exert its action via the activation of ion channels (e.g., TRPV1 and voltage gated sodium and potassium channels) and via transcription factors (e.g., PPARγ), thereby targeting multiple pathways and mechanisms of action which may contribute to different therapeutic applications.

Animal and human brain disease models have been used to investigate the behavioral effects of CBD. Also, brain imaging methods such as fMRI and EEG have permitted the assessment of the CBD's influence on brain functioning. A review of the available behavioral and neuroimaging data indicates that a drug concept is viable. Current research indicates that CBD is effective in managing epilepsy, especially when administered at an early stage, and can be used to manage anxiety and depression effectively. However, while the concept is there that CBD can be a solution for neurogenerative disorders, psychosis, and chronic pain, the research that has been done is still inconclusive and too often based on preclinical studies. Furthermore, more research is needed to define the right dosage. Ensuring that the dosage is respected is an actual challenge, as the medication might be abused.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Glossary

α-syn	α-synuclein
2-AG	2-Arachidonoylglycerol
3NP	3-nitropropionic acid
5-HT1A	5-Hydroxytryptamine Receptor 1A
5-HT1A	Serotonin 1A Receptor
AD	Alzheimer's Disease
AEA	Anandamide
Akt	Protein kinase B
BDNF	Brain-Derived Neurotrophic Factor
CB1	Cannabinoid Receptor Type 1
CB2	Cannabinoid Receptor Type 2
CBD	Cannabidiol
CNS	Central Nervous System
ECS	Endocannabinoid System
EEG	Electroencephalogram
FAAH	Fatty Acid Amide Hydrolase
FDA	Food and Drug Administration
fMRI	Functional Magnetic Resonance Imaging
GABA	Gamma-Aminobutyric Acid
$GABA_A$	Gamma-Aminobutyric Acid Type A
GPCR	G Protein-Coupled Receptor
GPR55	G Protein-Coupled Receptor 55
HD	Huntington's Disease
I-DOPA	L-Dihydroxyphenylalanine
IEDs	Interictal Epileptiform Discharges
IL-6	Interleukin 6
KCNQ2	potassium voltage-gated channel subfamily Q member 2
(f)MRI	Magnetic Resonance Imaging
NAEs	N-acylethanolamines
OEA	Oleoylethanolamide
PD	Parkinson's Disease
PEA	Palmitoylethanolamide
PET	Positron Emission Tomography
PPARα	Peroxisome Proliferator-Activated Receptor Alpha
PPARγ	Peroxisome Proliferator-Activated Receptor GammaPI3K - Phosphoinositide 3-kinase
PTSD	Post-Traumatic Stress Disorder
RBD	REM Sleep Behavior Disorder
RCT	Randomized Controlled Trial
(N) REM	(Non-)Rapid Eye Movement
SPECT	Single-Photon Emission Computed Tomography
SPST	Simulated Public Speaking Test
TD	Tardive dyskinesia
THC	Tetrahydrocannabinol
TNFα	Tumor Necrosis Factor Alpha
TRPV (1, 1a, 2)	Transient Receptor Potential Vanilloid (1, 1a, 2)
TRPM8	Transient receptor potential melastin channel 8
UPDRS	Unified Parkinson Disease Rating Scale

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Caffeine intake and anxiety: a meta-analysis

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The results from studies on relationship between caffeine intake and risk of anxiety remains controversial, so we conducted a meta-analysis to summarize the evidence about the association between caffeine intake and risk of anxiety. Relevant articles were identified by researching PubMed, Web of Science, Cochrane library, Embase, CNKI, WANFANG DATA, SinoMed and VIP from the inception to December, 2022. Three investigators independently sifted through the literature, extracted the data, and evaluated the quality of the included studies based on predetermined selection criteria and assessed articles with Risk of bias assessment tool for Cochrane systematic reviews and analytical cross-sectional study quality assessment tool from JBI PACES. After assessing the quality of the literature, meta-analysis was performed using Revman 5.4 and Stata 12.0. Data were obtained from eight articles, and 546 participants from 14 studies in eight articles from healthy populations were included in the caffeine-anxiety analyses. As the scales used to assess anxiety vary in the literature, we chose standardized mean difference as the outcome indicator. In terms of overall effect, the results of the meta-analysis showed that caffeine intake increased the risk of anxiety [SMD = 0.94, 95% CI = (0.28, 1.60), $p < 0.05$]. After suspecting that dose size might be responsible for the heterogeneity by sensitivity analysis, we performed subgroup analysis according to dose size and found that low-dose caffeine intake moderately increased the risk of anxiety [SMD = 0.61, 95%CI = (0.42, 0.79), $p < 0.05$], whereas high-dose caffeine intake had a highly significant increase in the risk of anxiety [SMD = 2.86, 95%CI = (2.50, 3.22), $p < 0.05$]. The results confirm that caffeine intake is associated with an elevated risk of anxiety in healthy individuals without psychiatric disorders, especially when the intake dose is greater than 400 mg.

KEYWORDS

coffee, caffeine, anxiety, meta-analysis, healthy population

Introduction

Coffee is one of the best-selling drinks in the world which has a powerful impact on long-term health. According to the Washington Post (2015), the world consumes 2 billion cups of coffee every day. Coffee consumption in China is also increasing year by year. Coffee consists of many bioactive substances with potential antioxidant, anti-inflammatory or anti-cancer effects such as chlorogenic acid, caffeine, alkaloids, caffeol (Cano-Marquina et al., 2013; Ludwig et al., 2014), among which caffeine is classified as a central nervous system (CNS) stimulant and an organic molecule known as methylxanthine.

Caffeine has three different mechanisms of action on the central nervous system to produce a psychostimulant effect, one of which involves the stimulatory antagonism of

methylxanthines at the level of adenosine receptors. Four receptors, A1, A2A, A2B, and A3, comprise the adenosine system, of which A1 and A2A bind to caffeine with high affinity and in a reversible manner at normal physiologic doses. Adenosine receptors are mainly located in the hippocampus, amygdala and prefrontal cortex. Adenosine A1 and A2A receptors act as neuromodulators that modulate the activity of other neurotransmitters such as glutamate, gamma-aminobutyric acid (GABA), acetylcholine, 5-hydroxytryptophan, and dopamine (Fredholm et al., 2005), which have been implicated in anxiety. A1 and A2A receptors are also involved in physiological mechanisms such as vasoconstriction and microglial cell functioning (Fredholm et al., 2005) as well as the modulation of a variety of psychological functions including sleep, arousal, memory and anxiety (Ribeiro et al., 2002; Wei et al., 2014). In summary, studies have shown that adenosine receptor blockade can cause anxiety (Maximino et al., 2011), and that caffeine, a methylxanthine substance, is an antagonist of adenosine receptors, which increases energy metabolism in the brain but at the same time reduces cerebral blood flow, leading to relative hypoperfusion, activation of norepinephrine neurons, and effects on the local release of dopamine, leading to anxiety (Nehlig et al., 1992).

According to the World Health Organization (WHO), the global prevalence of anxiety disorders has increased by 15 percent since 2005, with nearly 264 million people suffering from anxiety disorders in 2015 (World Health Organization, 2017). In addition, an estimated 25% increase in the global prevalence of anxiety disorders was recently reported during the 2019 coronavirus disease (COVID-19) pandemic (COVID-19 Mental Disorders Collaborators, 2021). Home confinement during an epidemic has an impact on the population, including changes in sleeping habits and eating habits. People consume unhealthy foods and develop unhealthy habits, including increased caffeine intake, while anxiety and stress levels are among the most reported in studies of physical and mental health (Ammar et al., 2020; Taheri et al., 2023). People with anxiety disorders often experience intense and excessive fear and worry, and these feelings are often accompanied by physical tension and other behavioral and cognitive symptoms that are difficult and distressing to control. If left untreated, they can last for a long time. Anxiety disorders interfere with daily activities and can jeopardize a person's family, social, school or work life, so research into the relationship between caffeine and anxiety has important public health implications.

Several epidemiologic studies have found a link between caffeine and anxiety, but the results of the existing literature are inconsistent. For example: In a cross-sectional analysis conducted in Iran to examine the association between caffeine intake and symptoms of psychological disorders in adults, the trial demonstrated a significantly lower probability of experiencing anxiety symptoms with weekly or more coffee consumption compared with no coffee consumption, consistent with the findings of a prospective cohort study on the association between tea consumption and anxiety symptoms conducted in Singapore (Chan et al., 2018; Nouri-Majd et al., 2022), while opposite conclusions were reached in a cross-sectional study investigating whether there is a correlation between caffeine intake and anxiety among college students at Florida State University, a randomized controlled trial of the effects of caffeine on mood performance among college student volunteers at the University of Bristol, and a cohort study using the resources of a UK Biobank (Smith et al., 1999; Bertasi et al., 2021; Min et al., 2023). As previous individual studies may not have been of sufficient quality to obtain reliable data, there is still a lack of meta-analysis between caffeine intake and anxiety in healthy populations, although there have been some meta-analysis on the effects of caffeine and anxiety episodes in patients with panic attacks and on the relationship between caffeine intake and symptoms of depression (Klevebrant and Frick, 2022; Torabynasab et al., 2023).

Controversy exists regarding the relationship between caffeine intake and the risk of anxiety, and in order to elucidate the relationship between caffeine and anxiety in healthy populations, meta-analysis of the literature on caffeine intake and anxiety that met predetermined inclusion and exclusion criteria were selected for this study to provide evidence-based evidence for anxiety prevention.

Methods

Search strategy

This study performs meta-analysis and reports findings in accordance with Systematic Reviews and Meta-analysis (PRISMA) Statement (Liberati et al., 2009). We used computer searches of PubMed, Web of Science, Cochrane library, Embase, CNKI, WANFANG DATA, SinoMed and VIP databases to retrieve qualified randomized controlled studies and observational studies on caffeine and anxiety. The computer search included keywords such as "coffee," "caffeine" and "anxiety," and the search format was a combination of thematic and free-form terms. PubMed database search formula: ("Coffee" [MeSH Terms] OR ("Caffeine" [MeSH Terms] OR "Vivarin" [Title/Abstract])) AND ("Anxiety" [MeSH Terms] OR ("Angst" [Title/Abstract] OR "Nervousness" [Title/Abstract] OR "Anxiousness" [Title/Abstract]) OR ("Anxiety Disorders" [MeSH Terms])). The search strategy was customized slightly for different databases. We searched for studies and related papers published before December 1, 2022.

Literature inclusion and exclusion criteria

Studies were included in this meta-analysis if they met the following criteria: (1) the included studies were randomized clinical trial, prospective cohort studies, case–control studies and cross-sectional studies; (2) the study population was healthy population without psychiatric disorders; (3) the intervention was caffeine consumption. The trial group received total coffee or caffeinated beverage and the control group received decaffeinated coffee or placebo and (4) the mean and standard deviation of anxiety scale scores were used as outcome indicators.

Studies were excluded if (1) subjects already suffered from anxiety or were in a specific stressful situation; (2) presence of other psychotropic drug interventions; (3) republished studies with the most detailed data were selected; (4) a study in which data were ambiguous, incomplete, or unable to be transformed or merged; (5) lack of original data.

Literature and data extraction

The systematic literature search was performed following the PRISMA guidelines (Moher et al., 2009). Two reviewers sifted through

the study independently, and any disagreement was resolved by discussion between the two reviewers. If consensus could not be reached, a third reviewer was consulted. After eliminating duplicates, the titles and abstracts were read first to exclude obvious irrelevant literature, and the full text was read further to determine final inclusion. If necessary, authors were contacted by email or telephone for unidentified if necessary, authors were contacted by email or telephone to obtain unidentified but important information relevant to the study. The extracts will include authors, year of publication, study region, basic characteristics of the study population, total number of people studied, type of study, caffeine consumption, methods of outcome assessment, outcome events, mean and SD of each scale score, and adjustment factors.

Assessment of methodological quality

We utilized the Cochrane collaboration's tool for assessing risk of bias to investigate the quality of the clinical trials included in this meta-analysis and JBI PACES to investigate the quality of cross-sectional trials (Higgins et al., 2011). The Cochrane collaboration's tool for RCT trials assesses risk of bias on the following domains: selection bias, performance bias, attrition bias, reporting bias, detection bias and other bias. For each criterion, risk of bias was assessed as (1) low risk of bias, (2) unclear risk of bias, (3) high risk of bias. The JBI evaluation criteria for cross-sectional studies consisted of 10 items, each rated on a scale of 0 to 2. A score of 0 indicated that the requirements were not met; a score of 1 indicated that it was only mentioned but not described in detail; and a score of 2 indicated that a detailed, comprehensive, and correct description was given. Conflicts of opinion were discussed with a third review author until consensus is reached.

Statistical analysis

The meta-analysis was performed by using Revman 5.4 and Stata version 12.0 software. Since all outcome indicators in this study were continuous variables and the scoring tools of anxiety scale were different, data were synthesized by using the standardized mean difference (SMD) with 95% confidence interval (CIs). For continuous outcomes, SMD with 95%CIs were calculated as the difference in means between groups divided by the pooled standard deviation (Cohen, 1988; Higgins and Green, 2010). Effect sizes with a value of $p < 0.05$ were considered significant. We utilized Dixon's Q-test and the I-squared (I^2) statistical tests to assess result heterogeneity. If there was no heterogeneity among the results ($p > 0.1$, $I^2 < 50\%$), the fixed-effect model was used for analysis. If heterogeneity existed ($p \leq 0.1$, $I^2 > 50\%$), random effects model was used for analysis, parallel subgroup analysis or sensitivity analysis (Higgins et al., 2011). $p < 0.05$ was considered a statistically significant difference. We utilized sensitivity analysis to examine the stability of the results by removing individual trials to determine whether the removed study had a particular impact. We created funnel plots and visually examined the signs of asymmetry to investigate publication bias. Where data was missing, we contacted the authors to request further information. If data could not be obtained, we did not include the study in the meta-analysis.

Cohen's categories were used to evaluate the magnitude of the overall effect size with (1) SMD = 0.2–0.5: small; (2) SMD = 0.5–0.8: medium, and (3) SMD > 0.8: large effect sizes (Cohen, 1988).

Results

Literature search and screening results

Our initial search identified 5,365 relevant articles, with 3,895 remaining after duplication. After screening titles and abstracts and excluding studies which were letters, reviews, meta-analysis, posters, and meetings, we identified 34 studies for further evaluation. Of the 34 initially included studies, we excluded 3 studies due to experimental animal studies, 2 studies with case control or case crossover design, 2 studies with a retrospective design, 10 studies with discontinuous data or unclear data and 9 studies with discordant outcome indicators. Eight studies remained in the meta-analysis (Figure 1).

Basic characteristics of the included studies

A total of eight papers (Quinlan et al., 1997; Souissi et al., 2012; Jin et al., 2016; Papakonstantinou et al., 2016; Distelberg et al., 2017; García et al., 2017; Giles et al., 2017; Chtourou et al., 2019) were included in this study. The included studies comprised approximately 546 study participants. Characteristics of these eight studies are shown in Table 1, including seven RCTS and one cross-sectional study. Regarding the study region, two studies were conducted in Europe, two in Tunisia, three in America and one in Korea. Eight studies included adults with elevated levels of anxiety by BAI, POMS, STAI, SAS scales. According to the methodological assessment of the included literature, seven RCTS reached a moderate risk of bias (Figures 2, 3). One CS scored 16 with a high quality.

Results of meta-analysis

Analysis of overall effects

Eight studies (Quinlan et al., 1997; Souissi et al., 2012; Jin et al., 2016; Papakonstantinou et al., 2016; Distelberg et al., 2017; García et al., 2017; Giles et al., 2017; Chtourou et al., 2019), including 546 people, were selected to evaluate the relationship between caffeine intake and anxiety. Meta-analysis of random effects models revealed that caffeine consumption significantly increased the risk of anxiety compared with the control group [SMD = 0.94, 95%Cl = (0.28, 1.60), $p < 0.05$; heterogeneity: $I^2 = 94.7\% > 50\%$, $p < 0.001$] (Figure 4). Based on Cohen's categories, these effects were of large size.

Sensitivity analysis and subgroup analysis

Sensitivity analysis demonstrated that the literature exhibits different sensitivity profiles based on caffeine consumption (Figure 5). Subgroup analysis was performed based on coffee

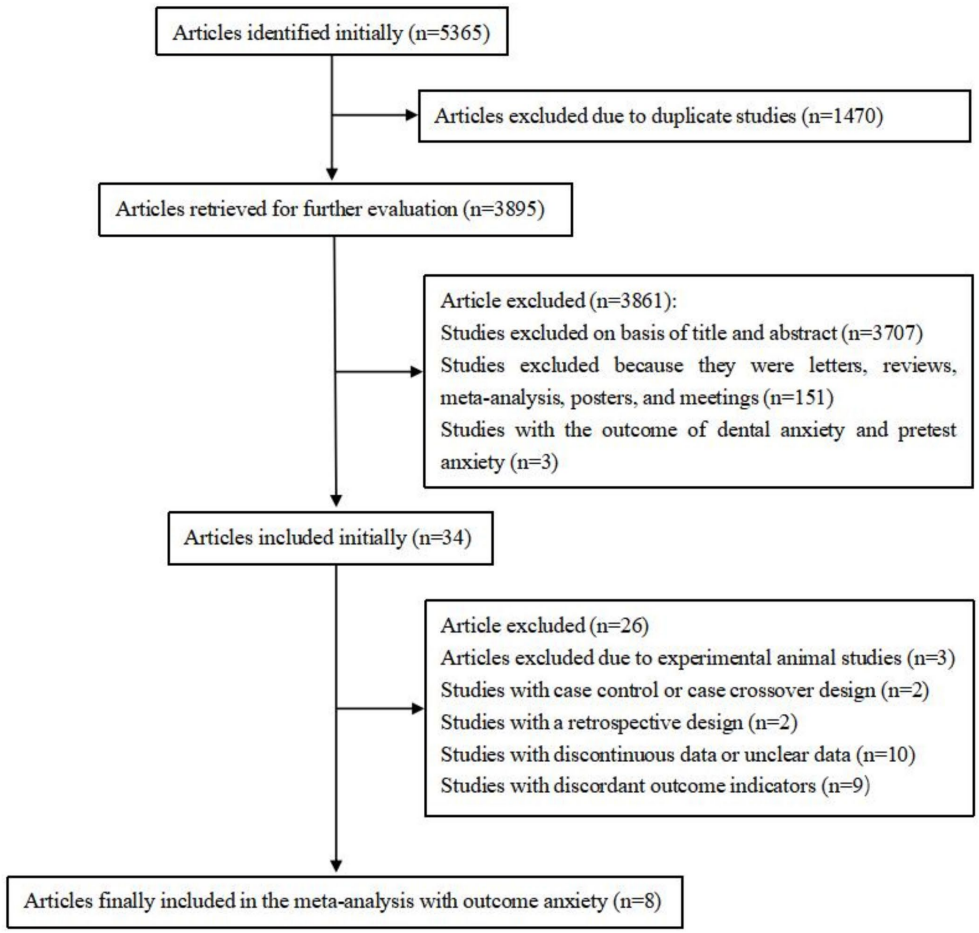

FIGURE 1
Flow diagram of included and excluded studies.

consumption divided into a high-dose caffeine intake group (≥400 mg) and a low-dose caffeine intake group (<400 mg), while the FDA recommended daily coffee intake was also <400 mg. By continuing the sensitivity analysis in an exclusion-by-exclusion manner, it was found that when (Souissi et al., 2012) was excluded from the high-dose caffeine intake group and (Papakonstantinou et al., 2016) filter coffee, (Quinlan et al., 1997; Chtourou et al., 2019) were excluded from the low-dose caffeine intake group, there was no heterogeneity in subgroup analysis. The results of the subgroup analysis of the fixed effects model showed a moderate increase in anxiety scores in the test group in the low-dose subgroup [SMD = 0.61, 95%Cl = (0.42, 0.79), $p < 0.05$; heterogeneity: $I^2 = 0\%$ <50, $p > 0.1$]. In the high-dose subgroup, the anxiety scores of the test group increased extremely significantly [SMD = 2.86, 95%Cl = (2.50, 3.22), $p < 0.05$; heterogeneity: $I^2 = 45.9\%$ <50, $p > 0.1$]. Detailed information can be found in Figure 6.

Publication bias assessment

The funnel plot of this study is basically symmetrical and it can be judged that there is no publication bias in the literature of this study (Figure 7).

Discussion

Our study was the meta-analysis of examining the association between caffeine intake and anxiety. This meta-analysis included 546 participants for caffeine intake and identified that caffeine intake was significantly associated with increased risk of anxiety in healthy people. Because of differences in baseline data collection at the time of the meta-analysis, heterogeneity between studies can easily occur, so we performed subgroup analyses based on the results of the sensitivity analyses, which showed that the high-dose caffeine (≥400 mg) group had a significantly higher increased risk of anxiety than the low-dose caffeine (<400 mg) group [SMD = 2.86, 95% Cl = (2.50, 3.22), $p < 0.05$]. Overall, this is also consistent with a number of findings from other literature (Chait, 1992; Kaplan et al., 1997) and, combined with quantitative analyses, suggests an anxietogenic effect of caffeine in healthy populations. It is noteworthy that polymorphisms of the A2A receptor influence the susceptibility of individuals to caffeine-induced anxiety (Alsene et al., 2003; Childs et al., 2008) but none of the included literature investigated A2A receptor polymorphisms, which is a potential factor for generating heterogeneity and may lead to biased results.

In discussing the clinical significance of the findings, we wondered if there was a dose–response relationship between caffeine and anxiety,

TABLE 1 Descriptive information for studies included in the meta-analysis.

Study	Study design	Sample size	Intervention/Exposure	Time of caffeine intake before the experimental session	Outcome assessment
Distelberg et al. (2017) the United States	RCT	49 Healthy participants	710 mL of either regular coffee (containing 450 mg caffeine) or decaffeinated coffee	5 day	BAI
Chtourou et al. (2019) Tunisia	RCT	19 Male physical-education students	Drinking 2 cans of "Red Bull' beverage (containing 160 mg caffeine) or drinking a placebo	1 h	POMS
García et al. (2017) Colombia	RCT	80 Medical students	The intake of 460 mL of either an energy drink or carbonated water	1 h	STAI
Giles et al. (2017) the United States	RCT	96 Adults	Consuming 400 mg caffeine or placebo	45 or 75 min	STAI
Jin et al. (2016) Korea	CS	234 Middle school students	Daily coffee intake >27.5 mg or <4 mg	1 month	BAI
Papakonstantinou et al. (2016) Greece	RCT	40 Healthy individuals	Randomly consumed four 200 mL coffee beverages containing 160 mg caffeine	1 h	SAS
Quinlan et al. (1997) the United Kingdom	RCT	16 Habitual caffeine consumers	Subjects ingested caffeine dose or placebo	1 h	STAI
Souissi et al. (2012) Tunisia	RCT	12 Elite judoists	Beverages were ingested with/without 100 mg caffeine	1 h	POMS

BAI, beck anxiety inventory; POMS, profile of mood states; STAI, state-trait anxiety inventory; SAS, self-rating anxiety scale.

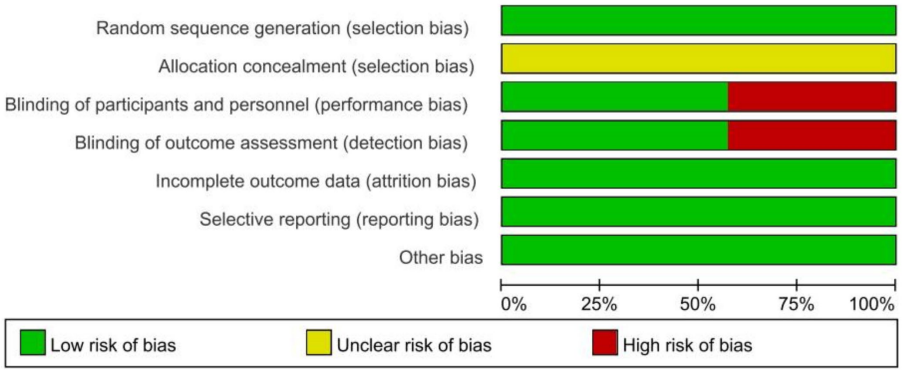

FIGURE 2
Methodological quality of included studies.

but due to the small amount of literature using doses of caffeine above 460 mg, the dose–response relationship could not be analyzed. Caffeine is a central nervous system stimulant and acts in the brain through adenosine receptors, influencing attention, alertness, and producing anxiogenic effects, which may cause anxiety disorders (Aniţei et al., 2011). It also interacts with dopaminergic transmission, which is considered a different interaction from other psychostimulants such as cocaine and amphetamines (Fredholm et al., 1999). Important factors to consider clinically are that caffeine interacts with treatment and the fact that caffeine is addictive and caffeine withdrawal has been added as a diagnostic category to the DSM-5 (Hasin et al., 2013). We recommend that clinicians consider the potential anxietogenic effects of caffeine in the treatment of psychiatric disorders, and that caffeine consumption be appropriately assessed for a more individualized treatment strategy. In addition, caffeine can produce anxiety or exacerbate anxiety in adults with pre-existing anxiety disorders (Nehlig, 2016); however, the doses associated with these effects are large (1–2 g of caffeine/day) and may be consumed by only a small percentage of caffeine consumers. It has been suggested that those who experience anxiety effects from caffeine may avoid the substance (James, 1991), and that the self-limiting nature of caffeine intake would reduce any likelihood of caffeine producing anxiety in adults (Nehlig, 2016), and therefore we recommend that caffeine intake in healthy populations does not exceed 400 mg per day.

The mechanism of the association between coffee or caffeine and anxiety was still not fully established. There have been several possible biological explanations so far: First of all, caffeine is a

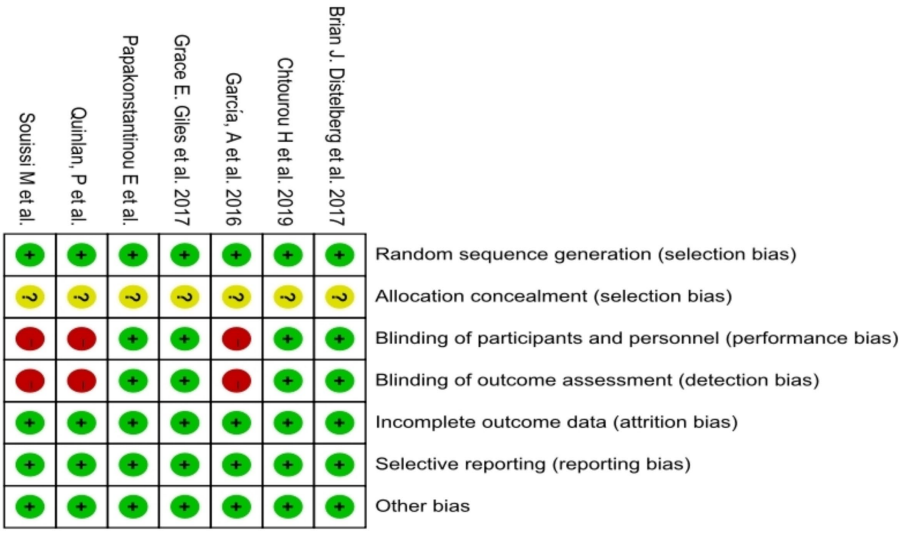

FIGURE 3
The distribution of the methodological quality of included studies.

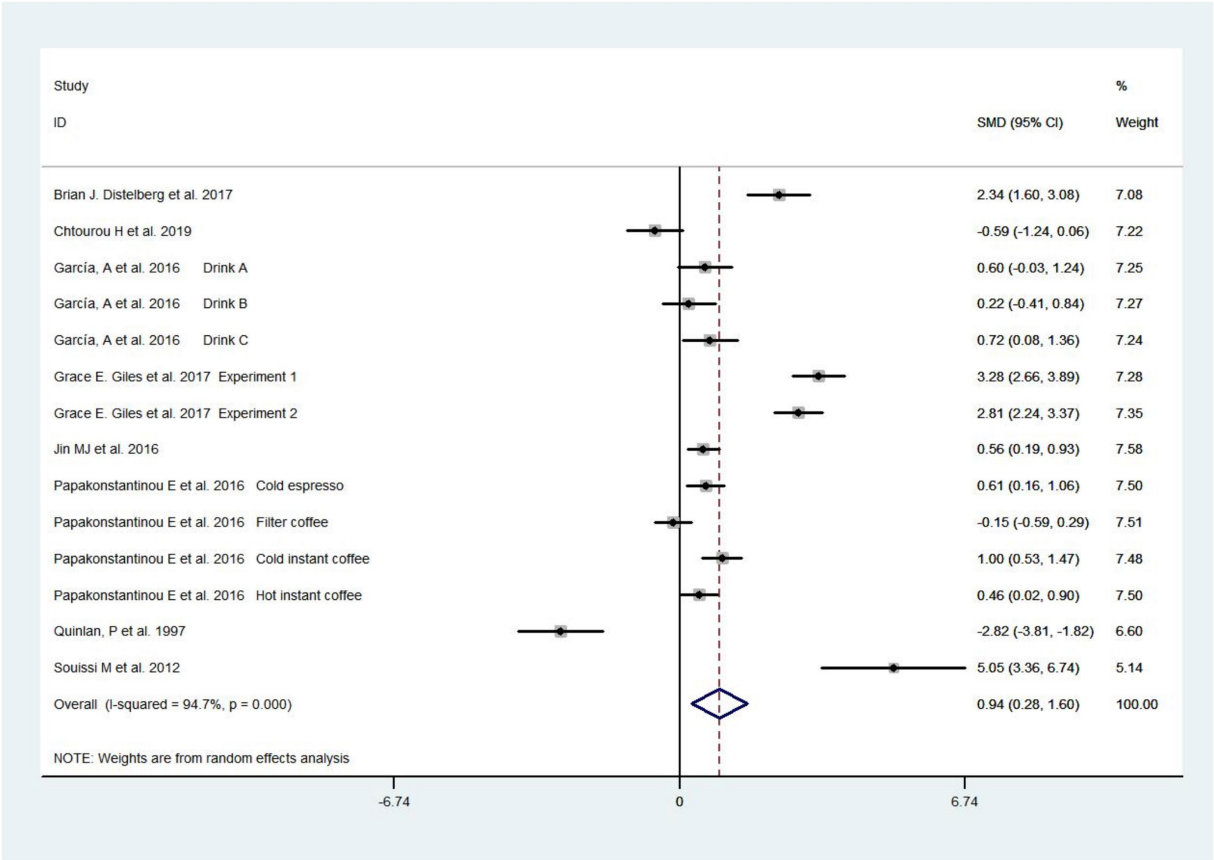

FIGURE 4
Forest plots of caffeine intake on anxiety.

xanthine with effects such as GABA inhibition, regulation of phosphodiesterases, activation of ryanodine receptors and non-selectively block adenosine receptors (Fredholm et al., 2005; Childs et al., 2008; Bodenmann et al., 2012), with the inhibitory A1 and predominantly excitatory A2A receptors being the most notable; the latter effects are most related to the systemic and local effects of

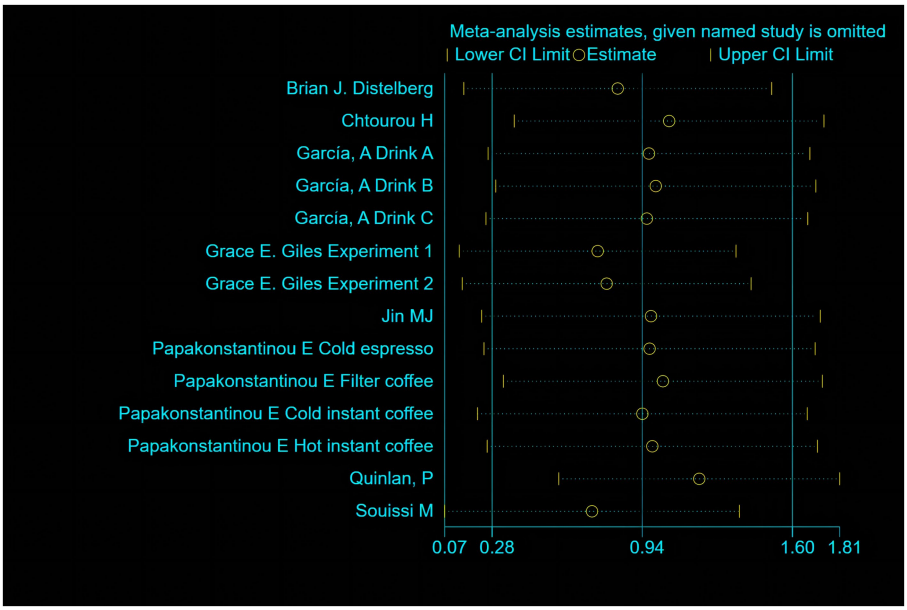

FIGURE 5
Sensitivity analysis of 8 literature.

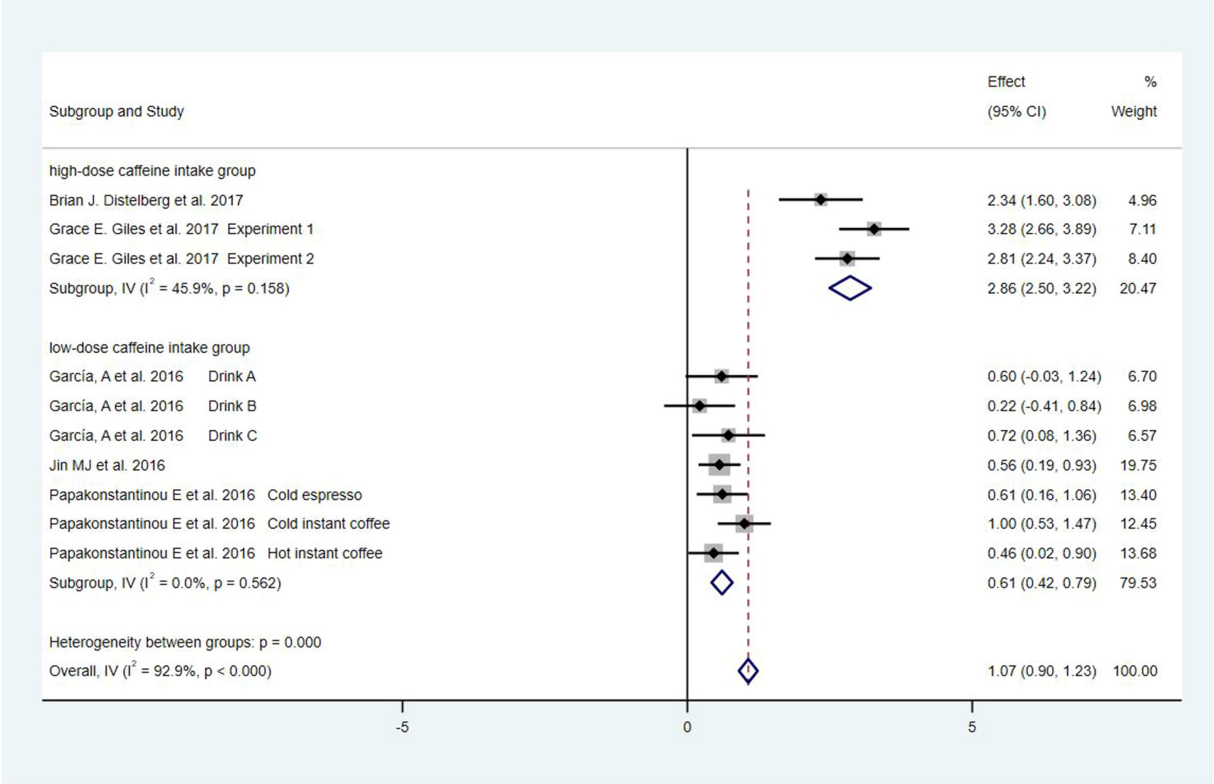

FIGURE 6
Forest plots of subgroup analysis.

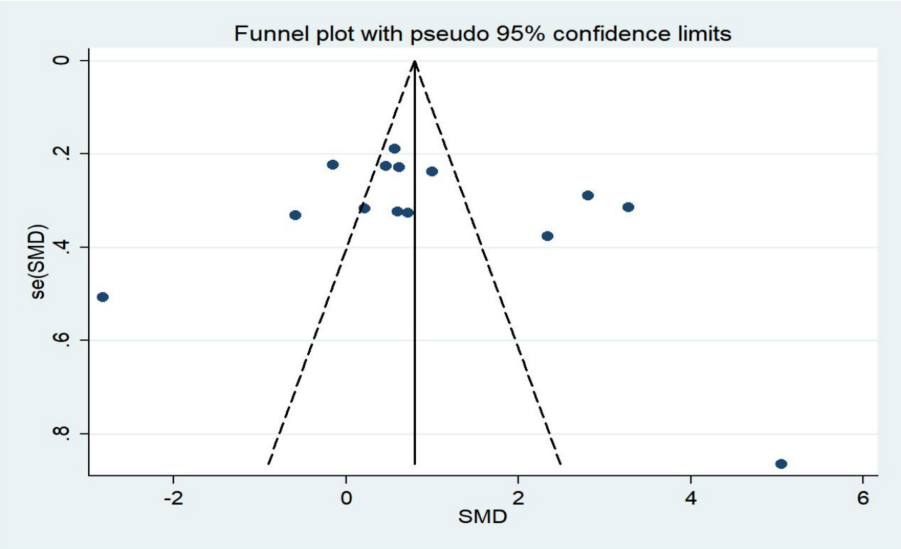

FIGURE 7
Funnel plot of caffeine intake on anxiety.

caffeine, such as increased alertness, decreased libido, wakefulness despite sleep deprivation, irritability, fatigue, headache, seizures, weakness, and sleep and mood disorders (Aniței et al., 2011). In this context, it is of interest that these receptors are involved in ground emotional processing. Adenosine A1 and A2A receptors are involved in the regulation of myocardial oxygen function and coronary blood flow, and antagonizing these receptors leads to increased heart rate. Psychologically, an increased heart rate may cause the body to believe that a disaster is imminent (Clark et al., 1997), meaning that the antagonistic effect caused by caffeine may cause anxiety, especially at higher doses, inducing more anxiety. Caffeine is a stimulant that affects the central nervous system, so consuming caffeinated beverages, such as coffee and tea, stimulates the central nervous system causing the body to produce and release adrenaline. This can cause a person to feel anxious or nervous (Nehlig et al., 1992). In addition, there has been no link between caffeine-induced anxiety and changes in brain activity and connectivity in healthy individuals, which could provide information on the mechanism of caffeine's anxiolytic effects.

Our study has some limitations. (1) Due to the limited amount of included literature, there was high between-study heterogeneity in the relationship between caffeine intake and anxiety, which was eliminated by excluding individual literature in the subgroup analysis. (2) Lack of consideration of confounding factors, and although we have excluded people with other psychiatric disorders and the presence of psychotropic drug interventions, factors such as gender were not controlled for, and there are studies suggesting that caffeine increases anxiety in males but not in females (Trapp et al., 2014; Richards and Smith, 2015; Kaur et al., 2020), which may bias our results. (3) The limited range of caffeine used [0–460 mg] prevented any meaningful analysis of the dose–response relationship. (4) Our results may also be confused by side effects.

Conclusion

In summary, the results of our meta-analysis suggest that caffeine consumption may have a detrimental effect on anxiety and may increase the risk of anxiety. This association was more pronounced at caffeine intake doses above 400 mg. Future studies should further elucidate the mechanisms of action between caffeine and anxiety from genetic risk polymorphisms to risk phenotypes. In addition, studies using a wider range of doses should be conducted to elucidate the dose–response relationship between caffeine and anxiety.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

CL: Methodology, Resources, Validation, Writing – original draft, Writing – review & editing. LW: Data curation, Software, Writing – original draft, Writing – review & editing. CZ: Data curation, Writing – review & editing. ZH: Software, Writing – review & editing. JT: Data curation, Writing – review & editing. JX: Data curation, Writing – review & editing. WL: Writing – review & editing, Supervision.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyg.2024.1270246/full#supplementary-material

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Brain reward function in people who use cannabis: a systematic review

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Rationale: Cannabis is one of the most widely used psychoactive substances globally. Cannabis use can be associated with alterations of reward processing, including affective flattening, apathy, anhedonia, and lower sensitivity to natural rewards in conjunction with higher sensitivity to cannabis-related rewards. Such alterations have been posited to be driven by changes in underlying brain reward pathways, as per prominent neuroscientific theories of addiction. Functional neuroimaging (fMRI) studies have examined brain reward function in cannabis users via the monetary incentive delay (MID) fMRI task; however, this evidence is yet to be systematically synthesised.

Objectives: We aimed to systematically integrate the evidence on brain reward function in cannabis users examined by the MID fMRI task; and in relation to metrics of cannabis exposure (e.g., dosage, frequency) and other behavioural variables.

Method: We pre-registered the review in PROSPERO and reported it using PRISMA guidelines. Literature searches were conducted in PsycINFO, PubMed, Medline, CINAHL, and Scopus.

Results: Nine studies were included, comprising 534 people with mean ages 16-to-28 years, of which 255 were people who use cannabis daily or almost daily, and 279 were controls. The fMRI literature to date led to largely non-significant group differences. A few studies reported group differences in the ventral striatum while participants anticipated rewards and losses; and in the caudate while participants received neutral outcomes. A few studies examined correlations between brain function and withdrawal, dosage, and age of onset; and reported inconsistent findings.

Conclusions: There is emerging but inconsistent evidence of altered brain reward function in cannabis users examined with the MID fMRI task. Future fMRI studies are required to confirm if the brain reward system is altered in vulnerable cannabis users who experience a Cannabis Use Disorder, as postulated by prominent neuroscientific theories of addiction.

KEYWORDS

cannabis, monetary incentive delay task (MIDT), reward processing, fMRI, neuroimaging, systematic review, functional neuroimaging

1 Introduction

Cannabis is one of the most widely used psychoactive substances globally, with over 219 million users (United Nations Office on Drugs and Crime, 2023). The regular use of cannabis can be associated with adverse psychosocial outcomes, including cannabis use disorders (CUD; Connor et al., 2021), mental health problems (Moore et al., 2007), impaired cognitive performance (Shrivastava et al., 2011), and hazardous behaviours (e.g., driving while intoxicated; Swift et al., 2010). Concerningly, cannabis use-related problems have been projected to rise with the increased accessibility and potency of cannabis products (Freeman et al., 2019). In order to develop effective preventative interventions in vulnerable people who use cannabis, it is important to understand the neurobiological mechanisms underlying cannabis use.

A key characteristic of regular cannabis use is altered processing of rewards (Pacheco-Colon et al., 2018). For example, people who use cannabis compared to controls show affective flattening, apathy, anhedonia, and decreased pleasure towards activities that are not related to cannabis use (Skumlien et al., 2021); as well as poorer cognitive performance during reward processing tasks (e.g., Iowa Gambling Task; Casey and Cservenka, 2020). In animal studies, repeated exposure to delta-9-tetrahydrocannabinol (THC; the main psychoactive compound of cannabis), leads to neuroadaptations within the brain's reward circuits, particularly within the mesolimbic dopamine system (Halbout et al., 2023).

Emerging functional neuroimaging evidence in human cannabis users has examined the neurobiology of reward processing (Balodis and Potenza, 2015; Skumlien et al., 2021). This work has been summarised by a recent high-quality systematic review (Skumlien et al., 2021). However, the review integrated evidence from varied functional Magnetic Resonance Imaging (fMRI) tasks (e.g., card guessing, coin toss, effort expenditure, and listening to music) with inconsistent cognitive load, complexity, and aspects of reward processing. As such, the findings could not be directly integrated. A separate review (Balodis and Potenza, 2015) synthesised findings from a specific reward processing fMRI task—the Monetary Incentive Delay (MID) task: the most consistently used and robust task to measure the function of the brain reward system (Oldham et al., 2018). However, the review included samples who use distinct substances (e.g., cannabis, alcohol, cocaine, nicotine; preventing the understanding of how the neurobiology of reward processing is affected by cannabis use specifically; Balodis and Potenza, 2015). Additionally, this review was published in 2015 and does not capture the most up-to-date literature (Balodis and Potenza, 2015). Furthermore, a systematic assessment of the *quality* of the fMRI literature of reward processing in cannabis users has yet to be conducted, which prevents a detailed interpretation of the evidence.

We aim to review the fMRI neuroimaging evidence to date that compared brain reward function between cannabis users and controls using the MID fMRI task. The secondary aim was to systematically synthesised the evidence on the association between brain reward function and metrics of cannabis use and related problems (e.g., dosage, frequency, and withdrawal), psychopathology symptom scores (e.g., anxiety, depression, and psychosis), and other variables (e.g., cognitive performance and other substance use).

2 Methods

This systematic review was pre-registered with PROSPERO (submitted 27/10/2022 and approved 17/11/2022; ID CRD42022354574) and was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (PRISMA).

2.1 Literature search

A comprehensive electronic database search was conducted using PsycINFO, MEDLINE, CINAHL, Scopus, and PubMed on June 10, 2022. The search strategy is outlined in Supplementary material and employed three concepts related to: (i) cannabis; (ii) functional neuroimaging; and (iii) reward processing. Medical Subject Headings (MeSH) and keywords (synonyms) were combined with Boolean OR/AND operators for each concept and were searched across the title and abstracts of the returned articles. All full-text articles from the database searches were imported into the reference manager software Covidence (www.covidence.org), and duplicates were removed.

2.2 Inclusion and exclusion criteria

Studies were included if they: (i) examined human participants; (ii) were written in the English language; (iii) were full-text peer-reviewed articles; (iv) assessed a sample of people who consume cannabis, as defined by each study criterion; (v) included a non-cannabis user control group, as defined by each study criterion; (vi) measured brain function during the MID fMRI task, and (vii) compared brain function between cannabis and control groups.

Studies were excluded if they: (i) examined non-human participants; (ii) were published in languages other than English; (iii) were non-peer-reviewed (e.g., conference abstracts only); (iv) were non-empirical (e.g., single case reports, dissertations, editorials, corrigendum, book chapters, letters to the editor, reviews, and meta-analyses); (v) measured brain integrity using neuroimaging techniques other than fMRI, for example: structural magnetic resonance imaging (sMRI), computed tomography scan (CT), electroencephalogram (EEG), positron emission tomography (PET), single-photon emission computerised tomography (SPECT); (vi) studies that included tasks other than the MID fMRI task; (vii) measured brain function during acute cannabis intoxication; (viii) examined a sample of participants who endorsed significant substance use other than cannabis, alcohol, and nicotine at a group level; (ix) included a sample of participants who endorsed a diagnosis of axis I mental health disorders at a group level; or (x) examined a sample with diagnoses of neurological disorders or major medical conditions that affect the central nervous system [e.g., epilepsy, human immunodeficiency virus (HIV)].

2.3 Manuscript screening

The title, abstract, and then full text of all retrieved articles were screened by two researchers (E.B. and S.A.) in accordance with the above inclusion/exclusion criteria to determine eligibility. The final list of studies was compared and resolved by the researchers via consensus; if consensus could not be reached it was resolved by the senior author (V.L.). Additionally, the reference lists of all selected studies were cross-referenced to aid the inclusion of relevant work.

2.4 Data extraction

The following data were extracted:

(i) Study characteristics (e.g., first author, year of publication, and study location);
(ii) Participant characteristics (e.g., sample size, age, sex, and handedness);
(iii) Cannabis use level (e.g., dosage, frequency, age-onset, duration, and hours of abstinence);
(iv) CUD/dependence (e.g., instrument used, level, and presence/absence);
(v) Experiment characteristics (e.g., study design, dropouts, and reasons for dropouts);
(vi) fMRI reward task characteristics, such as instructions, cognitive function targeted by fMRI task, fMRI task parameters (e.g., duration), fMRI task design (e.g., counterbalanced order);
(vii) fMRI data analysis approach [e.g., whole brain, region of interest (ROI) based, seed-based, and relevant regions]; and measure of brain function (e.g., activity/connectivity).
(viii) The group differences in patterns of brain function (e.g., location, direction), and the relevant contrasts used (e.g., reward anticipation vs. neutral anticipation, reward anticipation vs. loss anticipation).
(ix) The association between the level of brain function (e.g., location/direction) during fMRI reward processing tasks in cannabis users and behavioural measures such as: cannabis use levels (e.g., dosage, frequency, age-onset, and duration), psychopathology symptom scores (e.g., anxiety, depression, and psychosis), and other measures (e.g., cognition and/or behavioural metrics).

2.5 Assessment of risk of bias of the reviewed literature

The Joanna Briggs Institute Critical Appraisal Checklist for Analytical Cross-Sectional Studies (Munn et al., 2014) was used to assess the quality of the included studies using eight distinct criteria. The criteria were: (i) Were the criteria for inclusion in the sample clearly defined?; (ii) Were the study subjects and the setting described in detail?; (iii) Was the exposure measured in a valid and reliable way—*this criteria was not applicable and therefore removed from the table*; (iv) Were objective, standard criteria used for measurement of the condition? (v) Were confounding factors identified? (vi) Were strategies to deal with confounding factors stated? (vii) Were the outcomes measured in a valid and reliable way? and (viii) Was appropriate statistical analysis used? Bias ratings were assessed for each included study based on the criteria above (E.B. and H.T.).

A score for each paper was generated, whereby each criterion was scored either 1 = endorsed, 0 = not endorsed, or 0.5 = partially endorsed. Subsequently, the quality of each paper was rated either high (i.e., a score of $\geq$8) moderate (i.e., a score between 4 and 7.5), or low (i.e., a score of $\leq$ 3.5).

2.6 PRISMA flowchart

Figure 1 illustrates the PRISMA flowchart. The initial database search produced 1,835 articles. After duplicates were removed, 979 titles and abstracts were screened based on the inclusion and exclusion criteria. Of these, 45 articles underwent full-text review, and 36 of them were not eligible for inclusion. Overall, nine studies were included in this systematic review (Nestor et al., 2010, 2020; van Hell et al., 2010; Filbey et al., 2013; Jager et al., 2013; Yip et al., 2014; Enzi et al., 2015; Karoly et al., 2015; Skumlien et al., 2022).

3 Results

3.1 Overview of studies and samples socio-demographic, substance use, and other characteristics

3.1.1 Characteristics of the reviewed literature

Table 1 overviews the characteristics of the nine studies included in the review (Nestor et al., 2010, 2020; van Hell et al., 2010; Filbey et al., 2013; Jager et al., 2013; Yip et al., 2014; Enzi et al., 2015; Karoly et al., 2015; Skumlien et al., 2022). All studies were published in the past 13 years, between 2010 and 2022.

3.1.2 Overview of demographics of the reviewed samples

The reviewed samples comprised 534 participants (143 female and 391 males). Of these, 255 participants were people who use cannabis, and 279 were controls, with sample sizes ranging from 28 to 186. The average of the mean age of the samples was 22 years (range 16–28 years). The samples included both adolescents aged <18 years (three studies; Jager et al., 2013; Karoly et al., 2015; Nestor et al., 2020) and adults aged 18+ years (five studies; Nestor et al., 2010; van Hell et al., 2010; Filbey et al., 2013; Yip et al., 2014; Enzi et al., 2015) or both (1 study; Skumlien et al., 2022). Males were overrepresented in eight of the nine studies, and three studies recruited males only. See Supplementary material for recruitment sources and location of the reviewed studies.

3.1.3 Overview of levels of cannabis use

Table 1 outlines the level of cannabis consumption in the examined samples. The mean *age of cannabis use onset* was 16 years (range; 13-to-18 years). The level of mean *cannabis dosage* varied

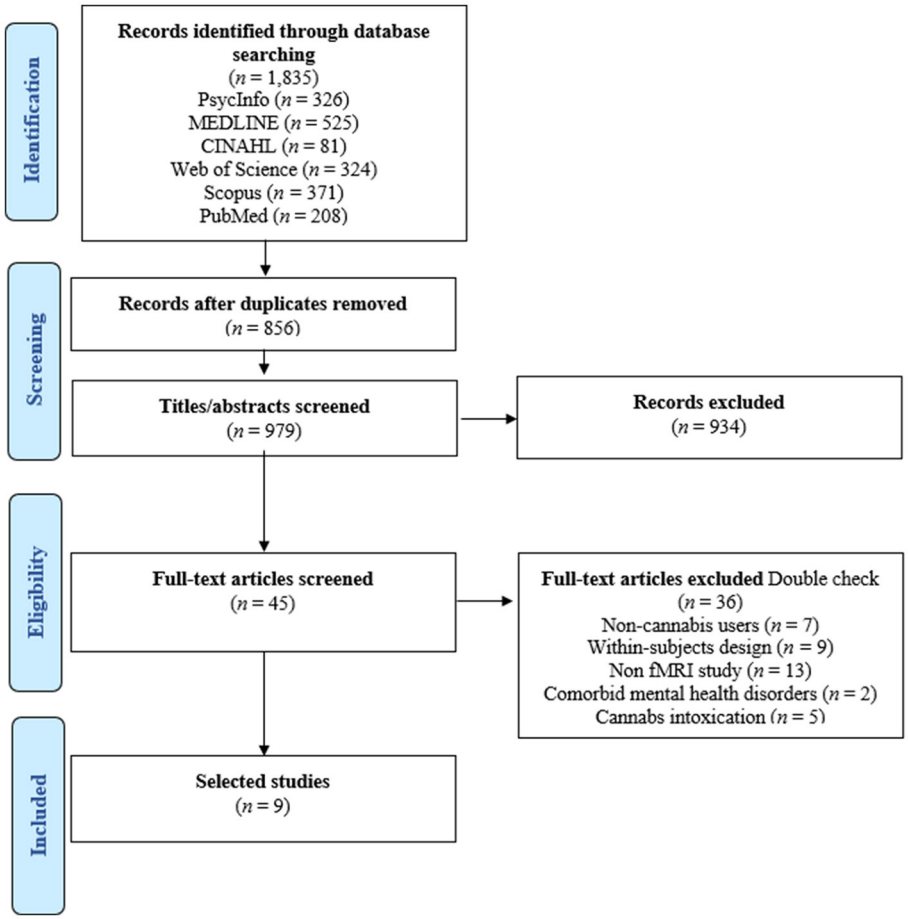

FIGURE 1
PRISMA flowchart.

across the reviewed samples: 20 joints/week (range; 13–44 (e.g., joints; joints/week), 1 gramme/week (Skumlien et al., 2022); and 14 cannabis hits/day (Karoly et al., 2015). All samples included a group of people with current cannabis use, except for two studies, where cannabis groups were abstinent for ~21 days (Yip et al., 2014) or 5 weeks (Jager et al., 2013). Two studies included additional control participants who used nicotine and participants who did not use nicotine (van Hell et al., 2010; Karoly et al., 2015).

3.2 Overview of methodologies used in the reviewed literature

3.2.1 Characteristics of the MID fMRI task

All nine studies used a modified version of the original MID fMRI task, developed by Knutson et al. (2001). The basic structure of the task included the following stages in this order: (i) *a reward cue*, singling a potential "win," "loss," or "no outcome" cue, (ii) a *target stimulus* where participants press a button to try to win or to avoid losing money, and (iii) a *feedback* stage where participants received feedback on either winning money, avoiding losing money, or losing money.

To note, studies used the terms win/reward or feedback/receipt interchangeably. As such, we use the terms "anticipation of reward/loss/neutral" and "feedback of reward/loss/neutral" for consistency and readability. Additional information on the parameters of the MID fMRI task is summarised in Supplementary material.

3.2.2 Overview of fMRI data analysis methods

The studies used different fMRI data analysis methods. They included: exploratory whole-brain analysis (three studies; Nestor et al., 2010; Filbey et al., 2013; Enzi et al., 2015), a priori region of interest (ROI) analysis focused on hypothesis-driven areas (two studies; Yip et al., 2014; Karoly et al., 2015). A total of two studies focused on the striatal ROIs: the ventral striatum (Yip et al., 2014; Skumlien et al., 2022). Other studies focused on the nucleus accumbens (NAcc; van Hell et al., 2010; Karoly et al., 2015), and caudate (Jager et al., 2013; Yip et al., 2014). Individual studies used other ROIs: the

TABLE 1 Overview of demographics and cannabis use characteristics for the reviewed samples.

References	Sub-groups	Sample *N* total (female) Age, *yrs*				Cannabis use level				Behavioral group differences in MIDT performance		
		Cannabis	Control	Cannabis	Control	Age onset, yrs	Duration, yrs	Days/mo	Dosage	Abstinence duration	Accuracy	Reaction time
Skumlien et al. (2022)	Adolescent	32 (16)	31 (15)	17 (1)	17 (0)	15 (1)	-	12 (8)	1 (1) *grammes p/w*	44 h	CB = HC (% correct) *No group-by-age interaction*	CB = HC *No group-by-age interaction*
	Adults	31 (14)	31 (16)	28 (1)	27 (1)	18 (3)	-	16 (8)		42 h	-	-
Nestor et al. (2020)		18 (1)	18 (1)	17 (0)	16 (0)	13 (0)	-	-	44 (9) *joints p/w*	Before scan	CB > HC CB = HC for relative motivational value (% accuracy during reward or loss/neutral)	CB = HC
Enzi et al. (2015)		15 (0)	15 (0)	26 (3)	27 (4)	16 (3)	8 (3)	-	13 (7) *joints p/w*	24 h	-	CB = HC
Karoly et al. (2015)	No-nicotine	14 (3)	38 (14)	16 (1)	16 (1)	13 (2)	-	20 (9)	98 (77) *hits p/w*	3 h	CB = HC & CB+tobacco, CB+alcohol +tobacco, tobacco, alcohol (% hits)	CB = HC and CB+tobacco, CB+alcohol +tobacco, tobacco, alcohol
	Nicotine	17 (4)	34 (13)	16 (1)	16 (1)	11 (2)	-	25 (7)	133 (175) *hits p/w*			
Yip et al. (2014)		20 (0)	20 (0)	27 (2)	29 (2)	13 (0)	14 (3)	16 (3)	-	24 h or 21 days	CB = HC (% correct hits)	CB = HC
Filbey et al. (2013)		59 (13)	27 (22)	23 (6)	30 (10)	15 (3)	9 (6)	28 (4)	-	72 h	CB = HC (% correct) CB = HC ($ won/lost)	CB = HC In CB, reward < loss, with $5 incentive
Jager et al. (2013)		21 (0)	24 (0)	17 (1)	17 (1)	13 (2)	-	-	15 (16) *joints p/w*	5 wks	CB = HC ($ won)	CB > HC, trend
Nestor et al. (2010)		14 (2)	14 (3)	22 (1)	23 (1)	16 (0)	6 (1)	20 (3)	16 (3) *joints p/w*	108 h	CB = HC (% correct)	CB = HC
van Hell et al. (2010)	Nicotine	14 (1)	14 (3)	24 (4)	25 (5)	-	-	-	11 (8) *joints p/w*	1 wk	CB = HC ($ won)	CB = HC
	Non-nicotine		13 (2)		24 (3)	-	-	-				

Hrs, hours; MIDT, monetary incentive delay task; m/o, months; p/w, per week; SD, standard deviation; wk, week; yrs, years.
Findings from Nestor et al. (2010), van Hell et al. (2010), Filbey et al. (2013), and Nestor et al. (2020) accounted for trial type (e.g.).

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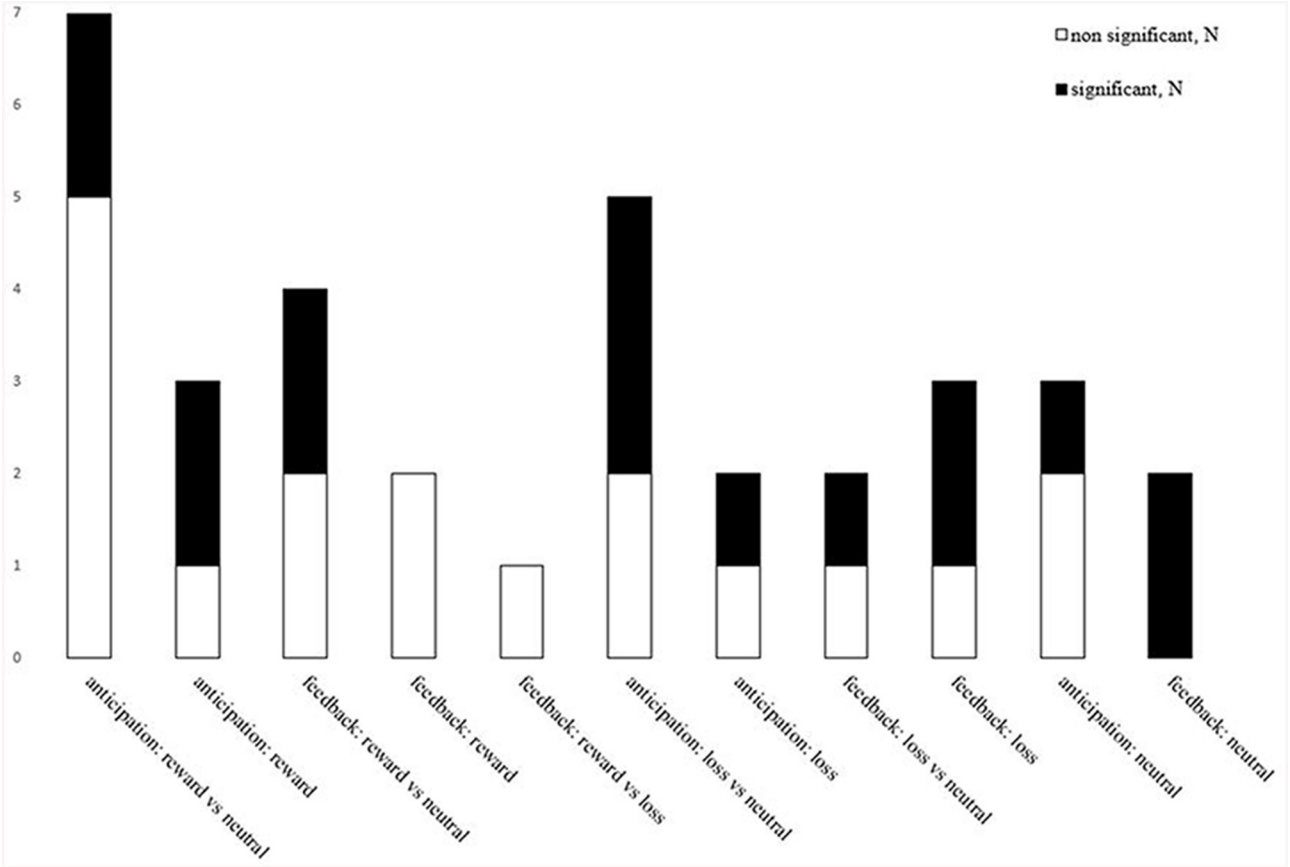

FIGURE 2
Overview of studies examining specific conditions/contrasts and of significant and non-significant findings. The total number of studies that found significant (black) and non-significant (white) findings between cannabis users compared to controls across the differing MID fMRI task contrasts. Anticipation: reward vs. neutral (seven studies; three significant): reward (three studies; two significant); loss vs. neutral (five studies; three significant); loss (two studies; one significant); neutral outcomes (three studies; one significant). Feedback: reward vs. neutral (four studies; two significant); reward (two studies; zero significant); reward vs. loss (one study; zero significant); loss vs. neutral (two studies; one significant); loss (three studies; two significant); neutral outcomes (two studies; two significant).

putamen (Jager et al., 2013), and the ventromedial prefrontal cortex (vmPFC; Skumlien et al., 2022). A total of three studies used both whole-brain and ROI approaches (van Hell et al., 2010; Yip et al., 2014; Skumlien et al., 2022). An individual study used graph theory ROI-to-ROI functional connectivity (Nestor et al., 2020).

3.3 Group differences in behavioural task performance

There were largely non-significant group differences in behavioural task performance, including accuracy and reaction times (Table 1). Only one of the nine studies reported that cannabis users were significantly more accurate than controls during loss vs. neutral trials (Nestor et al., 2020). One study found that cannabis users had a trend of slower reaction times than controls, during reward vs. neutral trials (Jager et al., 2013).

3.4 Brain functional differences during the MID fMRI task

This section overviews group differences in brain function during reward, loss, and neutral conditions (Figure 3). Out of 34 contrasts reported, 16 found significant group differences.

3.5 Group differences in brain function during reward trials

This section overviews group differences during the anticipation and receipt of rewards (Tables 2–4, respectively).

3.5.1 Group differences while anticipating monetary rewards vs. neutral outcomes

Seven studies compared groups by brain function during anticipation of *monetary rewards vs. neutral outcomes* (van Hell et al., 2010; Filbey et al., 2013; Jager et al., 2013; Yip et al., 2014;

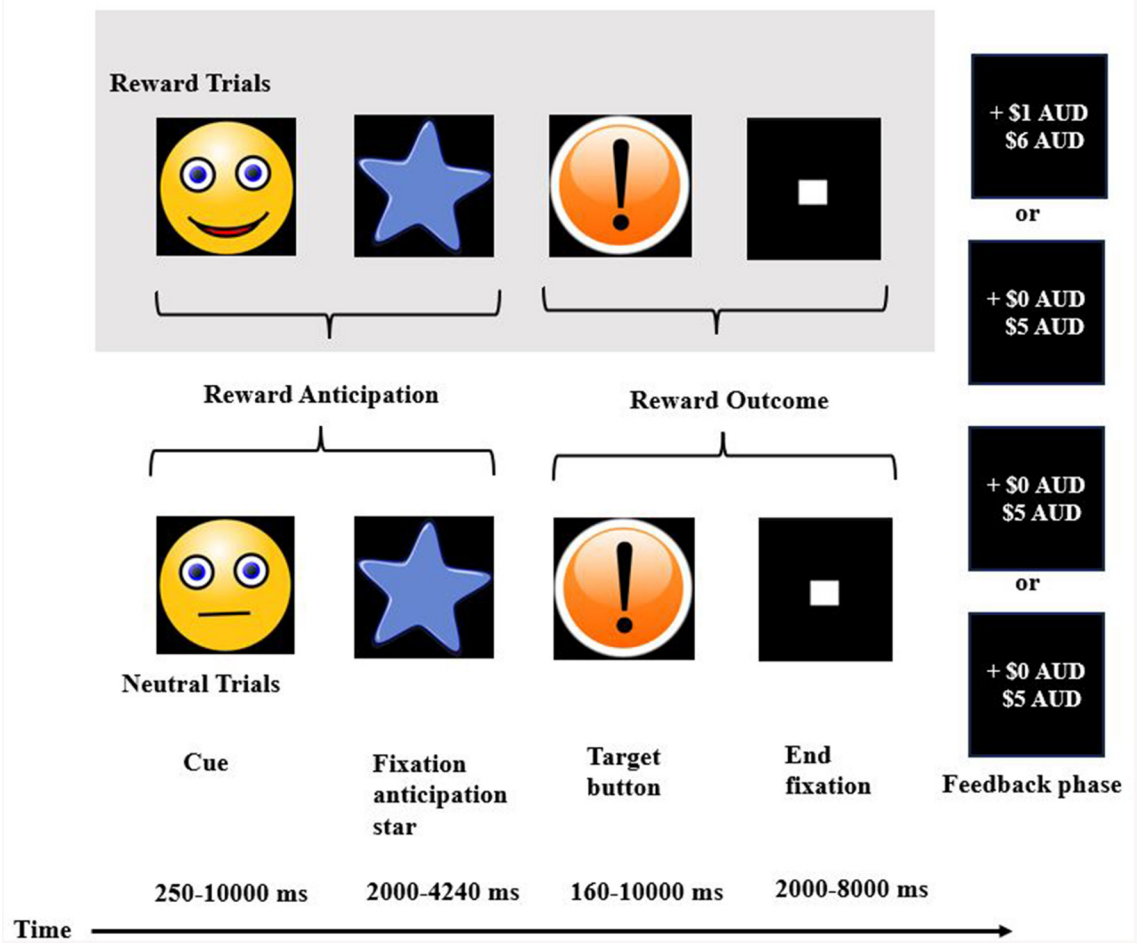

FIGURE 3
Example of the structure of the monetary incentive delay fMRI task.

Enzi et al., 2015; Karoly et al., 2015; Skumlien et al., 2022). Of these, five studies reported non-significant group differences. Only two studies reported significantly different brain function in the NAcc (van Hell et al., 2010; Karoly et al., 2015). An individual study reported group differences in other prefrontal-striatal regions (e.g., superior frontal cortex; van Hell et al., 2010).

Only three studies examined the association between brain function during *reward anticipation vs. neutral anticipation*, and cannabis use metrics. Two of the three studies reported significant correlations in opposite directions (Filbey et al., 2013; Skumlien et al., 2022). Two studies reported negative correlations between CUD symptoms, withdrawal, and brain function across the prefrontal-striatal regions, and one significant positive correlation between craving and middle frontal/lingual function (Table 2).

3.5.2 Group differences while anticipating monetary rewards

Two of three studies that examined group differences while participants *anticipated monetary rewards*, reported significant results in the ventral striatum (Nestor et al., 2010, 2020). One study reported different brain function in additional regions (e.g., insula, PFC, amygdala; Nestor et al., 2020).

Two studies reported significant associations between brain function during *reward anticipation* and the age of cannabis use onset (e.g., cluster efficiency), dosage (e.g., ventral striatum), and withdrawal (e.g., medial frontal gyrus; Nestor et al., 2010, 2020) (Table 2).

3.5.3 Group differences while receiving monetary rewards vs. neutral feedback

Four studies examined group differences while participants *received rewards vs. neutral outcomes* (van Hell et al., 2010; Yip et al., 2014; Enzi et al., 2015; Skumlien et al., 2022). Of these, two studies reported that cannabis users had altered brain function in prefrontal, striatal, and parietal regions (e.g., caudate, putamen, MFG, and precuneus); and no study reported significant correlations between CUD symptoms, cannabis craving, and nicotine use (Table 3).

TABLE 2 Group differences in brain function during anticipation of *monetary rewards*.

References	fMRI data analysis		Brain functional results	
	Type	Thresholding	Group examined/compared	Brain behaviour associations
Anticipation of *rewarding outcomes vs. neutral outcomes*				
Skumlien et al. (2022)	Whole brain	$p < 0.001$, $z = 3.1$, clusterwise corrected	CB = HC: both adults and adolescents	-
	ROI (ventral striatum, vmPFC)	-	CB = HC: both adults and adolescents *accounting for RT, depression (BDI), maternal education, use of alcohol, cigarette, and other drugs*	***Neg. corr***. CUD & ventral striatum ***Non-sig. corr.*** CUDIT, frequency (day/wk), dosage (daily grammes), age onset (first use, weekly use), hours last use
Karoly et al. (2015)	ROI (NAcc)	-	CB > HC: NAcc CB+alcohol+tobacco > tobacco: NAcc CB = HC and alcohol, CB+tobacco, CB+tobacco+alcohol	-
Enzi et al. (2015)	Whole brain	$p < 0.0015$, $k = 10$, uncorrected	CB = HC	-
Yip et al. (2014)	ROI (ventral striatum)	-	CB = HC	-
Jager et al. (2013)	Whole brain	$p < 0.05$, FWE	CB = HC	-
Filbey et al. (2013)	Whole brain	$p < 0.05$, $z = 1.96$, clusterwise corrected	CB = HC	***Neg. corr.*** withdrawal and OFC, ACC, *controlling for age onset and duration.* ***Sig. corr.*** craving and lingual gyrus, MFG ***Non-sig. corr.*** SCID
van Hell et al. (2010)	Whole brain	$p < 0.05$, $t > 4.5$, corrected for multiple comparisons	CB < HC: NAcc, caudate, putamen, inferior medial/superior frontal and cingulate CB > HC: middle temporal, para-hippocampus, cuneus CB < tobacco: middle temporal CB > tobacco: NAcc, medial frontal, cingulate	-
	ROI (NAcc)	-	CB+tobacco >HC: NAcc *accounting for N cigarettes.*	***Non-sig. corr.*** N cigarettes and NAcc
Anticipation of *rewarding outcomes*				
Nestor et al. (2020)	Whole brain	$z > 2.3$, FWE, $p < 0.05$	CB=HC	-
	ROI-to-ROI connectivity	$t > 3.1$, $p < 0.05$, FWE clusterwise corrected, permutation testing (5,000 permutations)	CB > HC: graph subnetwork of 63 edges between 46 nodes: NAcc, insula, PFC areas (lateral/medial PFC, OFC, frontal pole), temporal areas (amygdala, hippocampus/para-hippocampus, temporal pole/cortex, temporal); other regions (central opercular, posterior cingulate, parietal opercular, intra/supra-calcarine, supplementary motor, superior parietal, posterior division, fusiform)	-
	Graph theory	Distinct k thresholds ($0.1 < k < 0.5$, increments of 0.1), *bonferroni-corrected at* $p < 0.004$	CB > HC: clustering coefficient CB < HC: path length, global efficiency	***Pos. corr.*** age onset and clustering coefficient/global efficiency ***Neg. corr.*** age onset and path length ***Non-sign. cor***. dosage (lifetime joints)
Jager et al. (2013)	ROI (caudate, putamen)	-	CB = HC	-

(Continued)

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TABLE 2 (Continued)

References	fMRI data analysis		Brain functional results		Brain behaviour associations
	Type	Thresholding	Group examined/compared		
Nestor et al. (2010)	Whole brain	$p < 0.05$, clusterwise corrected	CB > HC: ventral striatum caudate, putamen; ventral putamen and lentiform nucleus: MFG, fusiform: cerebellum (declive of vermis) CB < HC: fusiform		***Pos. corr.*** duration, dosage (lifetime joints) and ventral putamen, cingulate, cerebellum (declive of vermis); duration, dosage (lifetime joints) and MFG; cuneus, ventral striatum, putamen, cerebellum (declive of vermis) ***Neg. corr.*** withdrawal and fusiform gyrus; duration, dosage (lifetime joints)/withdrawal and MFG ***Non-sig. corr.*** craving, alcohol, other drug exposure

CB, cannabis users; HC, controls; N, number; Neg. corr, negative correlation; Non-sig. corr, non-significant correlation; Pos. corr, positive correlation; RT, randomised trial; wk, week. ACC, anterior cingulate cortex; BDI, Beck Depression Inventory; CUD, cannabis use disorder; CUDIT, cannabis use disorder identification test; MFG; middle frontal gyrus; NAcc, nucleus accumbens; OFC, orbitofrontal cortex; PFC, prefrontal cortex; SCID, structured clinical interview for DSM disorders.

3.5.4 Group differences while receiving monetary rewards

No study reported group differences in brain function while participants received *monetary rewards*; or explored correlations with behavioural measures (Table 3).

3.6 Group differences in brain function during monetary losses

This section overviews group differences in brain function during the anticipation and receipt of losses (Table 4).

3.6.1 Group differences while anticipating monetary losses vs. neutral outcomes

Only two of five studies found group differences during the *anticipation of monetary losses vs. neutral outcomes* (Nestor et al., 2010; Filbey et al., 2013; Yip et al., 2014; Enzi et al., 2015; Karoly et al., 2015), including greater activity in the ventral striatum (Yip et al., 2014), and the ventral putamen (Nestor et al., 2010).

A single study found that greater withdrawal correlated with lower activation in OFC, ventral striatal, and temporal regions (Filbey et al., 2013) (Table 4).

3.6.2 Group differences while anticipating monetary losses

Only one of two studies that examined brain function during *anticipation of monetary losses* (Nestor et al., 2010, 2020), reported greater brain activity in distinct regions (e.g., putamen, cerebellum; Nestor et al., 2010). Neither study found significant correlations between cannabis use metrics and brain function (Table 4).

3.6.3 Group differences while receiving monetary losses vs. neutral feedback

One of two studies reported different brain function during *monetary loss feedback vs. neutral feedback* (Yip et al., 2014; Enzi et al., 2015), including greater activity of the ventral striatum, thalamus, and brainstem (Yip et al., 2014). Neither study examined brain-behaviour correlations (Table 5).

3.6.4 Group differences while receiving monetary losses

Two of three studies found that cannabis users had greater activity when receiving *monetary losses*, in distinct regions (e.g., caudate, inferior frontal gyrus and superior parietal lobule; Filbey et al., 2013; Enzi et al., 2015). Similarly, two of three studies found that brain function correlated with cannabis dosage (i.e., caudate, and superior parietal lobule; Nestor et al., 2010; Enzi et al., 2015).

One study reported non-significant group differences in brain function during *reward feedback* compared to *loss feedback* (Enzi et al., 2015) (Table 5).

TABLE 3 Group differences in brain function during the receipt of *monetary rewards*.

References	fMRI data analysis		Brain functional results	
	Type	Thresholding	Group examined/compared	Brain behaviour associations
Receipt of *rewarding outcomes vs. neutral outcomes*				
Skumlien et al. (2022)	Whole brain	$p < 0.001$, $z = 3.1$, cluster-wise corrected	CB > HC: frontal areas, parietal (supramarginal, angular) *in both adults and adolescents and age group*	-
	ROI (ventral striatum, vmPFC)	-	CB = HC: both adults and adolescents *accounting for RT, depression (BDI), maternal education, use of alcohol, cigarette, other drugs*	***Non-sig. corr.*** CUD, CUDIT, frequency (day/wk), dosage (daily grammes), hours last use, age onset (first use, weekly use)
Enzi et al. (2015)	Whole brain	$p < 0.001$, $k = 10$	CB = HC	-
Yip et al. (2014)	ROI (ventral striatum)	-	CB = HC: ventral striatum *across all win outcomes considered together ($5 vs. $0 and $1 vs. $0), & separately.*	-
van Hell et al. (2010)	Whole brain	$p < 0.05$, $t > 4.5$, corrected for multiple comparisons	CB > HC: ventral striatum (caudate, putamen) frontal areas (inferior/medial frontal, cingulate, and precentral), temporal areas (middle temporal, parahippocampus), parietal (postcentral, precuneus), middle occipital. CB < HC: frontal areas (middle frontal, and claustrum), cingulate/posterior cingulate, temporal gyrus (middle/superior), parietal (postcentral, inferior parietal lobule), occipital (lingual, fusiform, inferior occipital).	-
	ROI (NAcc)	-	CB = tobacco = HC: NAcc	***Non-sig. corr.*** cigarettes and NAcc
Receipt of *rewarding outcomes*				
Enzi et al. (2015)	Whole brain	$p < 0.001$, $k = 10$, uncorrected	CB = HC	-
Filbey et al. (2013)	Whole brain	$p < 0.05$, $z = 2.3$	CB = HC	-

CB, cannabis users; HC, controls; N, number; Non-sig. corr, non-significant correlation; RT, randomised trial; wk, week. BDI, Beck Depression Inventory; CUD, cannabis use disorder; CUIDT, cannabis use disorder identification test; NAcc, nucleus accumbens; SCID, structured clinical interview for DSM disorders.

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TABLE 4 Group differences during the *anticipation of monetary losses*.

References	fMRI data analysis		Brain functional results	
	Type	Thresholding	Group examined/compared	Brain behaviour associations
Anticipation of *loss outcomes vs. neutral outcomes*				
Enzi et al. (2015)	Whole brain	$p < 0.0015$, $k = 10$, uncorrected	CB = HC	-
Karoly et al. (2015)	ROI (NAcc)	-	CB = HC and CB+tobacco, CB+alcohol, CB+tobacco+alcohol	-
Yip et al. (2014)	ROI (ventral striatum)	-	CB > HC: ventral striatum	-
Filbey et al. (2013)	Whole brain	$p < 0.05$, $z = 1.96$, clusterwise corrected	CB = HC	***Neg. corr.*** withdrawal and OFC, ventral striatum, amygdala and hippocampus, *controlling for age onset and duration.* ***Non-sig. corr.*** SCID
Nestor et al. (2010)	Whole brain	$p < 0.05$, clusterwise corrected	CB > HC: ventral putamen	-
Anticipation of *loss outcomes*				
Nestor et al. (2020)	Whole brain	$z > 2.3$, FWE, $p < 0.05$	CB = HC	-
	ROI-to-ROI connectivity	$t > 3.1$, $p < 0.05$, FWE cluster corrected, permutation testing (5,000 permutations)	CB = HC	-
	Graph theory	Distinct K thresholds ($0.1 < k < 0.5$, increments of 0.1), bonferroni-corrected at $p < 0.004$	CB = HC	***Non-sign. corr.*** dose (lifetime joints), age onset
Nestor et al. (2010)	Whole brain	$p < 0.05$, clusterwise corrected	CB > HC: ventral putamen; cerebellum (declive of vermis)	***Non-sig. corr.*** craving, withdrawal, alcohol and other drug exposure

CB, cannabis users; HC, controls; Neg. corr, negative correlation; Non-sig. corr, non-significant correlation; OFC, orbitofrontal cortex; SCID, structured clinical interview for DSM disorders.

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TABLE 5 Brain function group difference between cannabis users and controls while people received *monetary losses*.

References	fMRI data analysis		Brain functional results	
	Type	Thresholding	Group examined/compared	Brain behaviour associations
Receipt of losses vs. receipt of neutral outcomes				
Enzi et al. (2015)	Whole brain	$p < 0.001$, $k = 10$	CB = HC	-
Yip et al. (2014)	Whole brain	$p < 0.05$, FWE	CB > HC: ventral striatum; caudate, putamen, thalamus, and brainstem	-
	ROI (ventral striatum)	-	CB > HC: ventral striatum	-
	ROI (caudate)	-	CB > HC: caudate CB = abstinent CB and HC: caudate	-
Receipt of losses				
Enzi et al. (2015)	Whole brain	$p < 0.001$, $k = 10$, uncorrected	CB > HC: caudate, inferior frontal gyrus	***Pos. corr.*** dose (lifetime joints) and caudate ***Non-sig. corr.*** THC-COOH, age onset, dosage (lifetime joints) and abstinence.
Filbey et al. (2013)	Whole brain	$p < 0.05$, $z = 2.3$	CB = HC	-
Nestor et al. (2010)	Whole brain	$p < 0.05$, clusterwise corrected	*Loss of 50c:* CB > HC: superior parietal lobule CB < HC: insula, precentral gyrus	***Neg. corr.*** dose (lifetime joints) and superior parietal lobule
	Whole brain	$p < 0.05$, clusterwise corrected	*Feedback that a loss of 5c was avoided:* CB < HC: insula	***Non-sig. corr.*** craving, withdrawal, alcohol, and other drug exposure.

CB, cannabis users; HC, controls; Neg. corr, negative correlation; Non-sig. corr, non-significant correlation; Pos. corr, positive correlation; THC-COOH, carboxy-Δ9-tetrahydrocannabinol.

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TABLE 6 Brain function group difference between cannabis users and controls during neutral trials.

References	fMRI data analysis		Brain functional results	
	Type	Thresholding	Group examined/compared	Brain behaviour associations
Anticipation of neutral outcomes				
Nestor et al. (2020)	Whole brain	$z > 2.3$, FWE, $p < 0.05$	CB = HC	-
	ROI-to-ROI connectivity	$t > 3.1$, $p < 0.05$, FWE clusterwise corrected, permutation testing (5,000 permutations)	CB = HC	-
	Graph theory	Distinct k thresholds ($0.1 < k < 0.5$, increments of 0.11 *Bonferronioni –corrected*	CB = HC	***Non-sign. corr.*** onset age and dose (lifetime joints)
Jager et al. (2013)	ROI (caudate, putamen)	-	CB > HC: caudate (trend) and putamen (trend)	***Neg. corr.*** onset age and caudate ***Non-sig. corr.*** onset age and putamen; dose (lifetime/past year joints) and caudate/putamen
Nestor et al. (2010)	Whole brain	$p < 0.05$, clusterwise corrected	CB = HC	-
Receipt of neutral outcomes				
Enzi et al. (2015)	Whole brain	$p < 0.001$, $k = 10$, uncorrected	CB > HC: caudate, IFG	***Non-sig. corr.*** onset age, dose (lifetime joints), THC-COOH, and abstinence
Nestor et al. (2010)	Whole brain	$p < 0.05$, clusterwise corrected	*Loss of 50c:* CB > HC: caudate, IFG, cingulate, MFG, SFG, STG, parahippocampus, precentral, postcentral, cuneus, culmen, middle occipital, and brainstem	-
	Whole brain	$p < 0.05$, clusterwise corrected	CB > HC: caudate, IFG, cingulate, parahippocampus, uncus, and cerebellum. CB < HC: paracentral lobule *Neutral win (win 50c)*	

CB, cannabis users; HC, controls; Neg. corr, negative correlation; Non-sig. corr, non-significant correlation; IFG, inferior frontal gyrus; MFG, middle frontal gyrus; THC-COOH, carboxy-Δ^9-tetrahydrocannabinol.

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3.7 Group differences in brain function during neutral trials

This section overviews group differences during anticipation and receipt of neutral outcomes (Table 6).

3.7.1 Group differences while anticipating neutral outcomes

Two of three studies found no significant group differences while anticipating neutral outcomes (Nestor et al., 2010, 2020). The other study reported trend-level greater activity of the putamen and caudate, and caudate activity correlated with earlier age of cannabis use onset (Jager et al., 2013). Other correlations led to non-significant results.

3.7.2 Group differences while receiving neutral outcomes

Both studies that examined brain function while receiving *neutral outcomes* reported significant differences in the caudate and inferior frontal gyrus (IFG; Nestor et al., 2010; Enzi et al., 2015). The only study to examine brain-behaviour correlations reported non-significant results (Enzi et al., 2015).

3.8 Overview of brain regions most consistently reported to differ between groups

Overall, the most consistently reported finding was altered NAcc activation in two of seven studies during reward anticipation vs. neutral anticipation (van Hell et al., 2010; Karoly et al., 2015), followed by the ventral striatum during loss anticipation vs. neutral anticipation (two of three studies; Nestor et al., 2010; Yip et al., 2014), and the caudate while participants received neutral outcomes (two of two studies; Nestor et al., 2010; Enzi et al., 2015).

4 Discussion

The fMRI evidence that brain reward function is altered in people who use cannabis is limited using the MID task, and led to largely non-significant or mixed findings, in contrast with prominent neuroscientific theories of addiction (Robinson and Berridge, 2001; Koob and Volkow, 2016). Yet within this, the few studies that reported significant group differences consistently identified changes in striatal regions underlying reward processing: the ventral striatum during anticipation of *monetary rewards* and losses; and the caudate while receiving neutral outcomes. There was emerging and inconsistent evidence that reward striatal function correlated with cannabis exposure and related problems (e.g., withdrawal, dosage, and age of onset).

There was no evidence of altered brain function in cannabis users during reward anticipation (van Hell et al., 2010; Karoly et al., 2015) and receipt (Nestor et al., 2010; Enzi et al., 2015). These findings contrast neuroimaging evidence of consistent striatal alterations during anticipation of rewards, in other substances, and in behavioural addictions (e.g., to gambling, cocaine, alcohol; Balodis and Potenza, 2015).

The discrepancy between the reviewed body of work in cannabis users and findings on other substances may additionally be attributed to distinct methodological issues. First, exposure to cannabis vs. other substances may exert a different effect on mesocorticolimbic pathways due to their distinct psychopharmacology (Oleson and Cheer, 2012). For example, exposure to cocaine robustly targets dopaminergic pathways to increase dopamine (Juarez and Han, 2016). Instead, THC induces only a modest dopamine increase (of 3.65%) within the limbic striatum; which is below the threshold of 5% of test-retest variability, meaning that the increase reported might reflect measurement error (Bossong et al., 2015). Thus, regular cannabis use might affect the reward circuitry less so than other drugs known to affect dopaminergic fronto-striatal pathways (e.g., cocaine). Alternatively, unmeasured variables entrenched with cannabis use may explain the emerging alterations in a portion of the studies. The variables might include: greater cannabis use related problems (Lorenzetti et al., 2016, 2020; Chye et al., 2019; Rossetti et al., 2021), poly-substance use (e.g., nicotine, alcohol; Brody et al., 2004), substance-related psychosocial variables (Jackson et al., 2020) and young age (mean = 22 years, range = 16–28 years in the reviewed literature), which may protect from the adverse impact of cannabis use on the brain (Lorenzetti et al., 2023). Notably, a paucity of studies measured potential moderators in relation to brain function (e.g., sex; Becker and Chartoff, 2019), and more evidence is required to test these notions.

Preliminary findings suggest that prefrontal (e.g., OFC, MFG) brain function during *reward anticipation* is associated with cannabis withdrawal (Nestor et al., 2010; Filbey et al., 2013). However, these correlations emerged in studies that reported no group difference in brain reward function. If greater withdrawal drove altered mesocorticolimbic function in cannabis users, it is possible that withdrawal levels were not severe enough in the reviewed samples to drive observable group differences. Indeed, most studies examined current cannabis users who were abstinent from cannabis on average between 1 and 7 days prior to testing. Further, withdrawal was largely unmeasured in the reviewed samples and is a key characteristic of cannabis dependence/CUD. Thus, future work should systematically examine the role of cannabis dependence/CUD and withdrawal in the neurobiology of reward processing.

Despite early findings of group differences in brain reward function, overall there was a lack of differences in behavioural task performance (e.g., reaction times, accuracy). Thus, emerging brain changes might have been insufficient to cause behavioural alterations, or might reflect a compensatory mechanism whereby cannabis users had to engage greater neural resources to perform similarly to controls (Mikulskaya and Martin, 2018).

There was emerging evidence that loss anticipation/receipt was underscored by different prefrontal-striatal function (e.g., IFG, putamen/caudate); and in correlation with greater dosage and earlier cannabis use onset. There is a paucity of evidence examining these variables. Therefore, we are not yet able to draw conclusions on the neurobiology of loss processing in cannabis users (e.g.,

anticipating, receiving, and avoiding negative outcomes); and in comparison with findings from normative samples (Oldham et al., 2018), and from samples who use substances other than cannabis (Morie et al., 2016).

Overall, the findings herein do not align with robust alterations of mesocorticolimbic pathways while processing non-drug related rewards and losses, as shown in substances other than cannabis and as postulated by prominent neuroscientific theories of addiction (e.g., incentive salience theory; Robinson and Berridge, 2001).

4.1 Limitations of the literature

The findings from the reviewed literature need to be interpreted in light of methodological limitations. First, the reviewed evidence is cross-sectional, and longitudinal work is required to determine if altered incentive salience predates or follows cannabis use onset. Second, to date, only nine fMRI studies used the MID task to examine brain reward function in people who use cannabis. The low number of studies precluded the running of a meta-analysis, which requires at least 17–20 studies employing an unbiased whole-brain approach (Muller et al., 2018). Instead, in the literature reviewed herein, the most consistently examined contrasts were reported only by five studies (i.e., *reward anticipation* vs. *neutral anticipation*) and three studies (e.g., *reward feedback vs. neutral feedback*; *loss anticipation vs. neutral anticipation*; and *loss feedback*) or <2 studies. Therefore, replication work is required to confirm how reward/loss processing plays a role in the neurobiology of cannabis use; and to explore which variables moderate such associations (e.g., presence of CUD/dependence, greater withdrawal, and exposure to cannabis and nicotine). Additionally, given the low number of studies, we could not systematically quantify differences in brain reward function between adolescents and adults. Furthermore, within the existing literature, there was no emerging evidence of consistent patterns of brain reward function between age groups. Moreover, one study which did directly compare adolescents vs. adults, found no differences in brain reward function (Skumlien et al., 2022). As such, future research is required to determine if age moderates group differences in brain reward function.

Third, assessment of CUD/cannabis dependence was lacking in half of the studies. Future work is required to demonstrate if processing non-drug related rewards affect the mesocorticolimbic circuitry differently in CUD/dependence vs. non-dependent use, as shown in other measures of neural integrity in cannabis users (Chye et al., 2019; Lorenzetti et al., 2020; Rossetti et al., 2021), and as postulated by neuroscientific theories of addiction (Robinson and Berridge, 2001; Koob and Volkow, 2016). Finally, assessment of metrics of exposure to cannabis/other substances and in relation to brain function is lacking. This issue precludes the understanding of which mechanisms may drive changes in reward brain function in people who use cannabis. To address this gap, future work should use robust metrics of substance use e.g., THC Unit, iCannToolkit (Freeman and Lorenzetti, 2020; Lorenzetti et al., 2022) and related problems using tools with diagnostic cutoffs (e.g., CUDIT; Myers et al., 2023).

4.2 Limitations of this review

The review focused on a single measure of reward processing i.e., the MID fMRI task. Perhaps, integrating other reward processing tasks, could have included other aspects of reward processing relevant to the neurobiology of cannabis use (e.g., reversal of learning contingencies with a reward learning task). Yet, the MID was the most consistently used fMRI reward processing task to date in cannabis users (and in normative samples; Oldham et al., 2018), therefore the focus on this task enabled the systematic integration of the findings. Future studies should use varied fMRI tasks to create a body of work examining how different facets of reward and loss processing are affected in people who use cannabis. Another limitation of the review is the exclusion of samples with comorbid mental health problems (e.g., schizophrenia, depression). While this approach enables the examination of cannabis-specific effects, the findings cannot be generalised to the most vulnerable people who use cannabis (Hasin and Walsh, 2020).

4.3 Conclusions

Overall, there exists largely non-significant evidence of brain alterations in cannabis users compared to controls, examined with the MID fMRI task. A subset of results reporting significant findings consistently identified significantly different *striatal* function during the anticipation of rewards and losses; and mixed results supporting associations between brain function and chronicity of cannabis use. Replication longitudinal neuroimaging studies of cannabis users are warranted to use robust metrics of substance use/mental health, and in relation to different types of rewards e.g., monetary, cannabis, and natural rewards (e.g., food). Such new evidence is required to identify with precision the neurobiology of reward processing in cannabis users and to enable comparison of the evidence in cannabis users with prominent neuroscientific theories of addiction.

Author contributions

EB: Conceptualisation, Data curation, Formal analysis, Investigation, Methodology, Writing—original draught. GP: Writing—review & editing. SA: Writing—review & editing, Methodology. HT: Methodology, Writing—review & editing. VL: Writing—review & editing, Conceptualisation, Supervision.

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Conflict of interest

GP was employed by Braincast Neurotechnologies.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Emerging therapeutic strategies in hypoxic-ischemic encephalopathy: a focus on cognitive outcomes

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Perinatal hypoxia-ischemia represents a significant risk to CNS development, leading to high mortality rates, diverse damages, and persistent neurological deficits. Despite advances in neonatal medicine in recent decades, the incidence of HIE remains substantial. Motor deficits can manifest early, while cognitive impairments may be diagnosed later, emphasizing the need for extended follow-up. This review aims to explore potential candidates for therapeutic interventions for hypoxic-ischemic encephalopathy (HIE), with a focus on cognitive deficits. We searched randomized clinical trials (RCT) that tested drug treatments for HIE and evaluated cognitive outcomes. The results included studies on erythropoietin, melatonin, magnesium sulfate, topiramate, and a combination of vitamin C and ibuprofen. Although there are several indications of the efficacy of these drugs among animal models, considering neuroprotective properties, the RCTs failed to provide complete effectiveness in the context of cognitive impairments derived from HIE. More robust RCTs are still needed to advance our knowledge and to establish standardized treatments for HIE.

KEYWORDS

perinatal insult, neurodevelopment, erythropoietin, ibuprofen, magnesium sulfate, melatonin, topiramate, vitamin C

Introduction

Pre- and perinatal injuries can disrupt the development of the central nervous system (CNS), resulting in multiple damages, dependent on the type and intensity of the insult, the developmental period in which they occur, and the affected area. While neonatal medicine has made significant advances in recent decades, a high incidence of neurological deficits in children following perinatal lesions still persists (McIntyre et al., 2013).

Perinatal hypoxia-ischemia (HI) is defined by transient or permanent disruption of blood flow and oxygen supply and is the most common type of insult in neonates, occurring in 3 out of 1,000 newborns before the 36th week of gestation (Hagberg et al., 2015). When it occurs from the 36th week onwards, the number of cases increases to 7 in 1,000 newborns (Chalak et al., 2012). HI events not only lead newborns to death but also constitute the main causative factors for encephalopathy and persistent brain damage in pediatric population (Johnston et al., 2009; Volpe, 2012; Lee and Glass, 2021). The incidence of hypoxic-ischemic encephalopathy (HIE) in developed countries is around 1.5 per 1,000 neonates (Glass, 2018) while this number reaches

26 occurrences per 1,000 newborns in developing countries (Li et al., 2017). Approximately 15%–20% of children affected with HIE die during the postnatal period, establishing HIE as a significant contributor to neonatal deaths. Futhermore, 25% of the survivors exhibit enduring neurophysiological impairments (Vannucci, 2000; Nelson et al., 2003; Volpe, 2008; Chen et al., 2009; Coq et al., 2016; Laptook, 2016).

Cerebral palsy (CP), one of the most severe clinical outcomes of HIE, is a debilitating, non-progressive disorder, mainly affecting the motor system and being strongly related to perinatal brain damage (Kuban and Leviton, 1994; Volpe, 2001; Nelson et al., 2003; Allen and Brandon, 2011). Cognitive deficits may arise in children who have undergone HIE, irrespective of the presence of motor deficiencies, although cognitive and neuromotor deficits have been strongly associated (Van Handel et al., 2007; Lee and Glass, 2021). The consequences can significantly impact the school phase since these youngsters have intelligence quotients (IQ) below average (Pappas et al., 2015) and learning difficulties, resulting in academic delays (Robertson and Perlman, 2006). In premature infants, learning problems are considered even more common, with a 3 to 5 times greater risk of deficits in reading, speech, mathematics, or writing (Aylward, 2002). In addition, impairments may endure into adolescence, accompanied by a decline in episodic memory (Gadian et al., 2000; Abily-Donval, et al., 2015) and poor performance in executive functions, as well as deficits in visual and verbal memory (Mañeru et al., 2001; Schreglmann, et al., 2020).

Most studies focused on evaluating children who have suffered HIE during early childhood indicate a close association with outcomes such as mortality, CP, and severe overall cognitive dysfunction. It is crucial to comprehend the entire range of neurodevelopmental consequences, including those without CP, as this enables professionals to recognize children in need of early intervention and continual monitoring.

HIE pathophysiology and key elements to therapeutical approaches

Although not fully understood yet, HIE pathophysiology has been reviewed elsewhere (Nair and Kumar, 2018; Molloy et al., 2023) and is presented in Figure 1. The damages start to be verified soon after the HI event and may last for months or years. In this context, different phases have been characterized. The first one, the acute phase, is related to rapid intracellular depletion of adenosine triphosphate (ATP) and change from aerobic to anaerobic metabolism (anaerobic glycolysis), leading to the first wave of neuronal death. In the latent phase, it is possible to observe the generation of reactive oxygen species (ROS), excitotoxicity mechanisms and neuroinflammation, besides mitochondrial dysfunction (Vannucci, 1990; Maiti et al., 2008; Abdel Baky et al., 2010; Forrester et al., 2018). This period has been shown to last from 6 to 15 h and is followed by the secondary and tertiary phases, in which cytotoxic mechanisms persist and cause a late phase of neuronal loss. Neuronal death itself is induced by the interplay of processes involving excitotoxicity, depolarization, inflammation, autophagy, and apoptosis. Particularly, it is prominent in regions of the brain recognized as vulnerable, such as the hippocampus and striatum (Lee et al., 2014). Apoptosis stands out as one of the main pathways that lead to cell death in cerebral ischemia and when it reaches the hippocampus, for example, it is one of the main agents that promote memory impairments (Abdel Baky et al., 2010; Cerbai et al., 2012; Lee et al., 2014). Regarding possible therapeutic approaches for newborns exposed to prenatal HI insults, a standardized and universally accepted therapy is still unknown. Therapeutic hypothermia (TH) has been extensively investigated in babies diagnosed with HIE (Simbruner et al., 2010; Higgins et al., 2011). Some studies demonstrate that the induction of moderate TH would be able to reduce mortality and motor damage, resulting in significant improvements for neonates who suffered moderate but not severe insults (Higgins et al., 2011). Despite this, other authors have concluded that such beneficial effects would be limited to full-term infants (Perlman, 2006; Rees et al., 2011) and, for great effectiveness, initiating treatment within the first 6 h after birth is crucial, signifying a 'window of opportunity' to minimize damages resulting from HI (Higgins et al., 2011). Chalak and collaborators highlight that despite an apparent consensus on the advantages of using TH, there are still controversies (Chalak et al., 2019). Due to its protocol, this intervention is not entirely benign, as neonates may have arrhythmias, thrombocytopenia, coagulopathy and necrosis of the subcutaneous adipose tissue (Zhang et al., 2020). Furthermore, these babies remain separated from their mothers, undergoing close monitoring in intensive care, invasive procedures, and occasional sedation to alleviate stress. However, it is important to underscore the risks and benefits offered by this treatment in each patient (Goswami et al., 2020). For these reasons, the search for therapies that improve maternal health and can significantly reduce the likelihood of infants developing HIE is of paramount importance.

To develop standardized strategies for preventing mortality and deficiencies, a comprehensive study of candidates for therapeutic intervention is essential. In this work, we review potential neuroprotective drugs that have been evaluated in HIE, with a focus on cognitive outcomes.

In September 2023, we searched the expression "Hypoxic-ischemic Encephalopathy AND (perinatal OR newborn OR neonate)" in the Embase database. We restricted the results to articles, randomized clinical trials, and entries with non-blank Emtree Drug Index Terms (Major Focus). After identifying 79 publications, we reviewed them and found 10 RCTs written in English that tested a drug treatment and its respective cognitive outcomes. One additional RCT was included in the table for being cited in an identified article. Table 1 summarizes the articles identified with the proposed therapeutic interventions.

Erythropoietin

Evidence that erythropoietin (EPO) may ameliorate neurodevelopmental outcomes after HIE was reviewed by Oorschot et al. (2020). EPO primarily regulates the production of red blood cells but also performs functions in the maintenance or recovery of general cells under stressful conditions, such as hypoxia (Ostrowski and Heinrich, 2018). The actions of EPO beyond the hematopoietic system, particularly in the CNS, have been documented since 1993 (Masuda et al., 1993; Li et al., 1996; Morishita et al., 1997; Juul et al., 1998; Marti, 2004), however, the presence of EPO receptors in the CNS was confirmed only in 2015 (Ott et al., 2015). As a component of the response to brain lesions, EPO receptors undergo upregulation in astrocytes, oligodendroglia, microglia, endothelial cells, and neurons, leading to EPO production (Ott et al., 2015)

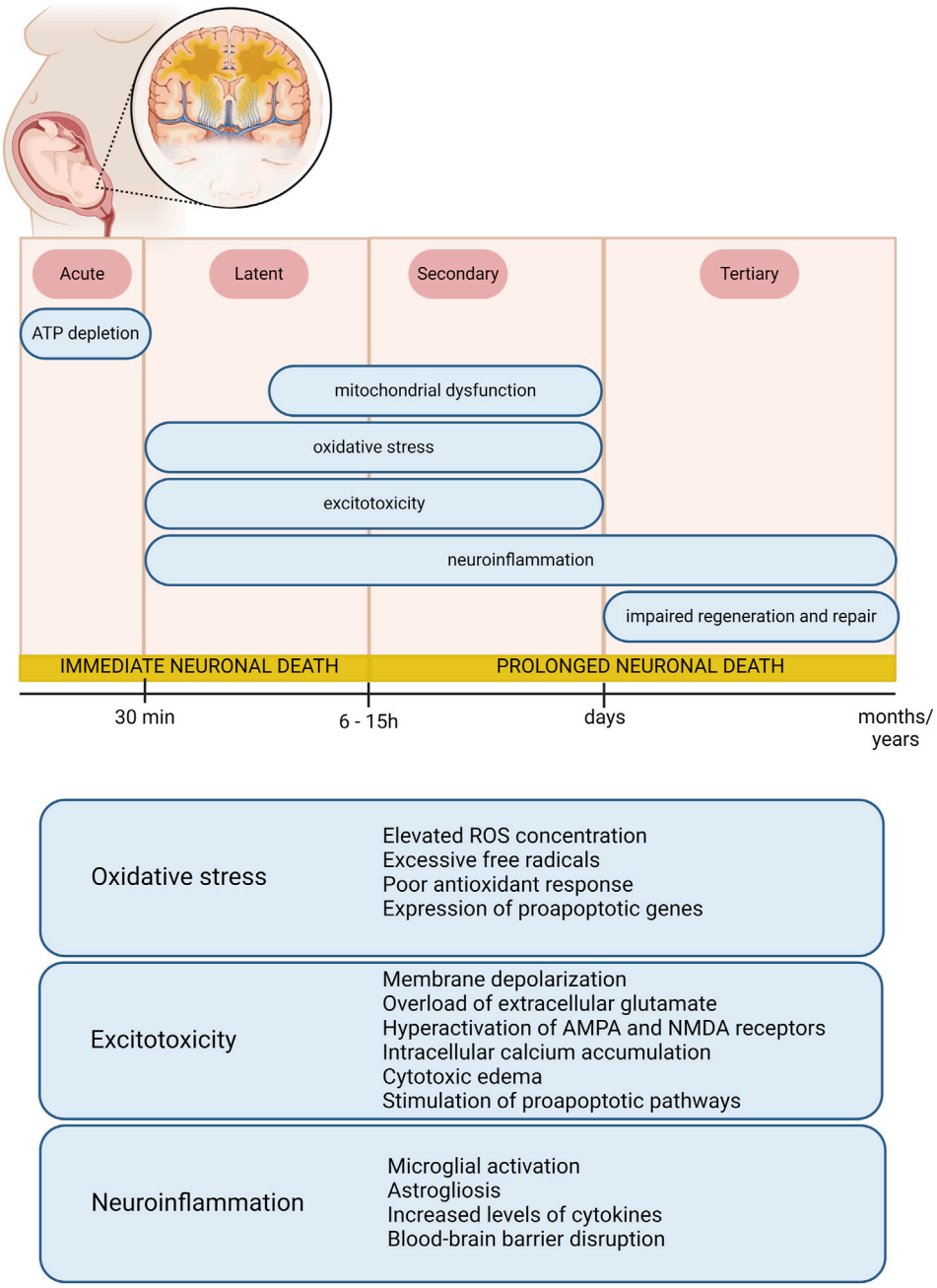

FIGURE 1
Schematic illustration of HIE pathophysiology, phases of injury and mechanisms involved. Figure created with BioRender.com.

and, consequently, contributes to enhanced ratios of oxygen utilization and retention. Hypoxia-inducible transcription factor (HIF)-1 is responsible for the oxygen-dependent regulation of EPO. A period of 30 min of hypoxia may trigger the expression of HIF-1 by cultured cells (Wang and Semenza, 1993).

Effects contributing to neuroprotection have been described: 1 - EPO decreases the hypoxic-induced NO surge (Kumral et al., 2004) and increases antioxidants (Genc et al., 2002; Kumral et al., 2005); 2—EPO inhibits glutamate release (Kawakami et al., 2001) and inhibits brain cell death (i.e., anti-apoptotic effect) (Juul et al., 1998; Xiong et al., 2011); 3—EPO decreases inflammation (Vannucci and Perlman, 1997). EPO also has neurorestorative effects promoting neurogenesis (Xiong et al., 2011) and oligodendrogenesis (Juul and Pet, 2015) and enhancing revascularization of the ischemic brain (Grasso et al., 2002; Xiong et al., 2011). The interactions between vascular endothelial growth factor (VEGF) and the capability of EPO to induce mitosis and migration of endothelial cells (Sola et al., 2005) corroborates its proangiogenic effects. All these EPO effects are dose- and timing-dependent (Xiong et al., 2011). Thus, EPO treatment stablished within the first 24 h of the insult could potentially be effective.

EPO has been studied in animal models of HIE since 1990, with accumulating evidence of benefits. Building on preclinical evidence,

TABLE 1 Table summarizing data from the RCTs discussed in this review.

Drug	Dosage	Study	Administration	Intervention group	Control group	Age during measurement	Scale	Evaluated outcome	Intervention group significantly favored?
Erythropoietin	1,000 U/kg	Wu et al. (2022)	IV	Drug + hypothermia	Hypothermia + placebo	22–36 months	BSID-III	Cognitive score <90	No
		Wu et al. (2016)	IV	Drug + hypothermia	Hypothermia + placebo	6 months	WIDEA	Mean score - Self-care; Mobility; Communication; Social	Yes
						12 months			No
	500 U/kg	Malla et al. (2017)	IV	Drug	Placebo	19 months	BSID-II	Mental Development Score Proportion (%) < 70; 70 to 84; >85	No
	200 U/kg	Lv et al. (2017)	IV	Drug + hypothermia	Hypothermia	9 months	GDS	Proportion (%) with good development; boundary situation or neurodevelopmental retardation - Adaptability; Language; Personal-social	No
Melatonin	10 mg/kg	Aly et al. (2015)	PO	Drug + hypothermia	Hypothermia	6 months	Neurological evaluation + DDST-II	Proportion (%) with normal neurological exam and at most one caution without delays in DDST-II	Yes
	5 mg/kg	Jerez-Calero et al. (2020)	IV	Drug + hypothermia	Hypothermia + placebo	6 months	BSID-III	Cognitive composite score	No
						18 months			Yes
Magnesium sulphate	250 mg/kg	Kumar et al. (2023)	IV	Drug + hypothermia	Hypothermia	12 months	DASII	Major neurodevelopmental disability score <70	No
		Prakash et al. (2016)	IV	Drug	Placebo	12 months	TDSC	Proportion (%) with developmental delay	No
Topiramate	10 mg/kg	Filippi et al. (2018)	PO	Drug + hypothermia	Hypothermia	18–24 months	BSID-III	Cognitive composite score <70 or <85	No
Ascorbic acid and ibuprofen	100 mg/kg/day (ascorbic acid); 10 mg/kg on day 1 and 5 mg/kg on days 2 and 3 (ibuprofen)	Aly et al. (2009)	IV (ascorbic acid) and PO (ibuprofen)	Drugs	Placebo	6 months	DDST-II	Neurodevelopmental outcome Proportion (%) with normal/ caution/delayed result	No

BSID-II, Bayley scales of infant and toddler development II; BSID-III, Bayley sales of ifant and toddler development III; DASII, developmental assessment scale for Indian infants; DDST-II, denver developmental screening test II; GDS, Gesell development scale; TDSC, trivandrum developmental screening chart; WIDEA, Warner initial developmental evaluation.

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Zhu et al. (2009) were pioneers in utilizing EPO for treating moderate to severe HIE in humans. They compared the effects of recombinant-human EPO (r-Hu-EPO) in a low dose to the conventional treatment at that time, which included respiratory and cardiovascular support, fluid infusion, anticonvulsants, reduction of intracranial pressure, and the correction of hypoglycemia, acidosis, and electrolyte imbalance. At 18 months of age, they described benefits restricted to the group that suffered moderate HIE and were treated with r-Hu-EPO. Interestingly, r-Hu-EPO treatment has shown no side effects (Zhu et al., 2009). Also examining neonates with moderate to severe HIE and administering EPO (500 U/kg IV) within the first 6 h of life, Malla et al. (2017) assessed the outcomes at 19 months of age. The study reported an improvement in the combined outcome of mortality or moderate to severe disability. These results were promising especially considering that many neonatal therapy centers do not have the necessary requirements to address HIE.

Clinical trials combining EPO administration and TH have been conducted by Wu et al. (2016), Wu et al. (2022). Despite the potential for a higher dose (1,000 U/Kg) in association with TH to reduce HIE brain injury and improve total developmental score at 6 months (Wu et al., 2016), a phase III study (Wu et al., 2022) found no beneficial effects on mortality or neurodevelopmental outcomes at a long-term period (2–3 years) in moderate or severe HIE term and near-term infants treated with multiple high doses of EPO. In addition, EPO adverse events have also been reported in infants, such as thrombosis or intracranial hemorrhage (Juul et al., 2023). Similar results were observed by Lv et al. (2017) combining a lower dose (200 U/kg i.v.) of EPO and TH treatment. The combined treatment is not superior to TH alone in improving the neurodevelopmental outcome of neonates with HIE at 9 months. Nevertheless, the authors described an elevation in the serum levels of tau protein in HIE neonates, an indicative of neuronal damage (Lv et al., 2015). In addition, the treatment was able to decrease tau levels in the subsequent period of 8–12 days compared to TH group. Therefore, despite the various neurorestorative mechanisms already described, clinical studies have not demonstrated efficacy with an EPO-TH combined treatment.

Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) (MT) stands out as one of main drugs investigated in clinical trials for HIE treatment, as monotherapy and associated with TH as well. MT, a hormone produced by the pineal gland, can penetrate the blood-brain barrier and access the intracellular compartment. It is a safe drug, its administration is feasible, and it has the potential to offer neuroprotection in HIE (Ahmed et al., 2021).

As an antioxidant and anti-inflammatory agent, MT may mitigate the activation of microglia and astrocytes. MT promotes the maintenance of mitochondrial integrity and upregulation of antioxidant enzymes, effectively preventing apoptosis (Cardinali, 2019; Tarocco et al., 2019). It is noteworthy that, since pineal MT production is not sufficiently developed at birth, newborns are especially vulnerable to HI brain damage (Gitto et al., 2009).

Besides MT neuroprotective role as a direct antioxidant, acting independently of receptor signaling, it also binds to MT1, MT2 (transmembrane receptors), MT3 (cytosolic) and nuclear receptors, which also mediate protective roles (Tarocco et al., 2019). Interestingly, MT may exhibit beneficial actions across multiple stages of HI injury cascade, encompassing latent, secondary, and tertiary phases (Cardinali, 2019; Paprocka et al., 2019).

Research on animal models have revealed significant improvements facilitated by MT treatment, such as the attenuation of CP severity in rats (Robertson et al., 2013; Cardinali, 2019). However, these animal studies also suggest that MT may have a limited therapeutic window of just 10 min to 2 h following the HI event. Thus, an early administration of MT is crucial to attain therapeutic levels effective for neuroprotection (Robertson et al., 2013; Robertson et al., 2019). At present, there is no established MT protocol; however pre-clinical evidence recommends a dose of 20–30 mg/kg promptly administered to the neonate (Pang et al., 2020).

Clinical studies that evaluate neuroprotective effects in newborns with HIE combined MT with TH in the first 6 h after birth. Solely one study (Jerez-Calero et al., 2020), which employed MT in a daily dose of 5 mg/kg, i.v., for 3 days, documented beneficial developmental outcomes in a long-term follow-up. Although the authors did not find any significant differences in the cognitive scale at 6 months, they reported a higher composite cognitive score ($p < 0.05$) in the MT + TH group at 18 months. There were no differences regarding the other components of neurologic development assessment, encompassing language and motor skills, at both 6 and 18 months.

Aly et al. (2015) administered MT using an oral route, in a dose of 10 mg/kg, daily, for 5 days. Blood measurements of nitric oxide (NO) levels and superoxide dismutase (SOD) activity were performed and an increase in these parameters were described in HIE newborns. The combination of MT + TH treatment caused a significant reduction in the NO concentration and in SOD activity at the end of treatment. After a 6-month follow-up, the assessment of developmental progress by the Denver Developmental screening test (DDST II) resulted in a markedly improved performance of the MT + TH group. Neither of the two RCTs described the incidence of adverse effects attributed to MT administration. A common limitation in both of them is the small number of patients.

Magnesium sulfate

Magnesium sulfate ($MgSO_4$) has diversified therapeutic uses. Its roles include anticonvulsant action in eclampsia/preeclampsia, laxative properties, use as a soaking solution, and in the treatment of hypomagnesemia and pediatric acute nephritis. There are also non-FDA-approved indications, such as asthma exacerbations, torsades de pointes, and prevention of preterm labor (Hicks and Tyagi, 2023).

This drug can be considered one of the most frequently prescribed medications in obstetrics for eclampsia and fetal neuroprotection (Brookfield and Mbata, 2023). Although not a typical anticonvulsant, $MgSO_4$ is the first-line treatment for eclampsia seizure and prophylaxis of eclampsia seizure recurrence (Laskowska, 2023). Several studies have demonstrated the effectiveness of $MgSO_4$ in the context of eclampsia, supporting WHO Recommendations (2011) (Altman et al., 2002; Azria et al., 2004; Duley et al., 2010), however, its mechanism of action remains not fully explained. Authors have suggested vasodilation properties as it is a calcium antagonist, which would reduce peripheral vascular resistance, lower systemic blood pressure, and dilate small distal brain capillaries, finally leading to reverse brain hypoxia (Euser and Cipolla, 2009). Calcium antagonism is

also suggested as a mechanism impacting BBB dynamics, since the decrease of tight junction permeability would limit cerebral edema formation (Euser et al., 2008). Moreover, $MgSO_4$ provides a non-competitive inhibition of NMDA receptors and so it may prevent glutamate excitotoxicity (Hoffman et al., 1994) and decrease proinflammatory cytokines and free radicals (Shogi et al., 2003), important factors in the context of HIE and inflammatory diseases of pregnancy.

Concerning its neuroprotective properties, a number of studies focused on the effects of $MgSO_4$ treatment on prenatal HI have presented conflicting results. Crowther et al. (2017), in a systematic review, concluded that $MgSO_4$ significantly decreased the risk of death or CP considering studies with follow-up durations of 18 or 24 months. A retrospective study showed a significant decrease in mortality and lower severity of cognitive impairment in premature infants, an important risk group for HI and CP. Ichiba et al. (2006) investigated the neurodevelopmental outcomes at 18 months after $MgSO_4$ infusion (in combination with dopamine). They reported that 73% of the treated infants (22 out of 30) showed normal neurodevelopmental outcomes, although the study lacked a control group for comparisons.

The RCT implemented by Iqbal et al. (2021) presented significant effects in a cohort of near-term neonates with had experienced moderate-to-severe birth asphyxia and received $MgSO_4$, such as control of seizures, shortened hospital stay, and early initiation of feeding, although mortality and neurodevelopmental outcomes at 6 months of age were not improved and cognitive assessment was not performed. Regarding cognitive development, an RCT conducted in India (Kumar et al., 2023) compared neonatal mortality and neurodevelopmental outcomes in infants treated with $MgSO_4$ along with TH or cooling alone. They found no significant effects between the groups at 1 year of age considering either the developmental assessment or the levels of serum malondialdehyde and total antioxidant status at baseline or after 72 h of life.

Prakash et al. (2016) administered $MgSO_4$ or placebo to term asphyxiated neonates diagnosed with mild, moderate, or severe HIE and evaluated the outcomes of mortality or disability, developmental delay, and neuromotor tone at 12 months follow-up. The authors reported no significant beneficial or adverse effect of $MgSO_4$ treatment on any of the outcomes measured. One of the relevant limitations in this study refers to the elicited follow-up duration, which should be extended to 18–24 months for an improved cognitive assessment, as highlighted by the authors (Prakash et al., 2016), a restriction that has been verified in many other trials that assess the neuroprotective effects of drugs on HIE.

Topiramate

Topiramate is a second-generation anticonvulsant approved by the FDA in 1996. It is indicated as monotherapy or adjuvant for generalized- or focal-onset seizures, and for the management of migraine and chronic weight conditions. Also, it has been used as an off-label medication for a variety of neurological and psychiatric conditions (Arnone, 2005; Soyka and Müller, 2017).

Its mechanisms of action seem to be multiple and include either the reduction of excitation or the increase of inhibitory neurotransmission (Sankaraneni and Lachhwani, 2015). It blocks voltage-gated sodium and calcium channels, antagonizes glutamate receptors, and enhances GABA receptors besides inhibiting carbonic anhydrase (Pearl et al., 2023).

Based on these actions, which would target the prevention of glutamate excitotoxicity, topiramate has gained attention as a putative neuroprotective agent in HIE in different animal models (Follett et al., 2004; Kaminski et al., 2004; Koh et al., 2004; Angehagen et al., 2005; Schubert et al., 2005; Sfaello et al., 2005; Noh et al., 2006; Ozyener et al., 2012; Jiang et al., 2014).

In human neonates with a diagnosis of HIE, Nuñez-Ramiro et al. (2019) compared the effects of topiramate associated with TH versus placebo with TH. They reported that topiramate treatment elicited non-significant differences concerning mortality and seizure activity. The authors point out that the lack of the expected effects may be attributed to the dose of topiramate employed (loading: 5 mg/kg; maintenance: 3 mg/kg; for 5 days), that was chosen to reduce possible side effects.

Currently, there is no standardized dose of topiramate for neonates, however Glass et al. (2011) indicate safety with a dose of 10 mg/kg in a study with a limited number of newborns. Filippi et al. (2010) reported no significant short-term adverse effects with two different topiramate regimens in combination with TH: 5 mg/kg for 3 days or 5 mg/kg on the initial day followed by 3 mg/kg on the subsequent 2 days. They highlighted that topiramate safety profile may be influenced by TH in neonates with HIE, as it induces a slow absorption and elimination of the drug (Filippi et al., 2010).

Evaluated in a higher dose (10 mg/kg for 3 days), topiramate was confirmed as a safe and well-tolerated drug when administered with TH however the neuroprotective outcomes described in animal models could not be verified in neonates with HIE (Filippi et al., 2018). Topiramate co-treatment with TH did not decrease mortality or improve injuries, sensory deficits or neurodevelopmental disabilities, either in motor or cognitive scores (Filippi et al., 2018). Regarding the effects of topiramate on molecular injury mediators, there is still a need for appropriate assessment in the context of HIE in newborns to confirm the findings described in experimental animal models.

Vitamin C and ibuprofen

Vitamin C, also called ascorbic acid, acts as a powerful antioxidant, combating oxidative stress through electron transfer or donation. It exists in various active forms, with L-ascorbic acid being the most extensively researched and biologically active among them (Al-Niamini and Chang, 2017).

Ibuprofen, a frequently used non-steroidal anti-inflammatory drug (NSAID), effectively eases pain and diminishes inflammation by targeting the cyclo-oxygenase (COX) enzyme. This enzyme exists in two forms: COX-1, responsible for producing prostanoids and thromboxane A2 from arachidonic acid; and COX-2, which, while naturally present in specific tissues like brain, kidney, and the female reproductive tract, can also be induced. Its function involves generating prostaglandins, essential mediators of pain, inflammation, and fever (Paul and Walson, 2021).

Ascorbic acid, at a dose of 30 mg/kg, demonstrated neuroprotective effects in rats with HIE, yet its combined use with ibuprofen did not yield benefits for term infants. A dose of 100 mg/kg in neonates was deemed safe, devoid of pro-oxidant or hemolytic effects in preterm infants (Aly et al., 2009). However, the authors cautioned against higher doses until comprehensive safety data on a larger scale becomes accessible.

Ibuprofen has exhibited protective effects on the adult brain in models of focal and global ischemia in animals. Administered intravenously at a dose of 10 mg/kg in children, it effectively crosses the blood-brain barrier, as observed in its optimal penetration into cerebrospinal fluid. However, a study by Aly et al. (2009) investigating a combined regimen of ibuprofen and ascorbic acid in infants with HIE did not show any neurological improvement—a unique trial exploring this approach to our knowledge. Notably, infants in the intervention group displayed no significant alterations in renal functions or platelet counts, and no adverse events linked to either medication were reported in the study. Yet, it is crucial to note that ibuprofen, depending on the dosage and concurrent medication usage, may lead to adverse effects such as renal and hepatic injuries (Barbagallo and Sacerdote, 2019).

Despite the elevated serum levels of IL-6 and IL-1b observed in HIE neonates, correlating with the severity of lesions, the combined treatment could not reverse this effect. Therefore, based on the trial's findings, it seems that despite the involvement of oxidative stress and inflammatory cytokines in HIE, the early administration of ascorbic acid and ibuprofen does not mitigate mortality or enhance neurodevelopmental outcomes.

Challenges and future directions

Besides the complexity of HIE pathophysiology, which has not been completely deciphered yet, some points are presented as challenges regarding the investigation of drug treatments for neonates diagnosed with HIE.

Considering the population of patients affected by HIE, the heterogeneity adds more complexity to this matter. HIE cases may vary in terms of severity, underlying causes, and individual characteristics and comorbidities. This variability emphasizes more personalized and adaptive treatment protocols.

As the nature of HIE sequelae is also variable, these infants should be followed up in a long-term setting. Motor deficits are usually identified more prematurely while cognitive deficits may be verified later, sometimes only when the child is introduced to school education. Thus, studies aiming to assess cognitive impairments derived from HIE should consider an extended follow-up period. The lack of prolonged observation limits our comprehension of either the impacts of HIE on cognition or the putative benefits to be provided by new therapies.

One of the main limitations refers to the translation of promising results obtained from animal studies to humans. Given the biological interspecific variations, some encouraging preclinical findings may not be confirmed in human neonates, highlighting the need for more robust studies, either in animals or in humans.

Some animal models may not fully replicate the injuries presented in HIE. The most common experimental protocols are based on carotid artery ligation, as the Rice-Vannucci model (Rice et al., 1981), for instance. Since these models produce a focal ischemia, they do not consider the maternal-fetal interaction and the systemic effects of the human insult. Conversely, a few prenatal systemic HI models have also been explored, revealing cognitive outcomes (Delcour et al., 2012a; Delcour et al., 2012b; Cunha-Rodrigues et al., 2018). Despite of that, there remains a need for the evaluation of pharmacological therapeutic approaches using more suitable preclinical models (Victor et al., 2022).

Currently, there are some recruiting trials focused on drug development for the improvement of neurocognitive outcomes in neonates with HIE. One of them (NCT02621944), an early Phase 1 study, will test doses of 0.5 mg/kg, 3 mg/kg, and 5 mg/kg of melatonin on distinct populations. Since our identified RCTs only tested the doses 5 mg/kg and 10 mg/kg (Aly et al., 2015; Jerez-Calero et al., 2020), this new trial could then present how lower doses interact with the studied population.

Other drugs which were not mentioned in this paper have also been studied in ongoing trials. A Randomized Multicenter Phase 2 study (NCT05778188) is intended to evaluate the efficacy and safety of RLS-0071, an anti-inflammatory peptide, suggested to decrease brain damage in an animal model (Kumar et al., 2021). The endothelin B receptor agonist, sovateltide, was considered safe in a study with adult patients diagnosed with ischemic stroke (Gulati et al., 2021) and showed neural damage reduction in a rat model (Ramos et al., 2022). The recruiting new trial, a Randomized Phase 2 study, could also assess the efficacy and safety of the drug on neonates with HIE (NCT05514340). Some tests on animal models have suggested that allopurinol could provide neuroprotection to those affected by HIE, even though the results are inconclusive in humans, as reviewed by Annink et al. (2017). A Randomized Phase 3 study (NCT03162653) intends to evaluate the efficacy and safety of the drug in a large population.

In conclusion, there is still a need to search for effective therapeutic strategies to mitigate neurological deficits in the context of HIE. Collaborative efforts from researchers, clinicians, and policymakers are essential to advance our comprehension of the disease and to improve survival and the quality of life for newborns affected by HIE.

Author contributions

KM: Writing–original draft, Writing–review and editing. VR: Writing–original draft, Writing–review and editing. CB: Writing–original draft, Writing–review and editing. GM: Writing–original draft, Writing–review and editing. PB: Writing–original draft, Writing–review and editing. MC-R: Writing–original draft, Writing–review and editing.

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Conflict of interest

Author CB was employed by company Daiichi Sankyo Brazil.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Chronic exposure to inhaled vaporized cannabis high in Δ^9-THC suppresses Adderall-induced brain activity

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Background: There are increasing reports of the misuse of prescription psychostimulants for cognitive enhancement together with recreational cannabis. This raises a concern that chronic use of cannabis high in Δ^9-THC may alter the sensitivity to amphetamines. In this exploratory study we hypothesized chronic exposure to Δ^9-THC through vaporized cannabis would diminish the central nervous system (CNS) activity of Adderall.

Methods: To address this issue we exposed male and female mice to inhaled vaporized cannabis (10.3% Δ9-THC) or placebo for 30 min each day for ten consecutive days. After 24 h, mice were imaged fully awake for changes in BOLD signal following an IP injection of Adderall (60 µg) during the scanning session. After a 2-week washout, without any cannabis or placebo exposure, mice were again imaged and challenged with Adderall during the scanning session. The data were registered to a mouse 3D MRI atlas with 134 brain regions providing site-specific increases and decreases in global brain activity.

Results: Mice exposed to cannabis when compared to placebo showed a decrease in brain activation to Adderall. The blunted Adderall response was characterized by a decrease in positive BOLD signal and increase in negative BOLD. The prefrontal cortex, accumbens, ventral pallidum, caudate/putamen, and thalamus were most affected. After a 2-week wash out there were no significant differences between the cannabis and placebo groups when challenged with Adderall.

Summary: This exploratory study shows that short, daily exposures to inhaled cannabis, something equivalent to recreational use, affects the sensitivity to the psychostimulant Adderall. The reduced Adderall effect on brain activity, particularly circuitry associated with dopaminergic signaling raises concerns about escalation in psychostimulant use.

KEYWORDS

basal ganglia, functional MRI, awake animal imaging, BOLD, accumbens

Introduction

Medical emergencies associated to cannabis, the most widely self-administered psychoactive substance in the United States, has increased dramatically over the last decade. The Drug Abuse Warning Network estimated that in 2021, there were over 785,000 drug-related emergency department visits in the United States in which cannabis use was reported in the medical record (SAMHSA, 2022; SAMHSA, 2011). The prevalence of cannabis use coupled with the medical and legal movement to normalize its use in the United States is especially alarming given that a number of deleterious effects, including higher risk of depression, anxiety, addiction, and psychosis (e.g., (Bechtold et al., 2016; Patton et al., 2002; Han et al., 2019; De Aquino et al., 2018), have been associated with early age of initiation, frequency, and duration of use (Volkow, 2016; Volkow et al., 2014a). The fact that polysubstance use and abuse is the norm rather than the exception in the United States (Kidorf et al., 2018; Lee et al., 2018; Leri et al., 2003; Al-Tayyib et al., 2018; Jones and McCance-Katz, 2019; LaRue et al., 2019), has also raised concerns that a history of cannabis use, and abuse might render individuals more susceptible to consuming other psychoactive substances. Indeed, clinical studies have reported that 75% of cannabis users consume other psychoactive drugs during their lifetime and have a 50% higher cumulative risk in their lifetime of abusing other illicit substances (SAMHSA, 2022; SAMHSA, 2011; Secades-Villa et al., 2015). Of particular concern, is the high incidence of cannabis and psychostimulant use amongst adolescent and college students, including prescription psychostimulants like amphetamine (Adderall) that are typically used as medications for treating Attention-deficit/hyperactivity disorder (ADHD) (Lewis and Martinez, 2023; Asante and Atorkey, 2023).

Despite the expansive clinical and preclinical literature on the neurobiological actions of Δ-9-tetrahydrocannabinol (Δ^9-THC) – the psychoactive cannabinoid in cannabis (Huestis et al., 2001), remarkably little is known about how a history of cannabis use impacts the neurobiological effects of prescribed psychostimulants like Adderall. As the mesocorticolimbic dopamine (DA) system plays a key role in the abuse-related neurobiological and behavioral effects of psychoactive drugs, including Δ^9-THC, these neurobiological substrates continue to be a major focus of research for most psychoactive drugs. There is mounting evidence suggesting a complex neurochemical, behavioral, and pharmacological interaction between DA and the cannabinoid (CB) receptor 1 (CB_1) receptor systems in the brain (Maldonado, 2002; Tanda and Goldberg, 2003). However, next to nothing is known about how a history of chronic exposure to Δ^9-THC impacts the neurobiological processes involved in subsequent psychomotor stimulant use and abuse. In this regard, in preclinical and clinical studies, an acute dose of Δ^9-THC has been reported to elevate DA release in the ventral striatum (Bossong et al., 2009; Gardner, 2005; Lupica and Riegel, 2005; Bloomfield et al., 2016). In monkeys, chronic Δ^9-THC exposure increased DA_1 and DA_2 receptor expression in striatal neurons and altered the primate striatal DA_1 and DA_2 linked neuron phenotype and signaling (Hasbi et al., 2020). Moreover, some evidence also suggests that heavy, long-term cannabis users manifest reduced DA release following psychostimulant challenge and deficits in DA-related behavioral consequences that conceivably reflect Δ^9-THC-induced neuroadaptive changes in DA signaling (Volkow, 2016; Volkow et al., 2014a; Bloomfield et al., 2016). However, the link between levels of chronic Δ^9-THC exposure, dysfunctional DA systems, and Δ^9-THC-induced functional consequences of prescribed psychostimulant drugs like Adderall remain essentially unknown.

In this exploratory study we used BOLD functional imaging to address the question if chronic exposure to Δ^9-THC would affect the sensitivity to Adderall. To make the findings relevant to the human experience, mice were exposed to vaporized, cannabis high in Δ^9-THC once daily for 10 days matching blood levels of drug associated with recreational use. Furthermore, mice were imaged while fully awake when given Adderall during the scanning session. Overall, we found that the activation of the reward-related brain regions with strong dopaminergic innervation, including the prelimbic cortex, ventral pallidum, accumbens and thalamus, was dramatically reduced by Adderall in mice with a history of cannabis inhalation. Interestingly, after discontinuation of inhaled cannabis exposure, the Adderall-induced increases in brain activity in these regions was fully restored.

Methods and materials

Animal usage

Approximately 90-day-old female and male C57BL/6J mice (n = 10/sex), weighing between 22–25 g, were procured from Charles River Laboratories (Wilmington, Massachusetts, United States). The mice were subjected to a reverse 12: 12 light-dark cycle, with lights turned off at 07:00 h, and were provided with unrestricted access to food and water. All experiments were conducted between 08:00 AM and 06:00 PM to minimize circadian disturbances associated with the light-to-dark transition. The acquisition and care of the mice followed the guidelines outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publications No. 85–23, Revised 1985), adhering to the National Institutes of Health and the American Association for Laboratory Animal Science guidelines. The study protocols adhered to the regulations of the Institutional Animal Care and Use Committee at Northeastern University under protocol # 23-0407R, and the research methods complied with the ARRIVE guidelines for reporting *in vivo* experiments in animal research (Kilkenny et al., 2010).

Drug preparation and administration

Cannabis exposure

Ten mice (n = 5 female/n = 5 males) were subjected to cannabis with a high Δ^9-THC content (10.3% Δ^9-THC and 0.05% CBD), while another group of ten mice of females and males received placebo cannabis with less than 0.01% Δ^9-THC and 0.01% CBD. The cannabis was sourced from the National Institute on Drug Abuse (NIH/NIDA, Bethesda, MD) through the Research Triangle Institute (Research Triangle Park, NC). The mice were placed in a 38-L exposure chamber (60 cm × 45 cm × 20 cm), equipped with a

vapor inflow tube and several small air outflow holes. To familiarize the subjects with the environment and minimize stress, they were acclimated to the exposure chamber for 2 days before exposure.

A Volcano Vaporizer (Storz and Bickel, Tuttlingen, Germany) was employed to heat cannabis plant material below the point of complete combustion, vaporizing the active ingredient (Δ^9-THC) and reducing the formation of harmful free radicals like polycyclic aromatic hydrocarbons associated with the combustion of organic plant material. The vaporizer was preheated to approximately 210°C and loaded with 0.450 g of minced cannabis or placebo. Tubing connected the vaporizer to the exposure chamber, and the heating fan ran for a total of 60 s, filling the exposure chamber with vaporized cannabis aerosols. Following 30 min of passive exposure, the mice were removed from the chamber and returned to their cages. This exposure regimen was repeated daily for 10 consecutive days. The amount of minced cannabis used was determined based on prior studies demonstrating that this method produced similar serum Δ^9-THC concentrations (130–150 ng/mL) to those observed in human users (Farra et al., 2020; Sadaka et al., 2023). Mice were imaged within 24 h after the last exposure. For the administration of the drug during the imaging session from a distance, a polyethylene tube (PE-20) with an approximate length of 30 cm was inserted into the peritoneal cavity. Each mouse was given an injection of 60 μg of *d*-*l*-amphetamine sulfate (Adderall) (Sigma Aldrich) in a volume of 250 μL of saline vehicle during the scanning session. This dose (2.4 mg/kg) was based on previous preclinical studies that have reported changes in cognitive function and cross-sensitization with other stimulants (Santos et al., 2009; Doremus-Fitzwater and Spear, 2011; Sherrill et al., 2013). Mice were returned to their home cages following imaging and left undisturbed for 2 weeks. After this "washout" period they were imaged again and challenged with an IP injection of Adderall.

Awake mouse imaging and acclimation

A comprehensive account of the awake mouse imaging system and the acclimation procedure are detailed elsewhere (Ferris et al., 2014). Mice are acclimated for 1 week prior to imaging. Notably, we utilized a quadrature transmit/receive volume coil with a diameter of 38 mm, offering both high anatomical resolution and a superior signal-to-noise ratio for voxel-based BOLD fMRI. Additionally, the mouse holder's distinctive design from Ekam Imaging (Boston, MA) ensured complete head stabilization within a cushioned helmet, reducing discomfort associated with conventional ear bars and restraint systems commonly employed for immobilizing the head during awake animal imaging (Ferris, 2022). A visual representation of the mouse setup for awake imaging can be viewed at http://www.youtube.com/watch?v=W5Jup13isqw.

Acclimation

One week before the initial imaging session, all mice underwent a familiarization process with the head restraint and the sounds typical of the scanner. Initially, mice were gently secured in the holding system under 1%–2% isoflurane anesthesia. After regaining consciousness, the mice were positioned in a simulated MRI scanner setting for a duration of up to 30 min. This environment resembled a "mock MRI scanner" enclosed in a black box, featuring an audio recording of MRI pulses. The acclimation procedure was repeated daily over four consecutive days to mitigate autonomic nervous system-induced effects during awake animal imaging. Such effects include alterations in heart rate, respiration, corticosteroid levels, and motor movements. The overarching aim was to enhance contrast-to-noise ratios and improve image quality (King et al., 2005). Other research groups have alternatively emphasized more prolonged acclimation periods to minimize stress during awake imaging (Ferris, 2022; Stenroos et al., 2018; Chang et al., 2016).

Motion artifact

The awake mouse restraining system combined with acclimation can minimize motion artifact. Supplementary Figure S1 shows the motion artifact as a mean and SE for each of the 250 image acquisitions for all 10 subjects in the two experimental groups–placebo plus Adderall and cannabis plus Adderall. Adderall given to mice exposed to vaporized placebo showed no motion in any orthogonal direction outside 50 um (±) (5.000E-02). The in-plane resolution of a single voxel in this study is ca 187 um^2. These data show the restraining system, head holder and acclimation procedure effectively minimize any increase in motor activity that may be caused by Adderall. When mice are exposed to vaporized cannabis daily for 10 days and then withheld for 24 h–there is increased motion in X and Y due to slight rotation of the head, hence the mirror image of the red and black lines. These spikes between 60–70, 91–101 and plateau starting at 141 reach 100 um (±) (1.000E-01) are just over ½ of a voxel dimension. These changes are judged to be acceptable as most correction algorithms can adjust for motion artifact when movement is below the size of a voxel. The difference between the two experimental conditions is noteworthy because it may reflect withdrawal from chronic cannabis exposure, something not reported in the preclinical imaging literature. We observed a similar increase in motion artifact in another study following a 24 h hiatus from chronic oxycodone exposure (Iriah et al., 2019). In that case the motion exceed the dimensions of a voxel and the data were unusable. In the absence of additional data, this is purely speculative and will require further research.

BOLD image acquisition and pulse sequence

Experiments were conducted using a Bruker Biospec 7.0T/20-cm USR horizontal magnet (Bruker; Billerica, MA) and a 2 T/m magnetic field gradient insert (ID = 12 cm) capable of a 120-μsec rise time (Sadaka et al., 2021). At the beginning of each imaging session, a high-resolution anatomical data set was collected using the rapid acquisition relaxation enhancement (RARE) pulse sequence (RARE factor 8); (18 slices; 0.75 mm; field of view (FOV) 1.8 cm^2; data matrix 128 × 128; repetition time (TR) 2.1 s; echo time (TE) 12.4 ms; Effect TE 48 ms; number of excitations (NEX) 6; 6.5 min acquisition time). Functional images were acquired using a multi-slice Half Fourier Acquisition Single Shot Turbo Spin Echo (HASTE) pulse sequence (RARE factor 53); (18 slices; 0.75 mm; FOV 1.8 cm; data matrix 96 × 96; TR 6 s; TE 4 ms; Effective TE 24 ms; 25 min acquisition time; in-plane resolution 187.5 μm^2). This spatial resolution is enough to delineate the bilateral habenula (ca 4-5 voxels for each side) but not between lateral and medial habenula. The lateral habenula is involved in coordinating a response to aversive stimuli by affecting activity in the ventral tegmental area and substantia nigra (Stamatakis and Stuber, 2012). The medial habenula has no such role. We hold the habenula up as an example of the spatial limitations of

TABLE 1 Positive BOLD volume of activation: Adderall challenge (number of positive voxels).

Brain area	Placebo			Cannabis		P-val	Ω Sq
	Ave	SE		Ave	SE		
medullary reticular ventral n.	**31**	**6.6**	>	**6**	**3.8**	**0.009**	**0.331**
anterior pretectal thalamic n.	**18**	**2.6**	>	**7**	**2.0**	**0.014**	**0.285**
bed n. stria terminalis	**41**	**5.1**	>	**20**	**5.7**	**0.016**	**0.272**
posterior thalamic n.	**25**	**3.1**	>	**9**	**3.6**	**0.019**	**0.254**
pontine reticular n. oral	**110**	**14.7**	>	**45**	**18.1**	**0.022**	**0.241**
ventral pallidum	**56**	**7.5**	>	**24**	**9.3**	**0.023**	**0.234**
vestibular n.	**63**	**11.8**	>	**26**	**10.6**	**0.024**	**0.231**
anterior hypothalamic n.	**65**	**8.5**	>	**38**	**7.7**	**0.027**	**0.218**
lateral posterior thalamic n.	**21**	**2.7**	>	**7**	**3.3**	**0.029**	**0.017**
globus pallidus	**73**	**8.7**	>	**29**	**11.7**	**0.030**	**0.209**
insular rostral ctx	**159**	**19.8**	>	**88**	**23.1**	**0.030**	**0.208**
parabrachial n.	**16**	**2.5**	>	**7**	**3.4**	**0.031**	**0.207**
prelimbic ctx	**43**	**5.7**	>	**17**	**7.0**	**0.033**	**0.200**
ventral thalamic n.	**151**	**18.1**	>	**65**	**24.1**	**0.033**	**0.199**
dentate gyrus	**186**	**21.6**	>	**81**	**29.4**	**0.034**	**0.198**
primary somatosensory ctx	**579**	**71.9**	>	**299**	**91.4**	**0.034**	**0.198**
forceps minor corpus callosum	**7**	**1.4**	>	**2**	**0.9**	**0.035**	**0.194**
central medial thalamic n.	**14**	**2.1**	>	**6**	**2.4**	**0.035**	**0.194**
reticulotegmental n.	**25**	**3.9**	>	**9**	**4.4**	**0.035**	**0.193**
gigantocelllaris reticular n.	**123**	**22.9**	>	**53**	**22.2**	**0.037**	**0.190**
lateral septal n.	**67**	**8.7**	>	**34**	**10.1**	**0.037**	**0.189**
accumbens core	**36**	**5.0**	>	**14**	**6.0**	**0.039**	**0.183**
extended amygdala	**22**	**3.4**	>	**10**	**3.8**	**0.039**	**0.183**
orbital ctx	**157**	**21.3**	>	**65**	**26.7**	**0.041**	**0.179**
lateral rostral hypothalamic n.	**84**	**11.1**	>	**36**	**13.7**	**0.041**	**0.179**
CA3	**83**	**11.3**	>	**40**	**12.0**	**0.041**	**0.178**
dorsal hippocampal commissure	**8**	**1.0**	>	**4**	**1.3**	**0.043**	**0.175**
endopiriform n.	**23**	**2.9**	>	**10**	**4.0**	**0.046**	**0.167**
cerebellar nuclear n.	**35**	**6.0**	>	**14**	**6.8**	**0.049**	**0.163**
parietal ctx	**7**	**1.5**	>	**3**	**1.4**	**0.049**	**0.162**
cerebral peduncle	**136**	**19.7**	>	**61**	**24.0**	**0.050**	**0.160**
entorhinal ctx	**425**	**53.6**	>	**234**	**61.6**	**0.050**	**0.160**

preclinical fMRI. Each functional imaging session consisted of uninterrupted data acquisitions (whole brain scans) of 250 scan repetitions or acquisitions for a total elapsed time of 25 min. The control window included the first 50 scan acquisitions (18 slices acquired in each), covering a 5 min baseline. Following the control window, an I.P. injection of Adderall was given followed by another 200 acquisitions over a 20 min period.

Imaging data analysis

The impact of Adderall on brain activity was measured by quantifying positive and negative percent changes in the BOLD signal compared to the baseline. Initial analyses of signal changes in individual subjects involved comparing image acquisitions 125–225 to the baseline 1–45. The statistical significance of these changes was evaluated for each voxel (approximately 15,000 per

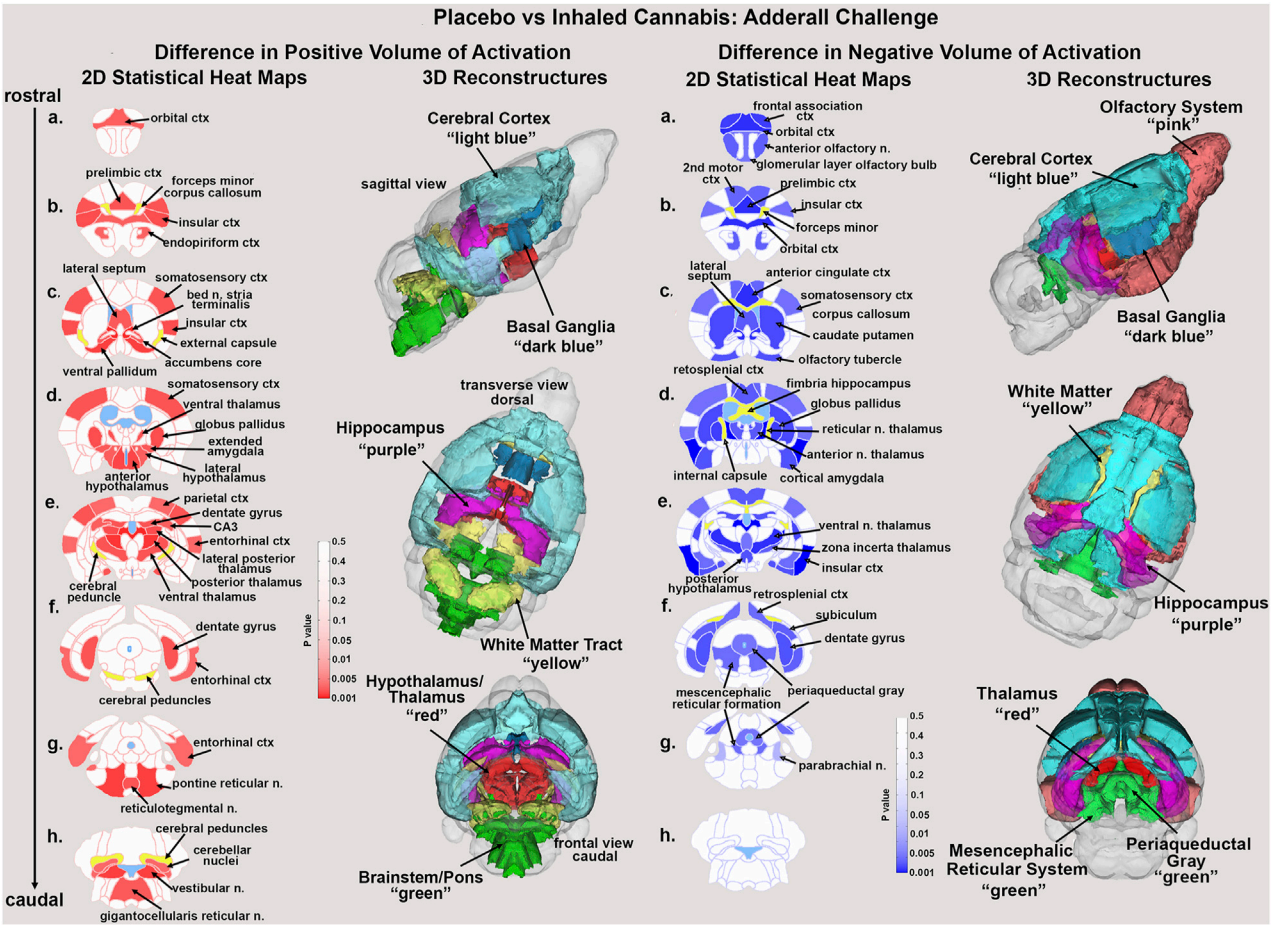

FIGURE 1
Statistical heat maps. Shown are 2D statistical heat maps for positive BOLD volume of activation (red) and negative BOLD volume of activation (blue). The areas shown were significantly different between mice exposed to placebo or cannabis and challenged with Adderall. The 3D color coded reconsrtuctions are a summary of the signficanlty affected areas from the 2D maps. ctx: cortex, n. nucleus.

subject in their original reference system) using independent Student t-tests, employing a 1% threshold to account for normal fluctuations of the BOLD signal in the awake rodent brain. The steps taken to control for multiple t-tests and false-positive detections have discussed in detail in previous studies (Sadaka et al., 2023; Sathe et al., 2023). Voxel-based percent changes in the BOLD signal, generated for individual subjects, were aggregated across subjects within the same group to construct representative functional maps. Image registration, alignment, and percentage change in BOLD signal for each voxel using a 3D Mouse Brain Atlas© with 134 segmented and annotated brain regions (Ekam Solutions; Boston, MA) has been described in previous studies (Sadaka et al., 2023; Sathe et al., 2023). The Kruskal-Wallis test statistic was employed to compare the number of activated voxels in each of the 134 regions between the placebo and cannabis groups.

Results

Tables reporting the positive and negative volumes of activation, i.e., number of voxels activated for all 134 brain areas for placebo and cannabis are provided in Supplementary Tables S1A, B. When the data from both groups are collapsed and separated in males and females there are no significant differences between sexes (Supplementary Material S1). There was a significant decrease in positive volume of activation following Adderall injection in mice with a history of cannabis exposure as shown in Table 1. This Adderall response affected 32/134 brain regions. These areas are ranked in order of their significance. Reported is the average (Ave) and standard error (SE) for placebo and cannabis groups together with probability values and the omega square (ω Sq) for effect size. The critical value was set at $p < 0.05$. A false discovery rate (FDR) for multi-comparisons gave a significant level of $p = 0.046$. The thalamus showed reduced activity with Adderall e.g., anterior pretectal, posterior, ventral, lateral posterior and central medial nuclei. Also affected were brain areas associated with the ascending reticular activating system and brain arousal e.g., medullary reticular n., pontine reticular n., parabrachial n. and gigantocellularis reticularis. It should also be noted that the accumbens and ventral pallidum, brain areas with dopaminergic efferent connections from the ventral tegmental area showed decreased activation with Adderall. The location of these areas and others from Table 1 are shown in 2D statistical heat maps in Figure 1.

TABLE 2 Negative volume of activation: Adderall challenge (number of negative voxels).

Brain area	Placebo			Cannabis		P-val	Ω Sq
	Ave	SE		Ave	SE		
reticular thalamic n.	1	0.8	<	8	2.9	0.006	0.342
rostral piriform ctx	6	5.6	<	51	21.6	0.006	0.330
internal capsule	2	1.9	<	15	4.5	0.008	0.316
lemniscal n.	0	0.0	<	7	3.4	0.008	0.281
anterior pretectal thalamic n.	1	0.6	<	6	1.6	0.009	0.318
orbital ctx	3	2.4	<	51	21.4	0.010	0.296
bed n. stria terminalis	2	2.3	<	9	3.6	0.011	0.297
prelimbic ctx	1	1.3	<	13	5.0	0.012	0.273
ventral thalamic n.	5	4.7	<	44	16.3	0.012	0.273
anterior cingulate n.	3	2.5	<	24	10.7	0.013	0.266
globus pallidus	2	1.3	<	24	9.2	0.013	0.275
frontal association ctx	4	3.8	<	31	10.5	0.016	0.259
caudate putamen	19	15.7	<	177	66.2	0.020	0.235
cortical amygdaloid n.	6	4.2	<	24	7.5	0.022	0.218
anterior amygdaloid n.	0	0.0	<	3	1.5	0.022	0.172
dentate gyrus	4	3.8	<	42	18.7	0.022	0.231
dorsal hippocampal commissure	0	0.0	<	3	1.4	0.022	0.188
forceps minor corpus callosum	0	0.0	<	3	1.3	0.022	0.194
olfactory tubercles	0	0.1	<	6	4.3	0.023	0.201
medial dorsal thalamic n.	1	1.2	<	5	1.5	0.024	0.206
caudal piriform ctx	8	6.2	<	37	10.7	0.025	0.209
medullary reticular ventral n.	4	3.6	<	12	4.9	0.027	0.216
retrosplenial rostral ctx	9	7.1	<	39	15.7	0.027	0.203
anterior thalamic n.	3	2.4	<	9	2.9	0.027	0.201
corpus callosum	7	4.9	<	38	15.6	0.027	0.204
lateral septal n.	2	1.6	<	18	7.1	0.027	0.208
subiculum	3	2.6	<	62	28.3	0.029	0.180
zona incerta	0	0.2	<	9	3.9	0.029	0.176
anterior olfactory n.	8	7.2	<	49	21.2	0.031	0.193
secondary motor ctx	6	5.4	<	54	21.4	0.037	0.172
medial geniculate	0	0.2	<	11	5.4	0.038	0.159
mesencephalic reticular formation	3	3.0	<	62	29.4	0.038	0.156
retrosplenial caudal ctx	9	9.3	<	46	20.1	0.038	0.161
basal amygdaloid n.	2	1.6	<	15	4.5	0.038	0.171
entorhinal ctx	23	14.9	<	111	41.1	0.038	0.178
fimbria hippocampus	6	3.1	<	17	4.8	0.038	0.173
flocculus cerebellum	11	5.9	<	39	12.5	0.038	0.173

(Continued on following page)

TABLE 2 (*Continued*) Negative volume of activation: Adderall challenge (number of negative voxels).

Brain area	Placebo			Cannabis		P-val	Ω Sq
	Ave	SE		Ave	SE		
primary somatosensory ctx	17	11.1	<	180	73.5	0.042	0.174
insular caudal ctx	2	1.4	<	15	6.2	0.042	0.162
periaqueductal gray	7	6.0	<	29	13.3	0.047	0.157
glomerular layer	11	6.3	<	70	27.2	0.047	0.158
parabrachial n.	1	1.2	<	6	2.2	0.048	0.147

Shown in Table 2 are the changes in negative volume of activation. There were 42/134 brain areas that showed a significant increase in negative BOLD volume of activation (FDR = 0.058). Several of these areas matched those in Table 1 e.g., prelimbic ctx, bed n. stria terminalis, dentate gyrus. Again the thalamus was well represented in addition to the prefrontal ctx, e.g., orbital, anterior cingulate, frontal association, prelimbic and 2nd motor cortices. The olfactory system also showed an increase in negative BOLD volume of activation e.g., glomerular layer of the olfactory bulb, anterior olfactory n., piriform cortices, and cortical amygdala.

Shown in Figure 1 are the anatomical localization of the areas listed in Tables 1, 2. The coronal sections are statistical heat maps labeled as sections a.- h. and arranged from rostral (top) to caudal (bottom). Light blue denotes the location of cerebroventricles and yellow denotes white matter tracts. The left side (red highlights) shows brain areas that had a decrease in positive BOLD signal with Adderall treatment while the right side shows areas that had an increase in negative BOLD with Adderall treatment. Together, this pattern of BOLD signal change would indicate chronic exposure to cannabis reduces the stimulant activity of Adderall. Sections (a. and b. left and right) highlight changes in the prefrontal cortex, e.g., 2nd motor, orbital, frontal association, prelimbic and insular cortices and the forceps minor, white matter projections to the prefrontal ctx. Section (c. left and right) highlights the accumbens core, ventral pallidum and caudate, all areas with dopaminergic efferent connections from the ventral tegmental area. Sections (d. and e. left and right) show the many thalamic areas affected by Adderall treatment. Sections (e. and f. left and right) highlights the hippocampus e.g., CA3, subiculum and dentate gyrus. Sections (g. and h.) show pons and brainstem. The 3D color coded reconstructions summarize the data from Tables 1, 2.

Shown in Figure 2 are time series of percentage change in BOLD signal over the 25 min scanning session for mice exposed to chronic placebo and challenged with Adderall before (black line) and after (blue line) the 2-week washout and mice exposed to chronic cannabis and challenged with Adderall before (gray line) and after (red line) washout. These time series were generated by averaging the BOLD signal at each image acquisition from the accumbens, caudate/putamen and ventral pallidum, areas comprising the basal ganglia highlighted in Tables 1, 2. Each mouse from each experimental condition (n = 9 for chronic cannabis; n = 10 for chronic placebo) was represented in the time series. Adderall was injected (arrow) at 5 min

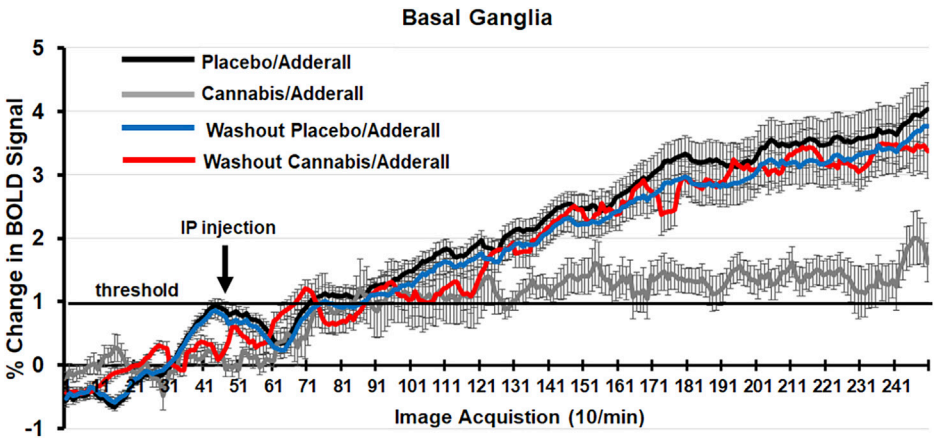

FIGURE 2
Change in BOLD signal over time. Shown are time series of percentage change in BOLD signal over the 25 min scanning session (250 image acquisitions) for mice exposed to chronic placebo and challenged with Adderall before (black line) and after (blue line) the 2-week washout and mice exposed to chronic cannabis and challenged with Adderall before (gray line) and after (red line) washout. These time series were generated by averaging the BOLD signal at each image acquisition from the accumbens, caudate/putamen and ventral pallidum, areas comprising the basal ganglia. Adderall was injected (arrow) at 5 min (50 acquisitions). The 1% threshold is highlighted by the black line to account for the normal fluctuations in BOLD signal in the awake mouse brain.

TABLE 3 Two-week washout: Positive and negative BOLD volume of activation with Adderall challenge. (Number of positive and negative voxels).

Positive volume of activation							
Brain n.	Placebo			Cannabis		P-val	Ω Sq
	Ave	SE		Ave	SE		
locus coeruleus	**1**	**0.4**	>	**0**	**0.0**	**0.001**	**1.000**
lateral lemniscus	**13**	**2.3**	<	**18**	**3.2**	**0.036**	**0.071**
frontal association ctx	**79**	**13.3**	>	**59**	**12.6**	**0.138**	**0.063**
Negative volume of activation							
Brain n.	Placebo			Cannabis		P-val	Ω Sq
	Ave	SE		Ave	SE		
anterior amygdaloid n.	**0**	**0.0**	<	**1**	**1.0**	**0.109**	**−0.055**
accumbens core	**0**	**0.0**	<	**1**	**1.1**	**0.109**	**−0.057**

(50 acquisitions). The 1% threshold is highlighted by the black line to account for the normal fluctuations in BOLD signal in the awake mouse brain. A two-way ANOVA showed a significant interaction between time and experimental condition for placebo/Adderall vs. cannabis/Adderall [$F_{(249,\ 18{,}177)} = 8.979$, $p < 0.0001$]. There were no significant differences between experimental conditions after washout (See Table 3).

Discussion

The global use of cannabis and amphetamines is an international public health problem (UNODC, 2016). Amongst adolescent and college students there is a high incidence of cannabis and amphetamine use (Lewis and Martinez, 2023; Asante and Atorkey, 2023). Considering the combined use of stimulants to enhance cognitive performance with the recreational use of cannabis, we sought to evaluate how chronic cannabis use will impact Adderall-induced changes on brain activity. Remarkably, we found that the effects of Adderall on BOLD functional activity in reward-related brain regions (e.g., prelimbic cortex, ventral pallidum, accumbens, and thalamus) was completely abolished in both male and female mice exposed to cannabis compared to placebo. However, after a 2-week discontinuation of Δ^9-THC, both the Δ^9-THC- and placebo-exposed mice showed robust and comparable Adderall-induced functional brain activity changes in these reward-related regions. Given the importance of DA in the effects of psychomotor stimulants, our data suggest that Δ^9-THC use likely triggers neurobiological adaptations that switch the DA reward-related brain regions off, rendering them unresponsive to Adderall, and that discontinuation of Δ^9-THC use "reactivates" these brain regions leading to a restoration of Adderall's responsiveness.

There have been numerous BOLD imaging studies in rats following changes in brain activity with amphetamine or methylphenidate (Ritalin) challenge (Easton et al., 2009; Easton et al., 2007; Preece et al., 2007; Chen et al., 1997; Dixon et al., 2005). While all these studies have been conducted under anesthesia, they report activation in the accumbens, caudate/putamen, thalamus and prefrontal cortex as demonstrated in this study on awake mice. Studies on human volunteers show amphetamine enhances activity in the prefrontal cortex and striatum through a DA mechanism (Slifstein et al., 2015; O'Daly et al., 2014). Psychostimulants enhance cognition and attention by activating the prefrontal cortex and the extended frontostriatal circuit (Spencer et al., 2015). In an earlier study, we treated juvenile rats with methylphenidate or amphetamine through the peripubertal period and discovered that the normal functional connections between the striatum, sensorimotor cortices and prefrontal cortex were reduced (Demaree et al., 2021). To the best of our knowledge, this is the first MRI study of chronic Δ^9-THC exposure and discontinuation on mice, awake or anesthetized, challenged with amphetamine.

It is noteworthy that all substances of abuse impact the functioning of brain regions with strong dopaminergic innervation, causing neuroadaptive alterations associated with drug reinforcement (Koob and Volkow, 2016). Prolonged cannabis use heightens the susceptibility to substance abuse and dependence (Volkow et al., 2014b; Ramesh et al., 2011). Cannabis has the shortest duration from first use to dependence, and earlier onset of use presents an elevated risk for developing dependence (Behrendt et al., 2009). There is ample evidence that the effects of chronic exposure to Δ^9-THC on tolerance and dependence is due to cannabinoid (CB) receptor 1 (CB_1) downregulation/desensitization in specific regions of the brain (Panagis et al., 2008; Singh et al., 2011; McMahon, 2011). Studies in humans have shown that frequent exposure to CB_1 agonists such as Δ^9-THC leads to physical dependence and withdrawal (Panagis et al., 2008; Singh et al., 2011; McMahon, 2011; Haney et al., 1999; Budney et al., 2004; Beardsley et al., 1986; Desai et al., 2013; Stewart and McMahon, 2010). Continuous exposure to Δ^9-THC in mice and rat's results in physical dependence as well (Manwell et al., 2014; Wilson et al., 2006; Bruijnzeel et al., 2016). Research by Freels and colleagues demonstrated that vaporized cannabis extracts possess reinforcing properties, supporting conditioned drug-seeking behavior in rats

(Freels et al., 2020). In previous experiments, we exposed both young adult and elderly mice to daily inhaled vaporized cannabis for two to three consecutive weeks. Utilizing voxel-based morphometry and diffusion-weighted imaging, we observed structural changes in the midbrain dopaminergic system (Sadaka et al., 2023; Taylor et al., 2023). The responsiveness of these brain areas to Δ^9-THC aligns with findings in preclinical literature (Kolb et al., 2006; Madularu et al., 2017).

In the present study, using BOLD functional imaging, we find the enhanced change in signal to Adderall is abolished in mice exposed to chronic inhaled cannabis. To be clear, when comparing the Adderall response in mice with a history of placebo and history of cannabis the positive volume of activation–the increase in number of positive BOLD voxels–decreased indicating that Adderall is less effective in stimulating brain activity. This decrease in brain activity to Adderall in mice with a history of cannabis exposure is complimented by an increase in negative BOLD volume of activation as these voxel numbers rise. In this case, Adderall is presumably reducing brain activity and decreasing blood flow. The accumbens, ventral pallidum and caudate/putamen, targets of DA efferents from the midbrain DA neurons, show reduced sensitivity to Adderall. This blunted response to Adderall also included the prefrontal cortex and thalamus. As we noted earlier, there is no established link between cannabis and amphetamine that could explain these results. The most plausible explanation is disruption in DA signaling. Indeed, the heightened motion artifact in the cannabis group as compared to placebo would be evidence to that effect. What was most interesting is the presumed flexibility, in that, following a 2-week discontinuation of cannabis exposure, the Adderall-induced changes in brain activity in the reward-related regions was fully restored, presumably due to a restoration of activity within the DA system. There is precedent for this flexibility in the DA system as reported in old mice exposed to vaporized cannabis for 4 weeks. Voxel based morphometry showed the areas comprising the DA system were smaller as compared to placebo. However, after a 2-week washout these measures of brain volume were reversed–now larger than placebo (Sadaka et al., 2023).

Data interpretation, limitations, considerations, and future studies

We recognize that the findings presented in this study raises several important and fundamental questions regarding chronic Δ^9-THC exposure and psychomotor stimulant use and abuse. For example, Adderall is believed to enhance cognitive function in the prefrontal cortex (PFC) by inhibiting the reuptake of monoamines, including DA, serotonin, and norepinephrine (Easton et al., 2009; Easton et al., 2007), thereby increasing neurotransmission of these chemical signals (Preece et al., 2007; Chen et al., 1997). It is worth noting, that these initial neuroimaging studies were designed to document changes in brain activity in reward-related brain regions rather than changes in monoamine levels, which would require another level of analysis (e.g., *in vivo* microdialysis) that was beyond the scope of this work. Although speculative in the absence of additional neurochemical data, the present findings suggest that short-term monoamine adaptations, especially in DA activity, are likely to occur in the reward-related brain regions. Also, the relationship between structural changes in the DA-related system due to chronic Δ^9-THC exposure, subsequent Adderall use, and cognitive function remains unclear. While the sensitivity to Adderall was significantly affected by chronic cannabis exposure, here we did not evaluate disruptions in cognitive performance and therefore it would be difficult for us to speculate how chronic Δ^9-THC and/or Adderall use impacts cognitive processes.

The observation that Adderall's responsiveness on brain activity was fully restored following cessation of Δ^9-THC was surprising and somewhat remarkable. At present, it is unclear whether this reflects neuroplasticity *per se*, given that chronic Δ^9-THC leads to differential tolerance or cross-tolerance among CB1-agonists across various behavioral, pharmacological, and physiologic endpoints that may reflect regional differences in CB1 receptor downregulation/desensitization in the CNS and/or pharmacological efficacy. Studies in humans and laboratory animals have also shown that frequent exposure to CB1 agonists such as Δ^9-THC leads to physical dependence and withdrawal. Although mounting evidence suggests that complex neurochemical, behavioral, and pharmacological interactions exists between DA–CB1 receptor systems in the brain, few studies have directly investigated the interplay between these systems, and next to nothing is known about how a history of heavy chronic exposure to Δ^9-THC impacts subsequent stimulant neurobiology in both sexes. Of interest, one interpretation of our data may be that neuroadaptations in dopaminergic activity may offer the opportunity for pharmacological interventions for substance use disorders; however, such an interpretation would be speculative as these experiments have not yet been conducted. An important consideration regarding our observed data is whether greater levels of cannabis exposure cause more long-term neuroadaptive changes in DA system that cannot be restored. Additional studies are needed to fully address these issues. To that end, postmortem histology studies would have helped to identify these changes at a molecular level within the monoamine systems in reward-related brain areas.

What would the Adderall effect have been if it followed a single exposure to vaporized cannabis? It would have been interesting to see how cannabis and Adderall interacted if they had been given together on a daily schedule for a prolonged period. The presentation of Adderall 24 h after a single exposure to inhaled cannabis would not be expected to interfere with the stimulant activities of this drug. The developmental studies we have reported in the literature giving cannabis for 2–3 weeks alters the DA system. There is no evidence this happens with a single exposure of cannabis.

With the legalization of cannabis in much of the US, its recreational use can be a daily routine among many individuals. Moreover, 75% of cannabis users consume other drugs of abuse and have a 50% higher cumulative risk in their lifetime of abusing other addictive substances. Indeed, it is common practice for individuals that use cannabis in high school, college, professional schools, and on the job to also use psychomotor stimulants [e.g., Adderall; amphetamines] either recreationally or therapeutically for treating ADHD and narcolepsy. Although both cannabis and

psychomotor stimulant polysubstance misuse is the norm rather than the exception, remarkably little is known about how exposure to Δ^9-THC with intermittent use of Adderall (i.e., recreational scenario), vs. chronic exposure of Δ^9-THC together with Adderall (i.e., therapeutic scenario) will, respectively, produce short- and long-lasting adaptations in DA-related neuroimaging and neurochemical signatures to impact cognition. Additional studies are needed to meaningfully address this issue.

Finally, blood concentrations of Δ^9-THC were not specifically gauged but were presumed to be similar to those reported in our prior studies using male and female mice who underwent inhalation in a chamber using the vaporization technique with a 10.3% Δ^9-THC cannabis mass (Sadaka et al., 2023). It is possible that the daily exposure to cannabis may have altered the pharmacokinetics of Δ^9-THC, such that a 24 h discontinuation may not have been sufficient to eliminate Δ^9-THC from brain and blood. Also, the Adderall effect would have the confound of Δ^9-THC on-board. Moreover, we did not measure plasma levels of amphetamine and show the equivalence to human usage as we did for Δ^9-THC.

Summary

This study provides clear evidence that the single exposure to cannabis each day for several days reduces the effect of Adderall on the prefrontal cortex, ventral striatal/caudate putamen, sensorimotor circuit. The use of vaporized cannabis that presumably produces blood levels of Δ^9-THC found in humans following smoked cannabis for recreational use reflects the human experience. These data have important clinical implications and inform the public regarding the risk of combining the recreational use of cannabis with psychostimulants to enhance cognitive performance or in the treatment of ADHD.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by Institutional Animal Care and Use Committee at Northeastern University protocol # 23-0407R. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JO: Writing–review and editing, Writing–original draft, Investigation. RID: Writing–review and editing, Writing–original draft, Conceptualization. PK: Writing–review and editing, Writing–original draft, Visualization, Software, Funding acquisition, Formal Analysis, Data curation. CF: Writing–review and editing, Writing–original draft, Resources, Funding acquisition, Conceptualization.

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Conflict of interest

CF and PK have a partnership interest in Ekam Solutions a company that develops 3D MRI atlases for animal research.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1413812/full#supplementary-material

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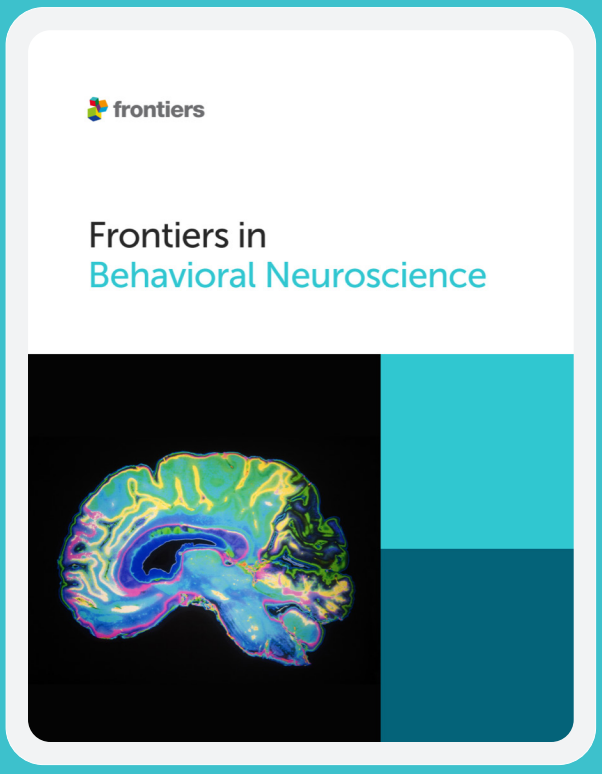

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