**Title:** Development and Validation of a Pictorial Cue Database for Cannabis Cue Reactivity: Insights from Behavioral and Neural Investigations

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**Abstract**

**Background:** Craving, a potent driving force behind drug-seeking and consumption behaviors, represents a dynamic emotional-motivational response primarily elicited by drug-related cues. In laboratory settings, the drug cue reactivity (DCR) paradigm is frequently employed to evoke craving and investigate the neural and behavioral responses to drug cues. This study adopts functional magnetic resonance imaging (fMRI) alongside behavioral assessments to establish a collection of validated pictorial cues encompassing both cannabis and neutral images.

**Methods:** 110 cannabis-related images were selected across cannabis flowers and powder, cannabis use methods, and paraphernalia categories. Male participants with a history of cannabis use were then asked to assess the selected images for craving, valence, and arousal using both the visual analog scale and the self-assessment Manikin. Using fMRI, the neural mechanisms underlying cannabis cue-reactivity were investigated at the whole-brain level and within Brainnetome atlas areas in a subgroup of 31 cannabis users.

**Results:** The selected cannabis-related images (n = 110) elicited significantly higher craving ($t = 6.56$; $p<0.001$) and arousal ($t = 17.46$; $p<0.001$) compared to the neutral ones (n = 30). 50 regular cannabis users (19.9 ± 4.8 years; 10 females and 40 males) with at least a one-year history of use were included in the fMRI study. Investigating blood oxygenation level-dependent (BOLD) responses to cannabis compared with neutral cues yielded significant activations in the inferior/medial frontal gyrus, fusiform gyrus, parahippocampal gyrus, orbital gyrus, postcentral gyrus, insula, precuneus, superior/middle temporal gyrus, and cerebellar tonsil.

**Conclusion:** This study provides a resource of ecologically validated cannabis-related images useful for both clinical and experimental studies applying DCR as interventions or assessments for cannabis users.

**Keywords:** fMRI, cannabis, cue-reactivity, craving, valence, arousal

## 1. Introduction

The drug cue reactivity (DCR) paradigm is commonly used in experimental studies for both assessments and interventions (Ekhtiari et al., 2019; Tsamitros et al., 2024). A "Cue" refers to a stimulus containing drug-related features presented through various sensory modalities such as visual, auditory, audiovisual, tactile, olfactory, or gustatory stimuli, which induce emotional responses in individuals with substance use disorders (SUDs). Craving, as an emotional response to drug-related conditioned cues, is experienced by individuals with various forms of SUDs (Addiction Cue-Reactivity Initiative (ACRI) Network et al., 2024; Ekhtiari et al., 2016), including cannabis use disorder (CUD) (Sehl et al., 2021; Sherman et al., 2018).

As a valuable tool, some evidence suggests that DCR could serve both as an underlying mechanism and as a predictor for drug use and relapse (Back et al., 2014; Chase et al., 2011; Ekhtiari et al., 2022; Tsamitros et al., 2024). Consequently, it is frequently utilized as a probe in neuroimaging and behavioral research to evaluate whether certain stimuli can evoke responses (e.g., the urge to use drugs), as reflected in brain activity or self-reports (Hill-Bowen et al., 2021). Previous research has explored the role of DCR as an intervention within exposure therapy (Dejoie et al., 2024; Goldstein et al., 2007) and memory reconsolidation paradigms (Ekhtiari et al., 2019). Cue exposure has been shown to elicit reward-related neural activation (Addiction Cue-Reactivity Initiative (ACRI) Network et al., 2024; Cousijn et al., 2013; Karoly et al., 2019), subsequently increasing subjective craving (Ekhtiari et al., 2016; Tsamitros et al., 2024; Vollstädt-Klein et al., 2011).

Given the importance of cue exposure, several studies have validated visual cues through databases (Ekhtiari et al., 2019; Macatee et al., 2021). The Normative Appetitive Picture System (NAPS) was the first published database specifically designed for limited sets of appetitive images, including 18 alcohol, 6 cigarettes, 12 food, and 12 non-alcoholic beverage-related images (Stritzke et al., 2004). Similarly, Billieux and colleagues validated alcohol-related images by asking participants to rate 60 alcohol-related images for valence, arousal, and dominance (Billieux et al., 2011; Vollstädt-Klein et al., 2011). Another study provided a validated database of pictorial cues for methamphetamine and opioids (Ekhtiari et al., 2019), which included 120 images for each substance rated by participants with a history of use. They also added 120 neutral images matched for their content (objects, hands, faces, and actions) with drug-related images to increase the potential for this database to be used in experimental DCR tasks (Ekhtiari et al., 2019). Additionally, Macatee and colleagues recently developed a database consisting of 280 cannabis-related images across four cannabis paraphernalia categories: bowl, bong, blunt/joint, and vaporizer. These images were rated by regular cannabis users with varying primary cannabis use methods. The database also includes 80 neutral images matched to the cannabis images based on key confounding elements and characteristics, such as the presence of human hands and faces (e.g., presence of human hands and faces) (Macatee et al., 2021).

DCR tasks utilized in functional magnetic resonance imaging (fMRI) studies represent an essential step toward integrating functional neuroimaging into clinical practice in addiction medicine (Addiction Cue-Reactivity Initiative (ACRI) Network et al., 2024; Ekhtiari et al., 2019). Cue-reactivity reflects increased motivational processing underlying continued substance use and relapse (Tsamitros et al., 2024; Wang et al., 2022). SUDs are associated with greater cue reactivity in brain regions such as the orbitofrontal cortex, anterior cingulate cortex, striatum, ventral tegmental area, and amygdala (Addiction Cue-Reactivity Initiative (ACRI) Network et al., 2024; Ekhtiari et al., 2016; Sahlem et al., 2024). Several fMRI studies have examined brain function in cannabis users exposed to cannabis vs neutral stimuli during cue-reactivity tasks (Karoly et al.,

2019; Sehl et al., 2021). Despite methodological heterogeneity, these studies relatively consistently demonstrate significant activations in response to cannabis stimuli, including in the amygdala, parietal, striatum, and prefrontal cortex (Cousijn et al., 2013; Karoly et al., 2019; Sehl et al., 2021). As cannabis use continues to rise globally, there is an increasing need for the development of therapeutic interventions and assessment tasks within cue reactivity paradigms. To our knowledge, there are only two cannabis pictorial databases currently available. The first, developed by Macatee et al. (Macatee et al., 2021), focuses on self-report measures such as craving, valence, and arousal. The second, created by Karoly et al. (Karoly et al., 2019), that measures craving induced by cannabis-related images using fMRI. However, there is a notable gap in the literature—a comprehensive cannabis-related image database that integrates both behavioral and neural measures is lacking. Developing such a database that also includes different types of categories (e.g., paraphernalia, action), would allow for more robust investigations using DCR for cannabis users. To address this gap, the present study utilized fMRI and behavioral data to provide a set of validated pictorial cues for cannabis and neutral images in a sample of regular cannabis users. The images were selected from cannabis alone, cannabis use methods, and three cannabis paraphernalia categories (blunt/joint, pipe/bowl, and bong). Cannabis users also rated 30 neutral images matched to the selected cannabis-related images based on important features, including the presence of hands and faces.

## 2. Methods

The present study consisted of three phases (Figure 1A): (a) preparatory phase (cannabis cue collection), (b) behavioral phase (cue validation), and (c) fMRI phase (cannabis cue reactivity), which are described below, respectively. For details on the method, see the Supplementary Materials.

- ***Preparatory Phase: Cannabis Cue Validation***

The preparatory study was conducted to collect and select a set of cannabis-related images. A sample of 10 regular cannabis users participated in this phase and presented with a set of 356 mages. They were asked to rate affective measures including arousal and valence for each image using a 5-point Likert scale and craving on a 0–100 mm Visual Analog Scale. Images were displayed on a 17-inch LCD monitor positioned approximately 70 cm away, using a laptop (images were presented by Photo Viewer for Win 10 1.0 for Windows).

- ***Behavioral Phase: Cue Validation***

The behavioral phase utilized 110 culturally competent, high-craving score images selected from the initial set of 356 (Table 1). These images were presented in two phases for cannabis users (n=50): (1) in an online behavioral phase, and (2) in a neural phase employing an fMRI cue-reactivity task.

- ***Neural Phase: Cannabis Cue Reactivity***

The neural phase involved using functional magnetic resonance imaging (fMRI) during a cannabis cue-reactivity task with the 110 selected images. This phase aimed to investigate neural responses to cannabis-related cues among regular cannabis users.

### *2.1. Participants*

The inclusion criteria for the three phases were as follows: (1) fluency in Farsi, (2) age between 18 and 30 years, and (3) regular cannabis users (i.e., individuals who used cannabis at least twice per week over the past year) (Young-Wolff et al., 2019). Participants were recruited via social media platforms such as Twitter and Instagram. Volunteers who met the inclusion criteria were selected and screened for eligibility. Additional inclusion criteria for participants in the fMRI study included: (1) abstinence from other substances and psychiatric prescription medication, (2) abstinence from cannabis for at least 12 hours prior to the scanning sessions (controlled by oral fluid testing), and (3) eligibility for MRI scanning.

For the preparatory study, participants were invited to the laboratory to perform the validation task and rate the images. For the online behavioral study, those who met the criteria received an online link containing questionnaires and a consent form prior to starting the cue validation task. All three phases of the study were conducted on the online Gorilla platform (https://gorilla.sc/).
Participants selected for the fMRI study were invited to the National Brain Mapping Lab, Tehran, Iran (https://www.nbml.ir) for imaging sessions. The study was approved by the Ethics Committee of the Iran University of Medical Sciences (Approval ID IR.IUMS.REC.1400.510), and all participants provided written informed consent before participation. Participants were monetarily compensated for their participation in the study.

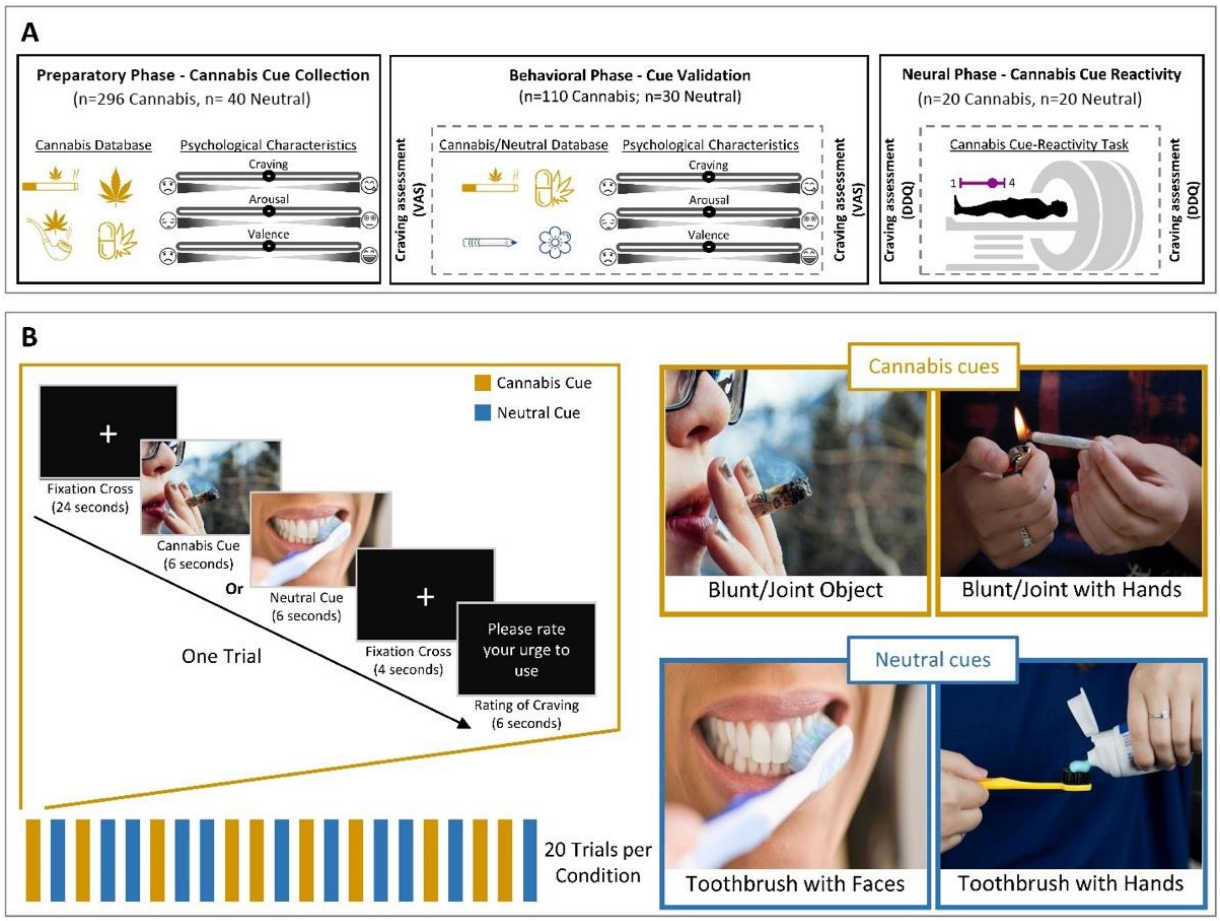


**Figure 1. Overview of the experimental procedures and cannabis cue-reactivity paradigm. (A)** Experimental procedure includes three phases: preparatory phase (cannabis cue validation), behavioral phase (cue validation), and fMRI phase (cannabis cue reactivity). In the cannabis cue validation phase (preparatory phase), participants (n = 10) were presented with cannabis-related cues and neutral cues (n = 356) and asked to rate the craving, arousal, and valence induced by each image. In the behavioral phase (cue validation), participants (n = 50) were presented with 140 images (cannabis-related cues and neutral cues) selected from the preparatory phase and rated the craving, arousal, and valence induced by each image. Immediately before and after the behavioral phase, participants rated their self-reported craving. In the fMRI phase (cannabis cue reactivity), participants (n = 31) underwent an MRI scan while completing cue-reactivity task. Immediately before and after the cue-reactivity task, participants completed the Desires for Drug Questionnaire (DDQ). **(B)** During the cue-reactivity task, participants were presented with cues in a random order for 6 seconds. Each image was followed by a fixation cross with a duration 4 seconds. Subsequently, participants rated their craving of the presented image on a scale from 1 to 4 (1 = not at all to 4 = extremely) for a duration 6 seconds using an MRI-compatible response box. In total, participants viewed 40 images (20 cannabis-related cues and 20 neutral cues) over 664 seconds.



### *2.2. Materials*

*Demographic data:* Participants were asked to fill out a questionnaire including information about age, gender, and education level, as well as history of cannabis use (i.e., duration of regular cannabis use and frequency of use per week). Additionally, participants confirmed their abstinence from other drugs, alcohol and tobacco, and psychiatric prescription medication.

*Cannabis-related images:* During the preparatory study, 356 cannabis-related images were selected from two databases validated by Macatee (Macatee et al., 2021) and Karoly (Karoly et al., 2019). In face-to-face sessions, the image sets were presented to 10 participants who rated

affective measures including craving, arousal, and valence for each image. Our small sample size was because this phase was preparatory, designed to select the most appropriate images for subsequent stages. From this study, vapor images (which are uncommon among Iranian users) and culturally incompatible images were excluded, resulting in the selection of 110 images for the online behavioral study. Out of these, 20 images depicted cannabis plant and powder (produced by grinding cannabis flowers)(Potter et al., 2008), while the remaining images portrayed specific methods of use and paraphernalia categories (i.e., vaporing, smoking). These images were categorized into Cannabis alone (subdivisions: cannabis flower and cannabis powder), Cannabis-related paraphernalia objects (subdivisions: blunt/joint, pipe/bowl, and bong), Cannabis-related paraphernalia with hands (subdivisions: blunt/joint with hand, pipe/bowl with hand, and bong with hand), and Cannabis-related paraphernalia activities with faces (subdivisions: blunt/joint with face, pipe/bowl with face, bong with face). Additionally, 30 toothbrush images (objects, with hands and toothbrush activities with faces) were selected as neutral images (Table 1).

**Table 1.** The number and types of selected cannabis and neutral images (n=140) within each category and subdivisions.

| **Categories (n)** | **Subdivisions (n)** |
|---|---|
| Cannabis alone (20) | Cannabis flower (10) |
| | Cannabis powder (10) |
| Cannabis-related paraphernalia objects (30) | Blunt/Joint objects (10) |
| | Pipe/Bowl objects (10) |
| | Bong objects (10) |
| Cannabis-related paraphernalia with hands (30) | Blunt/Joint with hand (10) |
| | Pipe/Bowl with hand (10) |
| | Bong with hand (10) |
| Cannabis-related paraphernalia activities with faces (30) | Blunt/Joint activities with faces (10) |
| | Pipe/Bowl activities with faces (10) |
| | Bong activities with faces (10) |
| Neutral (toothbrush) (30) | Toothbrush objects (10) |
| | Toothbrush with hands (10) |
| | Toothbrush activities with faces (10) |



*Valence and Arousal Scales:* The valence and arousal rating scales of the Self-Assessment Manikin (SAM) were used to assess the emotional valence and arousal levels associated with each presented image. Participants answer the question "How positive or negative the emotion is" as valence, and "How excited or apathetic the emotion is" as the arousal. In the preparatory study, participants rated valence and arousal on a 5-point Likert scale ranging from “1” to “5”. For the main study, a more detailed 9-point Likert scale was employed. On the valence scale, a minimum value of 1 was represented by a frowning, unhappy figure, indicating extreme unpleasantness, while the maximum value (5 or 9) was represented by a smiling, happy figure, representing extreme pleasantness (Bradley & Lang, 1994). The minimum value (1) on the arousal scale was

accompanied by a relaxed and sleepy figure, indicating a feeling of calmness, while the maximum value (5 or 9) was accompanied by an excited, wide-eyed figure, corresponding to feeling very excited and aroused (Bradley & Lang, 1994). Participants were instructed to rate their responses after being presented with the stimulus, providing valuable insight into the emotional responses elicited by the images.

*Craving:* In this study, we used two measures of craving, including the Visual Analog Scale (VAS) and the Desires for Drug Questionnaire (DDQ) (Franken et al., 2002). The VAS was used to visually measure the immediate desire for cannabis by participants in response to each image presented in both the preparatory and online behavioral studies. A 0–100 mm VAS was used to determine the intensity of cue-induced craving, where 0 indicated "no craving" and 100 indicated "extreme craving". Participants selected their answers on a ruler scale from 0 to 100. Inside the scanner, they responded by pressing the corresponding button on a 4-button response box, where 1 represented "not at all" and 4 represented "extremely" after each stimulus presentation. The DDQ is a self-report questionnaire comprising three subscales: desire and intention (7 questions), negative reinforcement (4 questions), and control (2 questions). This questionnaire has been validated for Iranian heroin users (Hassani-Abharian et al., 2016) and is widely used for different types of substances. Each question is rated on a 7-level Likert scale, where a score of 1 represents "completely disagree" and a score of 7 represents "completely agree". In this study, we used the DDQ before and after the fMRI scanning session.

*Oral Fluid Test sample:* Before each scanning session, a Six-panel multi-drug Saliva test kit (WONDFO biotech, USA) was used to screen the participants' substance use. This test kit screened for the presence of common drugs in Iran, including amphetamines, methamphetamine, methadone, morphine, benzodiazepines, and cannabis. This screening ensured that participants were not poly-drug users, enhancing the reliability of the study results.

*Cue reactivity fMRI paradigm:*
A visual fMRI cannabis cue-reactivity task was designed to examine differences in activation for cannabis vs. non-cannabis neutral images (toothbrush-related images) (Karoly et al., 2019; Vollstädt-Klein et al., 2011). Out of the 140 images (110 cannabis-related and 30 toothbrush-related) rated in the online behavioral study, 20 cannabis-related images with the highest craving scores and 20 neutral images (toothbrush-related, with the lowest craving scores) were selected for the DCR task. All images were of high resolution and scaled to similar dimensions to ensure a high-quality display in the MRI environment. After 24 seconds of resting-state, participants viewed images presented for 6 seconds in a random order, followed by a 4-second fixation period. Fixation is required to minimize the effect of previous images on the current one (Macatee et al., 2021). Subsequently, participants rated their craving for the presented image on a scale from 1 to 4 (1 = not at all to 4 = extremely) using an MRI-compatible response box placed under both hands (duration was 6 seconds). Each trial lasted 16 seconds, and the total duration of the fMRI task was 664 seconds. The fMRI cannabis cue-reactivity task design is shown in Figure 1B. Functional MRI images were collected using a Siemens MAGNETOM Prisma 3.0T scanner at the National Brain Mapping Laboratory. At first, we acquired a T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence of 4 min 12 sec (160 sagittal slices; repetition time (TR) = 1800 ms; echo time (TE) = 3.53 ms; inversion time (TI) = 1100 ms; flip angle (FA) = 7°; slice thickness = 1.0 mm; field of view (FOV) = 256 mm; voxel size = 1 × 1 × 1 mm). The T2*-weighted

gradient echo planar (EPI) sequence was acquired with 43 transversal slices oriented parallel to the AC–PC line (TR = 2000 ms; TE = 50 ms; FA = 90°; slice thickness 3.0 mm; FOV = 192 mm; voxel size = 3 × 3 × 3 mm) (TR = 2000 ms; TE = 50 ms; FA = 90°; slice thickness = 3.0 mm; FOV = 192 mm; voxel size = 3 × 3 × 3 mm).

### *2.3. Data Analysis*

To ensure that each image elicited at least moderate craving, one-sample t-test (Macatee et al., 2021) was used to compare each image's mean craving rating to 50, which represents the "moderate" point on the craving scale. Similarly, valence and arousal ratings were compared to 5, representing the "moderate" point on the valence and arousal scale, respectively. Behavioral data were analyzed using SPSS (Statistical Package of the Social Sciences, Version 29.0.2.0, SPSS, Inc., Chicago, Illinois).

The AFNI software package was used to preprocess the functional MRI data (National Institute of Mental Health, Bethesda, MD; https://afni.nimh.nih.gov/). The first three functional scans were discarded to ensure steady-state magnetization. The preprocessing pipeline includes despiking, slice-time correction, realignment, co-registration, spatial normalization to the Montreal Neurological Institute (MNI) standardized space, and spatial smoothing with a 4-mm full-width at half-maximum Gaussian kernel. Times of repetition (TRs) with motion above 3 mm were censored.

The preprocessed fMRI data were analyzed using a general linear model (GLM) created by modeling onset times for the cannabis conditions and for the neutral conditions with a 6-second boxcar function, convolved with a standard hemodynamic response function (HRF) to generate two regressors of interest. Six motion correction parameters from each subject were included in the first-level model as nuisance regressors. The differential contrasts directly comparing the cannabis with the neutral conditions were included for each subject in second-level mixed-effects models developed using AFNI's 3dMEMA. Based on Monte-Carlo simulations conducted in AFNI's 3dClustSim, all group-level results were cluster-level corrected for multiple comparisons ($P < 0.05$, cluster size > 60).

### *Group Factor Analysis*

We used group factor analysis (GFA) to investigate potential relationships between groups of variables with a sparsity constraint. GFA employs a sparse Bayesian estimation to find latent variables that either reflect a robust relationship between groups or explain away group-specific variation. Three variable groups were defined: (1) neural measures; (2) behavioral measures; and (3) demographic measures. For neural measures, cannabis minus neutral contrasts from 38 regions of interest (ROIs) (orbitofrontal cortex (47o_left, 47o_right, A11l_left, A11l_right), cingulate gyrus (A23c_left, A23c_right, A32p_left, A32p_right, A32sg_left, A32sg_right), precuneus (A31_left, A31_right, A5m_left, A5m_right, A7m_left, A7m_right, dmPOS_left, dmPOS_right), hippocampus (cHipp_left, cHipp_right, rHipp_left, rHipp_right), amygdala (lAmyg_left, lAmyg_right, mAmyg_left, mAmyg_right), basal ganglia (NAc_left, NAc_right, vCa_left, vCa_right, vmPu_left, vmPu_right, dlPu_left, dlPu_right), and insula (vIa_left, vIa_right, vIg_left, vIg_right), based on the results of meta-analysis were included as neural GFA group (Sehl et al., 2021).

The behavioral group consisted of 12 measures, including DDQ subscales (*Desire and intention, Negative reinforcement, Deficit of control*) both before and after scanning, as well as craving/thought/need self-reports before and after scanning. The demographic group comprised four measures: Age, Education, Cannabis use frequency, and Beck score. Therefore, the model included 38 ROI brain activation measures, 12 behavioral measures, and 4 demographic measures. The variables were z-normalized to have a zero mean and unit variance in order to provide a form appropriate for GFA. The GFA estimation process was repeated ten times to ensure the consistency of robust latent factors across the sample chains, minimizing the risk of identifying spurious latent factors.

We assessed potential bivariate relationships between neural and behavioral variables using Pearson's correlation tests. This served as a less reliable but complementary test for neuro-behavioral associations. Pearson's correlations and group factor analysis were conducted in statistical software R version 4.0.5. The GFA was conducted using the "gfa" function from the GFA package in R programming language.

## 3. Result

### *3.1. Preparatory Phase: Cannabis Cue Validation*

#### *3.1.1. Demographic and Cannabis Use Descriptive Data*

In the preparatory phase, 10 participants with a mean age of 19.71 years (SD = 6.8), who were regular cannabis users completed the single in-person session and rated cannabis related and neutral images (n=356). Of these, 3 participants were female and 7 were male, at the Bachelor's (n = 7) and Master's (n = 3) degree levels. The mean age of onset for cannabis use was 18.5 (SD = 2.9) years, with an average of 4.22 (SD = 3.3) years of regular cannabis use.

#### *3.1.2. Image Rating*

Table S1 shows the mean values (and standard deviations) of valence, arousal, and craving for 356 images. According to the preparatory phase, we selected the subcategories that elicited higher subjective craving scores from the participants. Out of 356 images, 140 images (110 cannabis-related cues and 30 neutral cues) were selected for the online behavioral study. The chosen images represented the top 10 highest mean craving scores for each category and demonstrated the greatest compatibility with Iranian cultural norms (those without any sexual content).

### *3.2. Behavioral Study (Cue Validation)*

#### *3.2.1. Demographic and Cannabis Use Characteristics Data*

Participants were cannabis users with a mean age of 25.9 years (SD = 4.8). Among them, 10 were female and 40 were male, distributed across Bachelor's (n = 30), Master's (n = 15), and Doctorate (n = 5) degree levels. The mean age of onset for cannabis use was 19.75 years (SD = 3.7), with an average of 4.18 years (SD = 2.8) of regular cannabis use. The demographics and cannabis use characteristics of the participants in the main study are summarized in Table 2.

**Table 2.** Participants' demographics and substance use characteristics in the behavioral phase (n = 50)

| Variables | Mean (SD)/ n (%) |
|---|---|
| Age (years) | 25.9 (4.8) |
| Sex | |
| Female | 10 (20%) |
| Male | 40 (80%) |
| Educational level | |
| Bachelor | 30 (60%) |
| Master | 15 (30%) |
| Doctorate | 5 (10%) |
| Cannabis use age of onset (years) | 19.75 (3.7) |
| Frequency of past year use of cannabis | |
| 2 times per week | 8 (16%) |
| 3-4 times per week | 9 (18%) |
| 5–6 times per week | 3 (6%) |
| Daily | 19 (38%) |
| Multiple times per day | 11 (22%) |
| Duration of regular cannabis use (in years) | 4.18 (2.8) |
| The common method of use | |
| Blunt/Joint | 50 (100%) |
| Pipe/ Bowl | 0 |
| Bong | 0 |
| DDQ Score | |
| Pre image rating | 23.71 (18.56) |
| Post image rating | 86.04 (15.31) |

*DDQ= Desire for Drug Questionnaire.*

### *3.2.2. Images Rating*

Mean values (SD) of ratings for each subcategory in terms of craving, valence, and arousal are presented in Table S2, for cannabis-related (n = 110) and control (n = 30) images. One-sample t-tests were used to compare each image's mean craving rating to 50 (moderate) in each category and subcategory (Macatee et al., 2021). The results are presented in Table 3. Figure 2 shows the five images with the highest reported craving (mean craving) from each of these subcategories.

**Table 3.** The comparison of each individual image's mean craving rating with 50 (moderate)

| Categories and subdivisions (n) | Craving (moderate=50) | | | |
|---|---|---|---|---|
| | t | df | p-value | Mean difference |
| Cannabis-related images (110) | 2.2 | 49 | 0.2 | -1.2 |
| Neutral (toothbrush) (30) | -46.18 | 49 | < 0.001 | -33.7 |
| Cannabis alone (20) | 6.66 | 49 | < 0.001 | 10.3 |
| Blunt/Joint (30) | 7.36 | 49 | < 0.001 | 6.3 |
| Pipe /Bowl (30) | -6.45 | 49 | < 0.001 | -6.7 |
| Bong (30) | -9.91 | 49 | < 0.001 | -8.61 |
| *Cannabis alone* | | | | |
| Cannabis powder (10) | 3.79 | 49 | $p = 0.004$ | 9.8 |
| Cannabis flower (10) | 5.67 | 49 | < 0.001 | 12.8 |
| *Cannabis-related paraphernalia objects* | | | | |
| Blunt/Joint objects (10) | 4.99 | 49 | $p = 0.001$ | 7.9 |
| Pipe /Bowl objects (10) | -5.88 | 49 | < 0.001 | -18.9 |
| Bong objects (10) | -3.83 | 49 | $p = 0.004$ | -11.55 |
| Toothbrush objects (10) | -21.01 | 49 | < 0.001 | -34.6 |
| *Cannabis-related paraphernalia with hands* | | | | |
| Blunt/Joint with hands (10) | 4.49 | 49 | $p = 0.002$ | 5.88 |
| Pipe /Bowl with hands (10) | -11.61 | 49 | < 0.001 | -24.1 |
| Bong with hands (10) | -16.42 | 49 | < 0.001 | -13.2 |
| *Toothbrush with hands (10)* | -32.99 | 49 | < 0.001 | -35.3 |
| *Cannabis-related paraphernalia activities with faces* | | | | |
| Blunt/Joint activities with faces (10) | 3.47 | 49 | $p = 0.007$ | 5.91 |
| Pipe /Bowl activities with faces (10) | -0.84 | 49 | 0.42 | -14.31 |
| Bong activities with faces (10) | -5.09 | 49 | 0.03 | -11.4 |
| *Toothbrush activities with faces (10)* | -32.18 | 49 | < 0.001 | -34 |

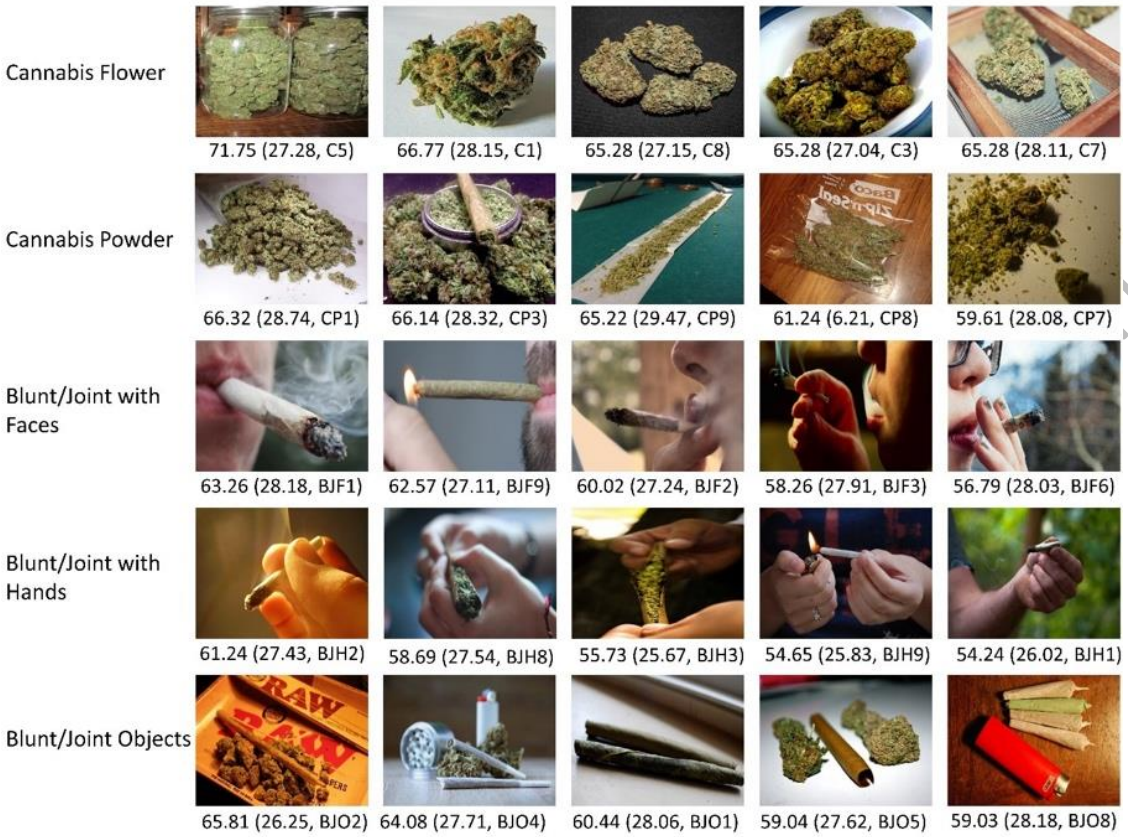


**Figure 2**. **Sample pictures from the cannabis cue database.** Note that, the images on each row represent the ones with the highest reported craving (mean (standard deviation, image number)) for each category.

Similarly, one-sample t-tests were used for each individual image's mean arousal score to 5 (moderate) within each category and subcategory (Macatee et al., 2021). The results showed that the subcategories of Cannabis powder (t = 2.62 and $p$ = 0.027), Cannabis flower (t = 4.72 and $p$ = 0.02), Blunt/Joint objects (t = 5.98 and $p < 0.001$), Blunt/Joint with hands (t = 2.4 and $p$ = 0.026), and Blunt/Joint activities with faces (t = 3.47 and $p$ = 0.006) had a mean arousal rating significantly higher than 5, indicating that all images in these categories elicited at least moderately intense arousal. The results are presented in Table 4.

**Table 4.** The comparison of each individual image's mean arousal rating with 5 (moderate)

| Categories and Subdivisions (n) | Arousal (moderate= 5) | | | |
|---|---|---|---|---|
| | t | df | p-value | Mean Difference |
| Cannabis related images (110) | -7.6 | 49 | 0.003 | -0.2 |
| Neutral (toothbrush) (30) | -44.21 | 49 | < 0.001 | -3.26 |
| Cannabis alone (20) | 4.23 | 49 | < 0.001 | 0.45 |
| Blunt/Joint (30) | 6.77 | 49 | < 0.001 | 0.75 |
| Pipe /Bowl (30) | -12.23 | 49 | < 0.001 | -1.2 |
| Bong (30) | -14.21 | 49 | < 0.001 | -1.1 |
| *Cannabis alone* | | | | |
| Cannabis powder (10) | 2.62 | 49 | 0.027 | 0.48 |
| Cannabis flower (10) | 4.72 | 49 | 0.002 | 0.42 |
| *Cannabis-related paraphernalia objects* | | | | |
| Blunt/Joint objects (10) | 5.98 | 49 | < 0.001 | 1.16 |
| Pipe /Bowl objects (10) | -5.4 | 49 | < 0.001 | -0.75 |
| Bong objects (10) | -8.19 | 49 | < 0.001 | -1.19 |
| Toothbrush objects (10) | -19.32 | 49 | < 0.001 | -3.4 |
| *Cannabis-related paraphernalia with hands* | | | | |
| Blunt / Joint with hands (10) | 2.46 | 49 | 0.026 | 0.58 |
| Pipe / Bowl with hands (10) | -8.41 | 49 | < 0.001 | -1.10 |
| Bong with hands (10) | -12.31 | 49 | < 0.001 | -1.11 |
| *Toothbrush with hands (10)* | -63.43 | 49 | < 0.001 | -3.07 |
| *Cannabis-related paraphernalia activities with faces* | | | | |
| Blunt/Joint activities with faces (10) | 3.47 | 49 | 0.006 | 0.51 |
| Pipe /Bowl activities with faces (10) | -8.4 | 49 | < 0.001 | -1.18 |
| Bong with activities faces (10) | -6.11 | 49 | < 0.001 | -1.03 |
| *Toothbrush activities with faces (10)* | -30.43 | 49 | < 0.001 | -3.12 |

Furthermore, one-sample t-tests were used for each individual image's mean valence score to 5 (moderate) in each category (Macatee et al., 2021). The results showed that the category of cannabis-related images (t = 10.3 and $p < 0.001$) and the subcategories of Cannabis powder (t = 3.31 and $p = 0.009$), Cannabis flower (t = 23.61 and $p < 0.001$), Blunt/Joint objects (t = 12.16 and $p < 0.001$), Pipe/Bowl objects (t = 3.61 and $p = 0.005$), Blunt/Joint with hands (t = 7.91 and $p < 0.001$), Bong with hands (t = 3.46 and $p = 0.007$), and Blunt/Joint activities with faces (t = 4.33 and $p = 0.002$) had a mean valence score significantly higher than 5, indicating that all images in these categories elicited at least moderately intense valence. The results are presented in Table 5.

**Table 5.** The comparison of each individual image's mean valence rating to 5 (moderate)

| Categories and Subdivisions (5) | Valence (moderate= 5) | | | |
|---|---|---|---|---|
| | **t** | **df** | **p-value** | **Mean Difference** |
| Cannabis-related images (110) | 10.3 | 49 | < 0.001 | 0.52 |
| Neutral (Toothbrush) (30) | -1.1 | 49 | 0.2 | -0.7 |
| Cannabis alone (20) | 7.92 | 49 | < 0.001 | 1.2 |
| Blunt/Joint (30) | 11.4 | 49 | < 0.001 | 0.91 |
| Pipe/Bowl (30) | 4.01 | 49 | < 0.001 | 0.34 |
| Bong (30) | 2.85 | 49 | 0.007 | 0.19 |
| *Cannabis alone* | | | | |
| Cannabis powder (10) | 3.31 | 49 | 0.009 | 0.92 |
| Cannabis flower (10) | 23.61 | 49 | < 0.001 | 1.62 |
| *Cannabis-related paraphernalia objects* | | | | |
| Blunt/Joint objects (10) | 12.16 | 49 | < 0.001 | 1.25 |
| Pip/Bowl objects (10) | 3.61 | 49 | 0.005 | 0.58 |
| Bong objects (10) | 0.78 | 49 | 0.45 | 0.12 |
| *Toothbrush objects (10)* | -0.024 | 49 | 0.98 | -0.01 |
| *Cannabis-related paraphernalia with hands* | | | | |
| Blunt/Joint with hands (10) | 7.91 | 49 | < 0.001 | 0.95 |
| Pipe/Bowl with hands (10) | 2.14 | 49 | 0.6 | 0.26 |
| Bong with hands (10) | 3.46 | 49 | 0.007 | 0.22 |
| *Toothbrush with hands (10)* | -3.66 | 49 | 0.005 | -1.07 |
| *Cannabis-related paraphernalia activities with faces* | | | | |
| Blunt/Joint activities with faces (10) | 4.33 | 49 | 0.002 | 0.65 |
| Pipe/Bowl activities with faces (10) | 1.25 | 49 | 0.23 | 0.17 |
| Bong activities with faces (10) | 1.71 | 49 | 0.10 | 0.25 |
| *Toothbrush activities with faces (10)* | 0.20 | 49 | 0.84 | 0.12 |

Additionally, independent paired-samples t-test was used to compare craving, arousal, and valence between cannabis-related images and neutral images. The results showed significant differences between all categories and subcategories with neutral images in terms of craving and arousal. The results are presented in Table 6.

**Table 6.** The comparison of craving, arousal, and valence between cannabis-related images and neutral (toothbrush) images.

| Categories and Subdivisions (n) | Craving (1-100) | Arousal (1-9) | Valence (1-9) |
|---|---|---|---|
| | t (p) | t (p) | t (p) |
| Cannabis images (110) vs. neutral (30) | 6.56 (< 0.001) | 17.46 (< 0.001) | 2.67 (0.08) |
| Cannabis (20) vs. neutral (30) | 20.9 (< 0.001) | 19.35 (< 0.001) | 3.27 (< 0.001) |
| Blunt/Joint (30) vs. neutral (30) | 26.19 (< 0.001) | 23.25 (< 0.001) | 4.22 (< 0.001) |
| Pipe/Bowl (30) vs. neutral (30) | 18.77 (< 0.001) | 19.81 (< 0.001) | 2.21 (0.32) |
| Bong (30) vs. neutral (30) | 20.40 (< 0.001) | 19.86 (< 0.001) | 1.71 (0.8) |
| Cannabis paraphernalia objects (30) vs. neutral objects (10) | 9.53 (< 0.001) | 8.28 (< 0.001) | 1.81 (0.06) |
| Blunt/Joint objects (10) vs. neutral objects (10) | 18.61 (< 0.001) | 9.9 (< 0.001) | 0.5 (0.62) |
| Pipe/Bowl objects (10) vs. neutral objects (10) | 7.91 (< 0.001) | 10.88 (< 0.001) | 0.53 (0.59) |
| Bong objects (10) vs. neutral objects (10) | 9.37 (< 0.001) | 9.71 (< 0.001) | 0.22 (0.82) |
| Cannabis paraphernalia with hands (30) vs. neutral with hands (10) | 11.18 (< 0.001) | 8.28 (< 0.001) | 6.99 (< 0.001) |
| Blunt/Joint with hands (10) vs. neutral with hands (10) | 24.08 (< 0.001) | 2.21 (0.04) | 0.33 (0.74) |
| Pipe/Bowl with hands (10) vs. neutral with hands (10) | 4.69 (< 0.001) | 6.36 (< 0.001) | 4.64 (0.001) |
| Bong with hands (10) vs. neutral with hands (10) | 19.47 (< 0.001) | 12.89 (< 0.001) | -3.32 (0.002) |
| Cannabis paraphernalia activities with faces (30) vs. neutral with faces (10) | 12.09 (< 0.001) | 8.97 (<0.001) | 0.66 (0.53) |
| Blunt/Joint activities with faces (10) vs. neutral activities with faces (10) | 19.42 (< 0.001) | 6.46 (<0.001) | 0.55 (0.4) |
| Pipe/Bowl activities with faces (10) vs. neutral activities with faces (10) | 10.31 (< 0.001) | 13.22 (<0.001) | 0.72 (0.47) |
| Bong activities with faces (10) vs. neutral activities with faces (10) | 16.69 (< 0.001) | 2.91 (0.04) | -2.05 (0.54) |

*Note: neutral images depict toothbrush, df=49*

Correlation analysis showed no significant correlations between craving and reaction time for cannabis (R = 0.09, p = 0.3; Pearson's correlation) and neutral (R = -0.36, p = 0.051; Pearson's correlation) cues (Figure 3A). These findings indicate that there is no correlation between craving scores and reaction time, meaning that higher craving scores do not necessarily accelerate decision-making or evaluation processes.

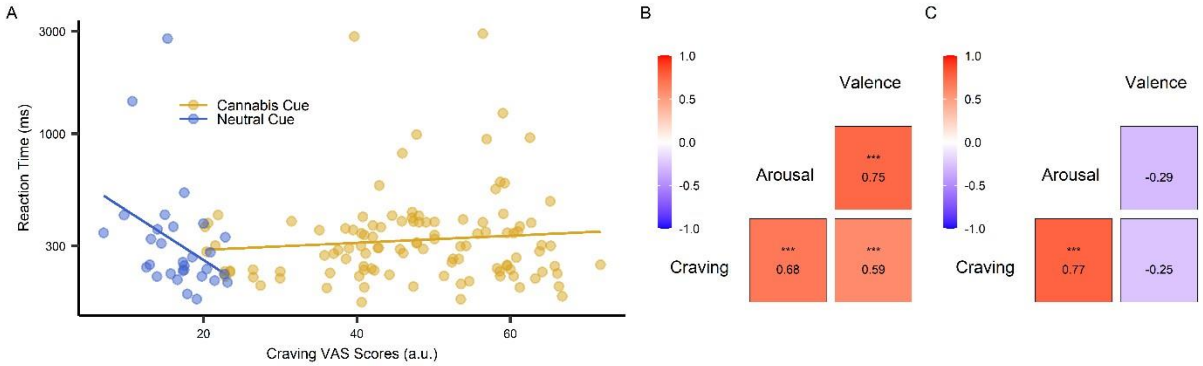


**Figure 3. Relations of behavioral responses to pictorial cannabis and neutral cues. (A)** Correlation between reaction time and craving scores. The scatterplot represents the relationship between reaction time and craving for cannabis (R = 0.09; *p* = 0.3; Pearson's correlation) and neutral (R = -0.36; *p* = 0.051; Pearson's correlation) cues. Each point presents data from the participants' average responses to each individual picture. **(B,C)** The corresponding correlation matrices between craving, valence, arousal for cannabis (B) and neutral cues (C).

Furthermore, we tested for bivariate correlations between psychological variables including craving, arousal, and valence. These tests revealed significant positive correlations between arousal and craving scores for cannabis ($r = 0.68$, $p < 0.001$; Spearman's correlation) (Figure 3B) and neutral ($r = 0.77$, $p < 0.001$; Spearman's correlation) cues (Figure 3C). Other significant correlations between valence and craving scores ($r = 0.59$, $p < 0.001$; Spearman's correlation) and between valence and arousal scores ($r = 0.75$, $p < 0.001$; Spearman's correlation) within cannabis cues (Figure 3B). Moreover, there were no significant correlations between valence and craving scores ($r = -0.25$, $p = 0.18$; Spearman's correlation) and between valence and arousal scores ($r = -0.29$, $p = 0.11$; Spearman's correlation) within neutral cues (Figure 3C). The distribution of craving in each category of pictures is shown in Figure 4. As our data did not follow a normal distribution, Spearman's rank correlation was used to test for bivariate correlations between psychological variables, including craving, arousal, and valence.

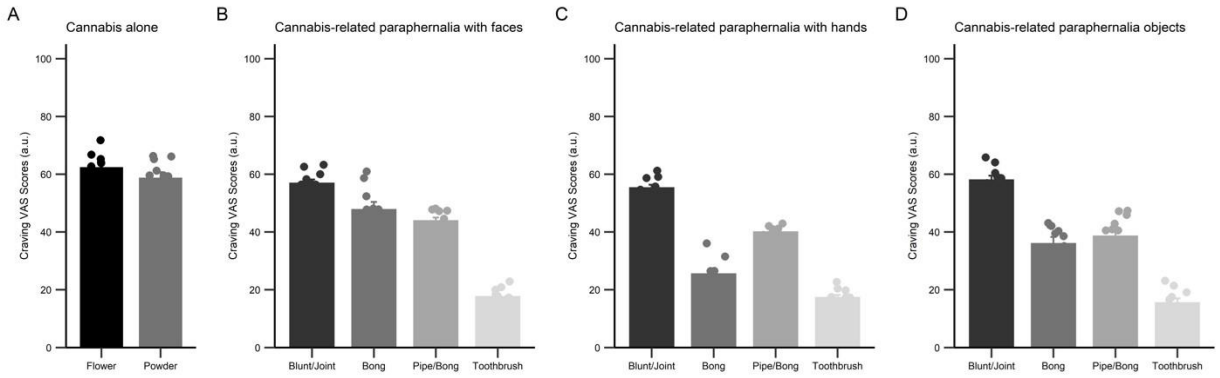


**Figure 4. Distribution of craving scores in four categories of the pictures.** Representative bar charts showing craving scores in four categories of the pictures (A) cannabis alone; (B) Cannabis-related paraphernalia with faces; (C) Cannabis-related paraphernalia with hands; and (D) Cannabis-related paraphernalia objects. Data in bar charts are represented as mean ± SEM.

### *3.3. Neural Study (Cannabis Cue Reactivity)*

#### *3.3.1. Demographic and Cannabis Use Descriptive in the Main Study*

Thirteen out of 50 participants were excluded from fMRI analyses due to positive COVID test result. In addition, four participants were excluded due to excessive motion (>3 mm), and two participants could not complete the fMRI task. The remaining sample consisted of 31 cannabis users, with a mean age of 26.1 years (SD = 3.34). Of these, 4 participants were female and 27 were male at the Bachelor's (n = 20), Master's (n = 10) and Doctorate (n=1) degree levels. The mean age of onset for cannabis use was 19.56 (SD = 3.55) years, with an average of 4.91 (SD = 2.45) years of regular cannabis use.

#### *3.3.2. Craving*

To test whether cue exposure increased participants' craving level by using on a 4-point Likert scale, a paired sample t-test was used. Our results indicated that craving after the cue exposure task significantly increased ($p < 0.001$, t= 7.61).

#### *3.3.3. fMRI Analysis*

To examine how cannabis cue-reactivity influenced the brain's circuitry, we analyzed BOLD activity measured during the cannabis cue-reactivity task at the whole-brain level using a GLM analysis (see Figure 5 and Table 7). As expected, the main effect of cue-reactivity (contrast: cannabis > neutral) was significant in several clusters. These clusters included regions in the inferior/medial frontal gyrus, fusiform gyrus, parahippocampal gyrus, orbital gyrus, postcentral gyrus, insula, precuneus, superior/middle temporal gyrus, and cerebellar tonsil (see Figure 5A). In addition, we also reported the brain activation results across the 246 subregions in the human Brainnetome Atlas (see Figure 5B).

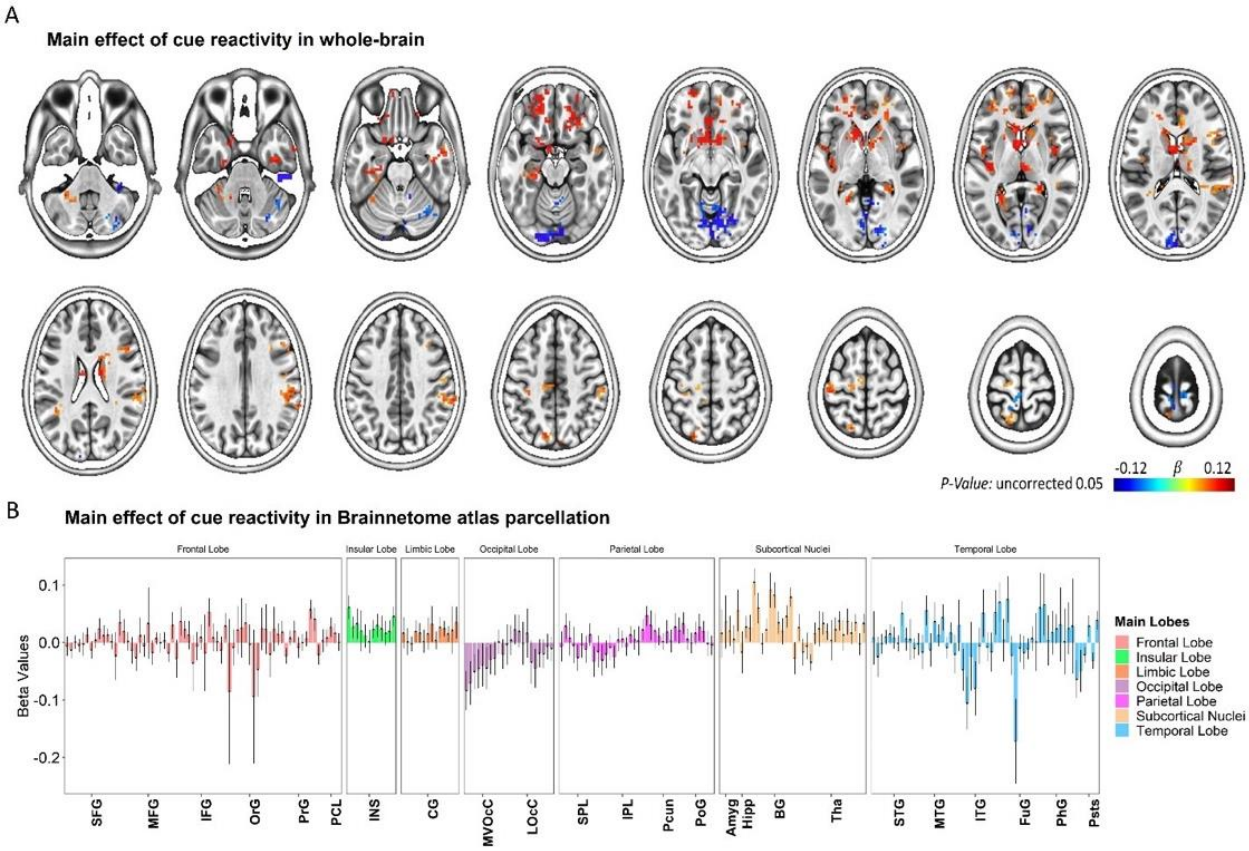


**Figure 5. Whole-brain response to the task-based fMRI in contrasts of Cannabis > Neutral. (A)** Brain activation maps and **(B)** changes in brain activation in Brainnetome (BNA) regions. Data in bar charts are represented as mean ± s.e.m. SFG, superior frontal gyrus; MFG, middle frontal gyrus; IFG: inferior frontal gyrus; OrG: orbital gyrus; PrG, precentral gyrus; PCL, paracentral lobule; STG, superior temporal gyrus; MTG, middle temporal gyrus; ITG, inferior temporal gyrus; FuG, fusiform gyrus; PhG, parahippocampal gyrus; pSTS, posterior superior temporal sulcus; SPL, superior parietal lobule; IPL, inferior parietal lobule; Pcun, precuneus; PoG, postcentral gyrus; INS, insular gyrus; CG, cingulate gyrus; MVOcC, medioventral occipital cortex; LOcC, lateral occipital cortex; Amyg, amygdala; Hipp, hippocampus; BG, basal ganglia; Tha, thalamus.

### *3.3.4. Brain-Behavior Relationships*

Two robust latent variables that collectively account for 15.12% of the variance across variable groups were found by employing group factor analysis (see Figure 6).

**Table 7.** Significant clusters for the main effects of cannabis cue reactivity in whole-brain analysis.

| Label | Side | Peak activation | | | Number of voxels | t-value |
|---|---|---|---|---|---|---|
| | | x | y | z | | |
| Inferior frontal gyrus | L | 15 | -12 | -33 | 960 | 2.55 |
| Fusiform Gyrus | L | 21 | 93 | -21 | 407 | -5.74 |
| Parahippocampal Gyrus | R | -24 | 42 | 3 | 293 | 3.81 |
| Orbital Gyrus | L | 18 | -30 | -30 | 194 | 2.48 |
| Postcentral Gyrus | L | 45 | 27 | 63 | 140 | 2.78 |
| Insula | R | -42 | 0 | 6 | 127 | 4.32 |
| Precuneus | R | -6 | 81 | 48 | 86 | 2.24 |
| Superior Temporal Gyrus | L | 48 | 3 | 3 | 84 | 4.10 |
| Parahippocampal Gyrus | L | 24 | 54 | 6 | 77 | 2.96 |
| Cerebellar Tonsil | R | -45 | 39 | -57 | 75 | -2.72 |
| Declive | R | -33 | 66 | -27 | 75 | -3.76 |
| Culmen | L | 39 | 36 | -39 | 73 | 2.17 |
| Uncus | L | 24 | 12 | -36 | 67 | 2.99 |
| Medial Frontal Gyrus | R | -3 | 30 | 84 | 63 | -3.36 |
| Middle Temporal Gyrus | R | -63 | 0 | -33 | 62 | 3.90 |

*Note. Whole-brain activations are clustered with a minimum cluster size of k = 60, which corresponds to a cluster-level alpha of p < 0.05 using NN2 clustering. L= left; R= right.*



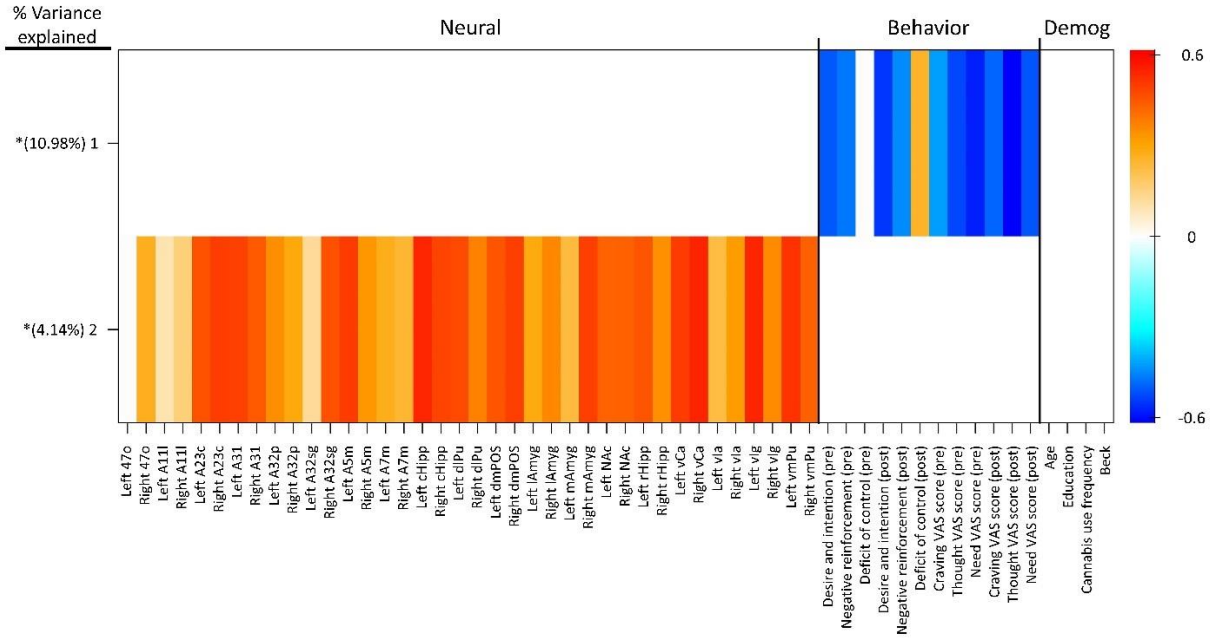


**Figure 6. GFA robust factor loadings.** Heatmap colors indicate the weight of each group variable loading. Robust group factors are sorted in descending order by mean % variance explained across all groups. Asterisks indicate group factors that contained at least one group variable loading whose 95% credible interval did not contain zero.

The mean-variance explained for the groups of behavioral and neural variables was 10.98 and 4.14%, respectively. There were no robust cross-unit latent variables identified between the neural group with behavioral and demographic groups. To put it another way, the GFA was unable to show any coherent relationship between the neural group with the behavioral and demographic groups in the latent variable space. In contrast, the significant bivariate relationships between neural and behavioral variables were found using the Pearson's correlation tests as a less reliable, complementary test for neuro-behavioral associations (Figure 7). These Pearson's correlation tests included the individual BOLD signal changes (contrast: cannabis vs. neutral) in the regions of interest and behavioral parameters (defined as changes in total DDQ score or DDQ subscales (*Desire and intention*, *Negative reinforcement*, *Deficit of control*), post-fMRI – pre-fMRI). Here, the individual BOLD signal changes in the left A7m subregion were positively and significantly with overall DDQ (R = 0.38, P = 0.034; Figure 7A) and *Deficit of control* subscale (R = 0.4; P = 0.024; Figure 7K). The individual BOLD signal changes in the right vmPu subregion were correlated with overall DDQ (R = -0.44, P = 0.014; Figure 7E), *Desire and intention* subscale (R = -0.39, P = 0.029; Figure 7G), and *Negative reinforcement* (R = -0.36, P = 0.045; 7J). The individual BOLD signal changes in the left dlPu subregion were negatively and significantly related to overall DDQ (R = -0.39; P = 0.029; Figure 7C) and *Negative reinforcement* (R = -0.44; P = 0.012; Figure 7H). The individual BOLD signal changes in the left mAmyg subregion were negatively and significantly related to overall DDQ (R = -0.46; P = 0.0098; Figure 7D) and *Negative reinforcement* (R = -0.39; P = 0.03; Figure 7I). There were other significant correlations between individual BOLD signal changes in the right cHipp subregion with overall DDQ (Figure 7B), in the right A32p subregion with *Desire and intention* subscale (Figure 7F), and in the right vIa subregion with *Deficit of control* subscale (Figure 7L).

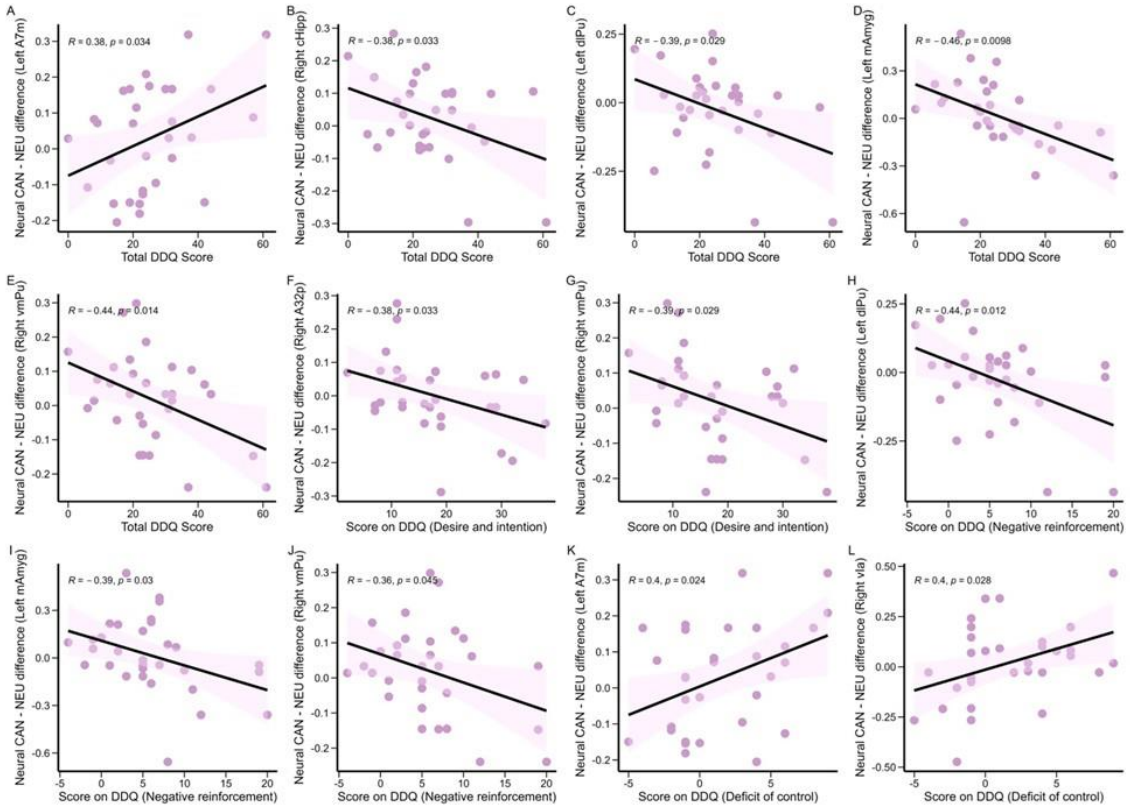


**Figure 7. Correlations between neural and behavioral findings.** Participants' total scores on the DDQ correlated with individual BOLD signal changes in the left A7m (A), right cHipp (B), left dlPu (C), left mAmyg (D), and right vmPu (E). Participants' scores on the Desire and intention subscale of the DDQ correlated with individual BOLD signal changes in the right A32p (F) and right vmPu (G). Participants' scores on the Negative reinforcement subscale of the DDQ correlated with individual BOLD signal changes in the left dlPu (H), left mAmyg (I), and right vmPu (J). Participants' scores on the Deficit of control subscale of the DDQ correlated with individual BOLD signal changes in the left A7m (K) and right vIa (L).



## 4. Discussion

Our study investigated cannabis cue reactivity in regular users using a combined behavioral and fMRI approach. We identified and validated cannabis-related images capable of inducing craving and activating reward-related brain regions. These findings contribute to the understanding of neural mechanisms underlying cannabis cue reactivity and have potential implications for treatment development. These results enhance our understanding of the neural processes that drive cannabis cue reactivity that is, the brain's response to cannabis-related cues, such as visual or sensory triggers that might prompt cravings or related responses. By identifying how specific brain regions and networks respond to these cues, this research offers insights into the biological and psychological factors that influence craving and reactivity. Such knowledge can guide the development of targeted treatments, potentially informing behavioral and pharmacological strategies to reduce cue-induced craving and help manage cannabis use disorders (Ekhtiari et al., 2020; Jafakesh et al., 2021).

### *4.1. Specificity of Cue-Elicited Responses*

Our study confirmed the potent nature of cannabis cues in triggering craving and motivational responses. Both behavioral data (significantly higher craving, arousal, and valence ratings) and fMRI data (increased activation in reward-related brain regions) provided converging evidence for cue-elicited reactivity. This aligns with previous research highlighting the ability of drug cues to elicit robust emotional and neurocognitive responses in individuals with SUDs (Ekhtiari et al., 2020; Sinha & Li, 2007; Volkow & Fowler, 2000). Notably, our study employed multiple measures to comprehensively assess cue reactivity, including subjective ratings, self-report questionnaires, and objective brain activity measures. This multimethod approach strengthens confidence in our findings and offers a more comprehensive understanding of cue reactivity compared to studies relying solely on self-report measures.

### *4.2. Decoding Reward Circuitry Activation*

Research on cue-induced brain activity in substance use disorders (SUDs) provides evidence for both activation and deactivation of brain regions during cue exposure. The present study identified activation in reward-related brain regions during exposure to cannabis cues, including the frontal gyrus, insula, and hippocampus. This aligns with current models of cue reactivity in SUDs, which posit that drug cues activate circuits associated with reward processing, memory, and salience attribution (Cousijn et al., 2013; Goldstein & Volkow, 2002; Karoly et al., 2019; Koob & Volkow, 2010). Specifically, the frontal gyrus plays a crucial role in decision-making and impulse control, the insula contributes to interoceptive awareness and craving generation, and the hippocampus mediates memory consolidation and emotional processing (Everitt & Robbins, 2016; Rolls & Grabenhorst, 2008). There is also evidence of reduced activity in areas associated with cognitive control, such as the Medial Frontal Gyrus (Dakhili et al., 2022) or Fusiform Gyrus (Pollard et al., 2023) that is involved in face recognition. This deactivation can impair the ability to resist cravings and inhibit compulsive behaviors, highlighting the dual impact of drug cues on neural circuits related to reward and self-regulation.

These findings further support the notion that cue reactivity involves coordinated engagement of multiple brain regions underlying various aspects of addictive behavior.

### *4.3. Understanding Individual Variability in Cue Reactivity and Moderating Factors*

While our study revealed overall trends in cue-elicited responses, the lack of robust relationships between neural and behavioral data in the latent variable space suggests significant individual variability in cue reactivity. This is consistent with growing evidence indicating individual differences in the neurobehavioral correlates of SUDs(Belin et al., 2008; Leggio et al., 2009). Future research should explore factors contributing to individual variability, such as genetic predispositions, personality traits, environmental influences, and individual differences in reward sensitivity. Additionally, it is crucial to explore moderating factors that might influence cue reactivity, such as current abstinence status, severity of dependence, and co-occurring psychiatric disorders (Ekhtiari et al., 2022). These investigations can further inform the development of personalized treatment approaches tailored to specific vulnerabilities and risk factors.

In studies exploring cannabis users' reactions to different types of cannabis paraphernalia, it is often observed that subjective responses to bowl and bong images are different compared to those elicited by blunt or joint images. This difference can be explained by variations in personal

experiences, social contexts, and emotional associations with these forms of cannabis consumption (Macatee et al., 2021).

Subjective responses include emotional reactions, cravings, and arousal when users view cannabis-related images. A less robust response to bowls and bongs might indicate that users have weaker emotional or psychological connections to these methods of consumption. Blunt or joint images typically elicit stronger responses, likely due to their association with more social and ritualistic settings, where cannabis is consumed in shared spaces. Blunts and joints are more often linked to collective experiences, which may heighten emotional associations, cravings, and arousal when users see these images. On the other hand, bowls and bongs are often used in more solitary settings or for more casual use, which may lead to a weaker emotional connection. This difference in context might result in lower subjective responses to these images. This finding reflects how drug-related cues can vary in their impact based on the user's personal history and the social meaning attached to different paraphernalia. In Iran, Blunts and join are more common than others for consumption cannabis use (Macatee et al., 2021).

Cannabis users often show less positive emotional reactions (valence) compared to their strong desire and physical response (craving and arousal) when viewing cannabis-related images versus neutral images. This pattern—where cannabis-related images trigger strong cravings and physical responses but a less positive emotional reaction—suggests that addiction leads to a separation between emotional enjoyment and the physiological and psychological drive to use the substance (Beraha et al., 2013).

### *4.4. Limitations and Future Directions*

We acknowledge the limitations of our study. Firstly, our participants were predominantly male, that could limit generalizability of results. Future investigations should explore potential sex differences in cue reactivity and include diverse samples considering age, ethnicity, and socioeconomic status. Secondly, relying on self-reported measures introduces potential biases. Future studies could incorporate objective physiological measures (e.g., heart rate, skin conductance), ecological momentary assessment methods (e.g., real-time craving reports), and implicit measures (e.g., implicit association tests) to enhance data sensitivity and ecological validity. Thirdly, our study lacked a control group of non-cannabis users. This limits our ability to definitively attribute the observed neural and behavioral responses to cannabis cue reactivity specifically. The observed differences could be due to pre-existing differences between cannabis users and non-users, rather than being directly caused by exposure to cannabis cues. Including a control group in future studies would allow for a more conclusive determination of the specific effects of cannabis cues on brain activity and subjective experience. Fourthly, although our study identified brain regions activated during cue exposure, further research is needed to elucidate the specific neurotransmitter pathways and cognitive processes mediating cue reactivity. This knowledge could inform the development of targeted interventions aimed at specific neural and cognitive mechanisms underlying relapse vulnerability. Sixth, we did not conduct the COVID-19 testing process for medical reasons; rather, individuals self-reported their COVID-19 status verbally.

### *4.5. Clinical Implications and Potential Interventions*

Our findings hold significant implications for the development of evidence-based interventions for CUD. The observed individual variability in cue reactivity underscores the need for personalized treatment approaches. Such approaches could involve tailored cognitive-behavioral therapy (CBT)

incorporating cue exposure therapy or mindfulness training focusing on individual vulnerabilities and reactivity patterns. Additionally, identifying specific brain regions and cognitive processes involved in cue reactivity could inform the development of targeted interventions such as:

- Neuromodulation techniques: Using transcranial magnetic stimulation (TMS) or transcranial electrical stimulation (tES) to modulate activity in specific brain regions implicated in cue reactivity.
- Pharmacological interventions: Developing medications targeting specific neurotransmitter pathways involved in reward processing and craving generation.
- Virtual reality exposure therapy: Utilizing VR technology to create immersive simulations of high-risk situations with cannabis cues, allowing individuals to practice coping skills in a safe and controlled environment.

Furthermore, understanding the triggers and mechanisms of cue reactivity can inform the development of preventative strategies, such as psychoeducational programs aimed at raising awareness about cue reactivity and teaching individuals coping skills to manage cravings in high-risk situations.

## 5. Conclusion

This study provides valuable insights into the neural and behavioral correlates of cannabis cue reactivity, as well as a pipeline for the cue validation process. We employed a multimethod approach to identify and validate cannabis cues capable of inducing craving and activating reward-related brain regions. Our findings highlight the role of individual variability and emphasize the need for personalized treatment approaches. By further exploring the specific mechanisms underlying cue reactivity and developing targeted interventions, future research can pave the way for more effective interventions and prevention strategies for CUD.

## Ethics Statement

This study was carried out in accordance with the recommendations of the ethics board of the Iran University of Medical Sciences with written informed consent from all subjects. The protocol was approved by the ethics board of the Iran University of Medical Sciences.


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## Author Contributions

The concept of the study was developed by HE and TR. All authors contributed to the study design. ZH and PGh managed data collection, analyzed data, and wrote the first draft of the manuscript. PR and PGh ran the fMRI data analyses and contributed to the fMRI data interpretations. All authors participated in the revising of the manuscript.

**Data Availability**
Publicly available datasets were analyzed in this study. This data can be found here: https://osf.io/85j6k/

**Conflict of Interest**
The authors have reported no conflict of interest.

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