Research article

Clinical utility of fMRI in evaluating of LSD effect on pain-related brain networks in healthy subjects

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ABSTRACT

Objective: We aimed to evaluate the effect of Lysergic acid diethylamide (LSD) on the pain neural network (PNN) in healthy subjects using functional magnetic resonance imaging (fMRI).

Methods: Twenty healthy volunteers participated in a balanced-order crossover study, receiving intravenous administration of LSD and placebo in two fMRI scanning sessions. Brain regions associated with pain processing were analyzed by amplitude of low-frequency fluctuation (ALFF), independent component analysis (ICA), functional connectivity and dynamic casual modeling (DCM).

Results: ALFF analysis demonstrated that LSD effectively relieves pain due to modulation in the neural network associated with pain processing. ICA analysis showed more active voxels in anterior cingulate cortex (ACC), thalamus (THL)-left, THL-right, insula cortex (IC)-right, parietal operculum (PO)-left, PO-right and frontal pole (FP)-right in the placebo session than the LSD session. There were more active voxels in FP-left and IC-left in the LSD session compared to the placebo session. Functional brain connectivity was observed between THL-left and PO-right and between PO-left with FP-left, FP-right and IC-left in the placebo session. In the LSD session, functional connectivity of PO-left with FP-left and FP-right was observed. The effective connectivity between left anterior insula cortex (lAIC)-lAIC, lAIC-dorsolateral prefrontal cortex (dlPFC) and secondary somatosensory cortex (SII)-dlPFC were significantly different. Finally, the correlation between fMRI biomarkers and clinical pain criteria was calculated.

Conclusion: This study enhances our understanding of the LSD effect on the architecture and neural behavior of pain in healthy subjects and provides great promise for future research in the field of cognitive science and pharmacology.

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1. Introduction

LSD is a well-known serotonergic hallucinogen or psychedelic drug that modulate human consciousness in a profound and novel way. It was first synthesized in 1938 and its extraordinary psychological properties were not discovered until 1943. However, increasing recreational use and its devastating impact on youth culture led to LSD became illegal in the late 1960s. As a result, studies on the effects of LSD on human cognition and perception came to a halt for half a century. Recently, there has been a resurgence of interest in the therapeutic effects of psychotropic agents (eg, psilocybin and ayahuasca) and in this regard a number of new reports on the psychological effects of LSD have also been published. On the other hand, the use of psychedelics to assist in the treatment of pain has been widely discussed, and a significant number of clinical studies over the past decade demonstrate that psychedelic drugs have exploratory value in mediating or treating chronic pain conditions. LSD has been shown to have therapeutic properties and play a role in pain management; however, how LSD treatment affects the brain network involved in pain processing is still unknown.

Modern functional neuroimaging techniques have mainly focused on the acute effects of psychedelic drugs with the aim of detecting the neural correlates of their psychedelic state [1]. On the other hand, functional neuroimaging studies have delineated brain network changes in chronic pain. Among different functional imaging techniques, fMRI scanning has been of great interest because of its high spatial resolution, ability to assess deep areas of the brain (most pain-related areas located deep in the brain), as well as remarkable anatomical resolution during brain activity mapping (3).

Recently, functional neuroimaging has provided a sensitive tool for investigation how LSD affects the brain. Previous fMRI studies have mainly focused on the effect of LSD on regional brain activity and neural substrate of emotional processing, self-processing, social cognition and concomitant subjective experience in healthy subjects (4–8). However, the LSD-induced changes in neural activity of the pain network have not yet been investigated [2,3].

Advanced data analysis includes: modelling of both functional connectivity and effective connectivity, modelling of information flow within brain networks, with the aim of identifying active brain regions that depend on inter-regional coherence or connectivity. In addition, these models can determine the interaction of intra-network regions, specific pathways in the cerebral cortex as well as the temporal dependence (causality) of regional brain activity [4]. In the current study, functional and effective connectivity modeling were used to deduce the connectivity patterns of LSD effect on brain regions that are associated with pain processing. In this regard, the effects of causality between those brain regions that have received more attention in previous research and their role in neuropsychiatric mechanisms are very common, were also investigated [5,6]. With this approach, healthy individuals were randomly selected to receive LSD and placebo, and the PNN that is thought to be primarily affected by LSD, including lAIC, thalamus, SII, ACC, and dlPFC, were investigated using advanced fMRI analysis methods.

2. Materials and methods

2.1. Patients

A total of 20 healthy volunteers participated in the study. The research design was explained in detail and participants signed written informed consent after screening for physical and mental health. Physical health screening included ECG analysis, routine blood test, urine drug test and pregnancy test. A psychiatric interview was conducted and participants presented their history of drug use. Main exclusion criteria include: age younger than 21 years old, any history of diagnosed psychiatric illnesses or a family history of psychotic disorders, previous experience with a classic psychedelic drug (e.g. LSD, mescaline, psilocybin/magic mushrooms or DMT/ ayahuasca), any use of psychedelic drug during the last 6 weeks before entering the study, pregnancy, problematic alcohol consumption (consuming more than 40 units/week), or any important medical condition that makes the participant unfit to enter the study. Data was also acquired from the Openneuro site (https://openneuro.org/datasets/ds003059/versions/1.0.0/1.0.0).

2.2. Study design

This research was approved by the National Research Ethics Service (NRES) committee London – West London and was conducted in accordance with the ethical standards of declaration of Helsinki (2000). Imperial College London sponsored the research and a Home Office license was obtained for research with schedule one drugs.

A balanced order and within subjects/cross over design was chosen to compare LSD with a carefully matched placebo. Twenty healthy volunteers participated in two scanning sessions, given at least 2 weeks apart. Sessions included an fMRI followed by a MEG scanning, each lasting 75 min. The resting-state (task free) data were acquired under eye-closed condition. LSD (75 μg in 10 mL saline) or placebo (10 mL saline) was administrated via intravenous (IV) infusion over 2 min. According to the study protocol, one day participants were given placebo and the next day LSD. The order of the conditions was balanced among the participants and the participants were blind to this order, but the investigators were not. Then, participants underwent two resting state arterial spin labeling (ASL) scans 100 min after medication administration, as the initial phase of pharmacological peak of LSD occurs at 120–150 min post infusion. In addition, for each session, two 14 min BOLD resting-state fMRI scans and two MEG resting state scans were acquired 135 min and 225 min post-infusion. Study procedures were explained in detail to the participants. Moreover, before the acquisition session, participants were screened for (psychotropic) drugs abuse and pregnancy (where relevant) by means of a urine test, as well as a breathalyzer test was performed for recent alcohol use. Also, participants in this study made visual analogue scale (VAS) ratings to rate the intensity of pain sensation using button presses and a projection screen visible from within the scanner. The 11-item altered states of consciousness (ASC) questionnaire (26) was completed at the end of each scan day. Closed visual hallucinations and other marked

changes in consciousness under LSD were reported by all participants (Fig. 1). It was observed that the intensity of the subjective effects of LSD was relatively stable for ASL and BOLD scans, while it decreased somewhat for MEG (Table 1). For more method details see SI Appendix [7].

2.3. fMRI data preprocessing

To minimize the effects of instability in the scanner magnetic moment, the first 5 frames were removed from each participant's imaging data. Then data were analyzed using the SPM 12 software package (Statistical Parametric Mapping, http://www.fil.ion.ucl.ac.uk/spm/) with the Functional Connectivity (CONN) toolbox (http://web.mit.edu/swg/software.htm) running on MATLAB (2016b). The following pre-processing steps were applied to each subject's time-series of fMRI: slice-timing, realignment, spatial co-registration, segmentation, normalization and smoothing. The time difference between slice image acquisitions was corrected by slice-timing. Subject motion in fMRI data was also corrected by realignment procedure so that the images were removed from the study with a displacement of more than 1.5 mm. In order to improve the image quality, the adverse effects of the tissues were removed during the segmentation stage. Anatomical images were co-registered on functional images and normalization was performed by transferring data to the anatomical standard MNI space. Finally, alterations in regional cerebral blood flow were estimated at the smoothing stage by applying a 6 mm wide Gaussian filter to the functional images.

2.4. Brain activity analysis

ALFF analysis determines regional brain activity by measuring changes in the intensity of blood oxygen level-dependent (BOLD) signal. The regional homogeneity (ReHo) approach explores the activated brain regions by calculating the similarity of the BOLD signal of voxels in the active region with those of its nearest neighboring regions in a voxel-wise way [8]. The CONN toolbox was used for seed-to-voxel analyses by computing the temporal low-frequency fluctuations of BOLD signals. To measure the ALFF, the BOLD signal of each brain voxel was filtered using a mid-pass or down-pass filter and its root-mean-square was calculated in CONN toolbox.

$$\text{ALFF}(\mathbf{x}) = \sqrt{\frac{1}{N} \sum \left(f(t) * S(x, t) \right)} \tag{eq 1}$$

N: number of time points, S: BOLD time series, and f: mid-pass or low-pass filter.

CONN network analysis summarizes the characteristics of voxel-to-voxel connectivity in the activated brain regions as a set of values in each voxel. For further study, See Tae et al. [9].
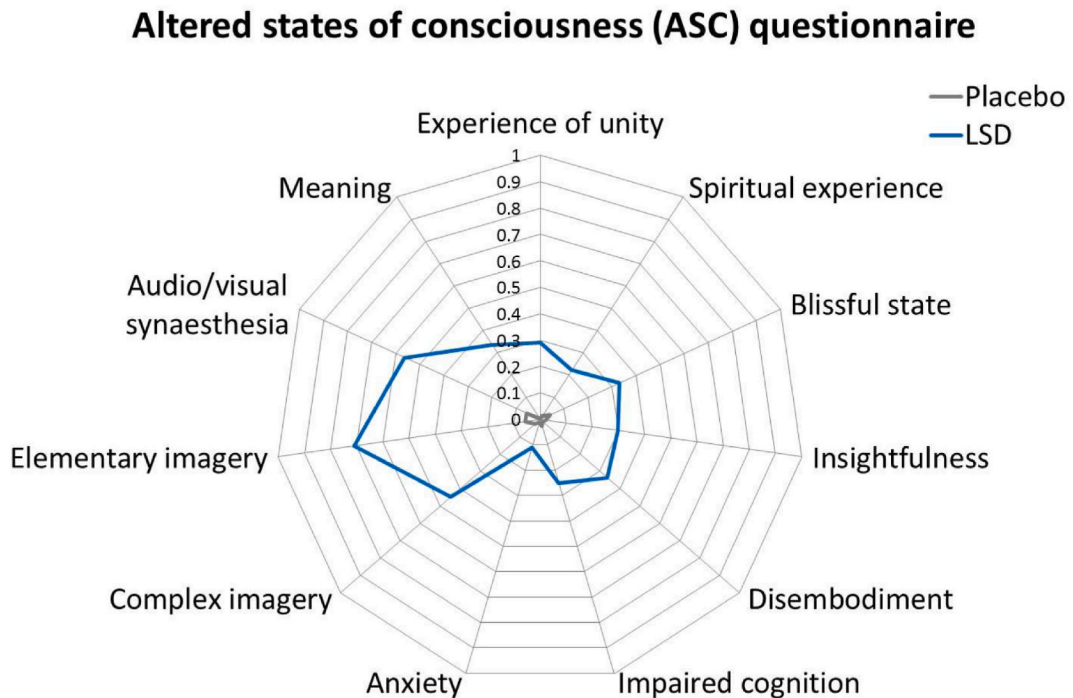

Fig. .1. The 11 ASC factors were completed at the end of the scan days and a radar plot with total mean values (0–1) was reported for the placebo (gray) and LSD (blue) conditions. It was found that under LSD, ten of the 11 factors were rated significantly higher than placebo with "anxiety" as an exception [7].

Table 1

Mean values (possible range = 0–20, steps = 1) and positive standard errors for VAS ratings at 3 different time points after LSD and placebo administration. Under LSD, all items were rated significantly higher than placebo in all 3 methods. (P < 0.05/6, Bonferonni correction, methods). EC = eyes closed [7].
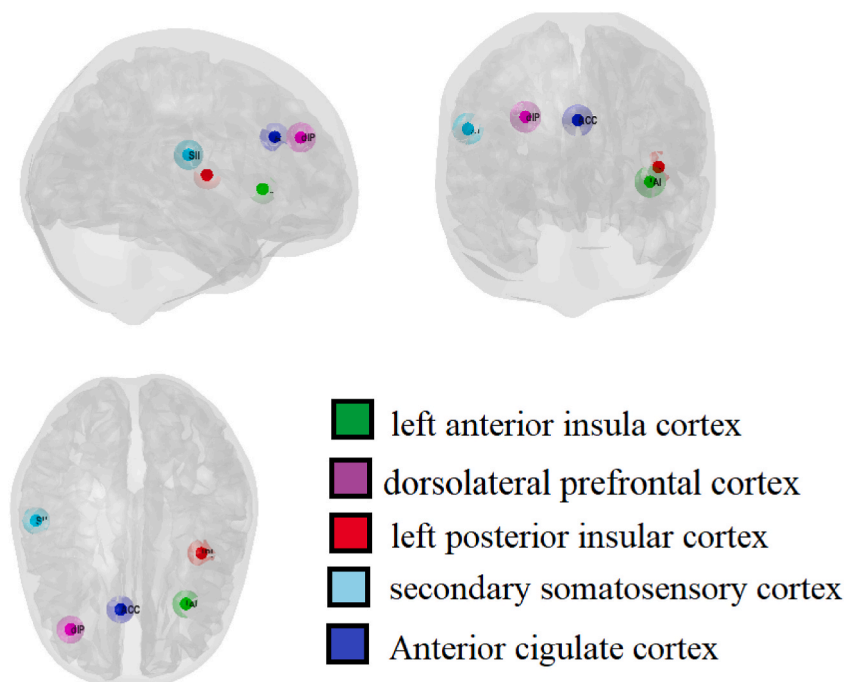
VAS Ratings		End of ASL scans (100 min post inj.)	End of BOLD scans (135 min post inj.)	End of EC MEG scans (135 min post inj.)
LSD + mean SEM)	Intensity	11.7 (0.8)	12.4 (0.9)	6.6 (0.8)
	Simple hallucinations	10.4 (1.7)	909 (1.5)	6.5 (1.3)
	Complex hallucinations	8.0 (1.6)	7.4 (1.7)	4.3 (1.1)
	Emotional arousal	1.06 (0.9)	11.2 (1.2)	6.4 (1)
	Positive mode	7.8 (1.4)	7.0 (1.5)	5.3 (1)
	Ego-dissolution	4.4 (1.3)	5.7 (1.7)	3.3 (1.1)
Placebo + mean SEM)	Intensity	0.2 (1.8)	0.2 (0.15)	0.17 (0.14)
	Simple hallucinations	0.17 (0.1)	0.17 (1.0)	0.1 (0.4)
	Complex hallucinations	0.1 (0.9)	0.03 (0.03)	0 (0)
	Emotional arousal	0.9 (0.3)	0.8 (0.3)	0.8 (0.35)
	Positive mode	0.7 (0.3)	0.3 (0.15)	0.4 (0.25)
	Ego-dissolution	0.2 (0.2)	0 (0)	0 (0)

2.5. Resting state networks (RSN) identification using ICA analysis

ICA is a data-driven, multivariate and model-free analysis that decomposes fMRI data of the whole brain into a number of statistically independent spatial components that are temporally correlated to each other. ICA is widely used in neural network analysis to investigate functional connectivity between activated brain regions and their susceptibility to a particular disease [8]. In this study, ICA calculation was performed using 64 components. Group-level spatial maps were extracted using the CONN toolbox and considering pain-related nodes including IC r, IC l, THL-right and THL-left, FP r, FP l, ACC, PO r, PO l.

2.6. ROI-to-ROI connectivity of pain neural network

Functional connectivity considers the brain as a complex network consisting of a set of nodes and edges to study the topological organization of its neural networks. Using mRRC method, the general and regional characteristics of the brain can be evaluated (12). At this stage, functional analysis was performed using the ROI-to-ROI function in the CONN toolbox. For two-way ROI-to-ROI analysis, we

- left anterior insula cortex
- dorsolateral prefrontal cortex
- left posterior insular cortex
- secondary somatosensory cortex
- Anterior cigulate cortex

Fig. .2. Selected brain regions associated with the PNN.

performed p-false discovery rate (FDR) estimation and permutation test (10,000 permutations) for IC r, IC l, FP r, FP l, ACC, PO r, PO l, and left/right thalamus. General and regional brain characteristics for different ROIs were obtained using functional connectivity analysis (mRRC) and calculation of correlation coefficients and their FDR-corrected values at a significant level of P ≤ 0.05.

2.7. Analysis of effective connectivity between brain regions using DCM

DCM is a computational approach used in neuroimaging, particularly in fMRI studies, to investigate the effective connectivity among brain regions [10–12]. It employs a Bayesian framework to quantify uncertainty, incorporate prior knowledge, make statistical inferences about the underlying neural dynamics, and estimate the most likely neural network model based on observed fMRI data [13]. DCM allows researchers to infer the causal relationships between brain regions and how these connections change over time in response to stimuli or tasks. By specifying a set of plausible neural models and comparing them against the fMRI data, DCM can help researchers identify the most likely network architecture and how it is modulated by experimental manipulations [14,15]. In this study, DCM analysis was used to determine significant changes in effective connectivity between activated brain regions. The DCM method involves selecting a set of regions, extracting related time series, model selection and estimation, as well as session analysis, which is outlined below.

2.7.1. Region selection and related time series extraction

In this step, specific brain regions of interest (ROIs) are selected based on previous studies and the opinions of neuroscientists about the neural network involved in pain processing. These regions serve as nodes in the connectivity model. Time series data are then extracted from these regions by averaging the fMRI signal across voxels within each region. These time series show LSD-induced neural activity in selected regions over time. The extracted time series are used as input data for the DCM analysis, where the effective connectivity between regions is estimated [16,17]. For this purpose, the ROIs of ACC, SII, dlPFC, lAIC, and left posterior insular cortex (lPIC), which are associated with pain processing, were selected according to Fig. 2 and Table 2. The time series of ROIs were also extracted using ALFF analysis.

2.7.2. DCM model determination

Effective connectivity analysis was performed using the method proposed by Friston. Friston's DCM method involves specifying a set of brain regions or nodes, defining the connections between them, and estimating the strength and direction of these connections [10]. Whit this approach, we tried to identify the best DCM model by considering cognitive science information, neurological assumptions, and Bayesian framework. Connectivity graphs in brain regions associated with the pain processing was generated using the selective model in Fig. 3.

2.7.3. DCM model estimation and session analysis

Model estimation was used to fit the selected neural network model to the fMRI data to estimate the parameters governing the effective connectivity between regions involved in pain processing. This process uses Bayesian inference to optimize the model parameters and generate estimates of how neural activity is influenced by connections within the network [18]. Also, the model estimated for several fMRI sessions was analyzed to investigate how the connectivity patterns changed after LSD administration [19]. In this regard, DCM model was estimated for all fMRI-data and effective connectivity between activated regions was extracted for all subjects. False Discovery Rate (FDR) method with a statistically significant value of P = 0.05 was used for session analysis. Correlating parameters were compared between the two sessions by Adopted Bayesian Parameter Averaging (BPA) method.

2.8. Determine the differences between brain effective connectivity using statistical analysis

All statistical procedures were conducted using SPPS software for Windows. Numerical values were examined for normal distribution using the Kolmogorov-Smirnov test at the significant level of α = 0.05. For values with normal distribution, *t*-test and for values with non-normal distribution, Man-Whitney test was used to compare the relationship between several values. (α = 0.05). The boxplots were drawn to evaluate the differences in the effective connectivity of activated brain regions between placebo and LSD sessions.

Table 2
Coordinates of the selected brain regions.

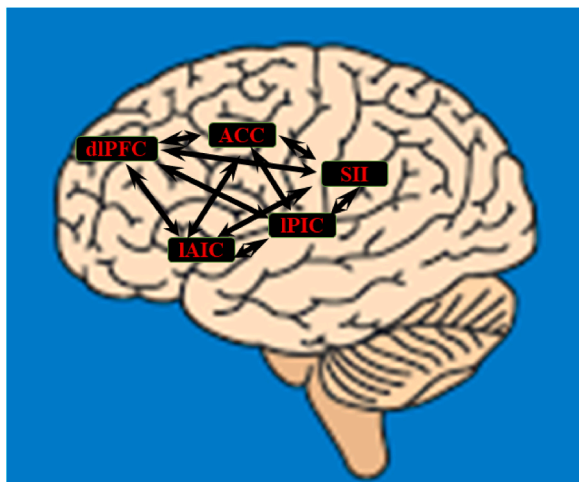| MNI Coordinates			
ROI	X	Y	Z
ACC	7	27	29
SII	56	−22	24
dlPFC	35	39	31
lAIC	−30	23	−2
lPIC	−38	−6	5

Fig. 3. Selective model for evaluating effective connectivity of the activated brain regions at resting state fMRI.

3. Results

3.1. ALFF analysis

Brain activation patterns in the PNN for the resting state were generated using ALFF analysis. Regional activities of the placebo and LSD sessions were shown in Figs. 4 and 5, respectively. Bar graphs display the mean and standard error of the activation in the corresponding brain regions. Fig. 6 shows the brain-activated regions caused by placebo > LSD state.

The number of activated voxels in placebo and LSD with a focus on the brain regions associated with pain processing is shown in Table 3.

As shown in Table 3, based on the ALFF analysis, the placebo session showed more activated voxels than the LSD session in all brain regions associated with pain sensation and processing. Moreover, in FP r, FP l and ACC regions, 42, 31 and 559 active voxels were obtained, respectively (P < 0.05).

RSN Identification using ICA Analysis.

The results of the ICA analysis for the placebo and LSD as well as the placebo > LSD mode in brain regions associated with pain processing were shown in Fig. 7 (a - i), 8(a - i), 9(a - i), and Table 4. The study regions were selected according to a neuroscientist. Additionally, brain activation maps associated with the PNN in the placebo and LSD sessions as well as the placebo > LSD mode were shown in Figs. 6–8 (P < 0.05) (see Fig. 9).

The statistical characteristics of brain regions associated with pain processing using ICA analysis in placebo, LSD, and placebo > LSD mode is given in Table 4.

According to Table 4, the number of activated regions in brain regions of ACC, thalamus left, thalamus right, IC r, PO l, PO r and FP r, in the placebo session is higher than in the LSD session. On the other hand, the number of activated voxels in brain regions of FP l and IC l, in the LSD session is more than the placebo session.

3.2. Functional connectivity analysis

The functional connectivity patterns determined based on Multivariate ROI-to-ROI Connectivity (mRRC) analysis in the PNN for the placebo and LSD sessions are shown in Figs. 10 and 11, and Table 5, respectively. In the placebo session, the left thalamus is functionally connected to the PO. In addition, the PO l is functionally connected to the set of regions FP l, FP r, and IC l (Fig. 10). In the LSD session, the PO l is functionally connected to the FP l and FP r (Fig. 11).

3.3. DCM analysis

DCM analysis was performed to compare the values of connectivity parameters between placebo and LSD sessions. Between-session comparisons of effective connectivity strength were performed using two-sample t-tests and the post hoc Mann-Whitney *U* test. As shown in Fig. 12, the effective connectivity between lAIC- lAIC, lAIC -dlPFC, and SII-dlPFC were significantly different (P < 0.05) (Table 6).

4. Discussion

In this study, we evaluated the effect of LSD, a potent psychedelic drug, and a placebo on changes in the function of the brain
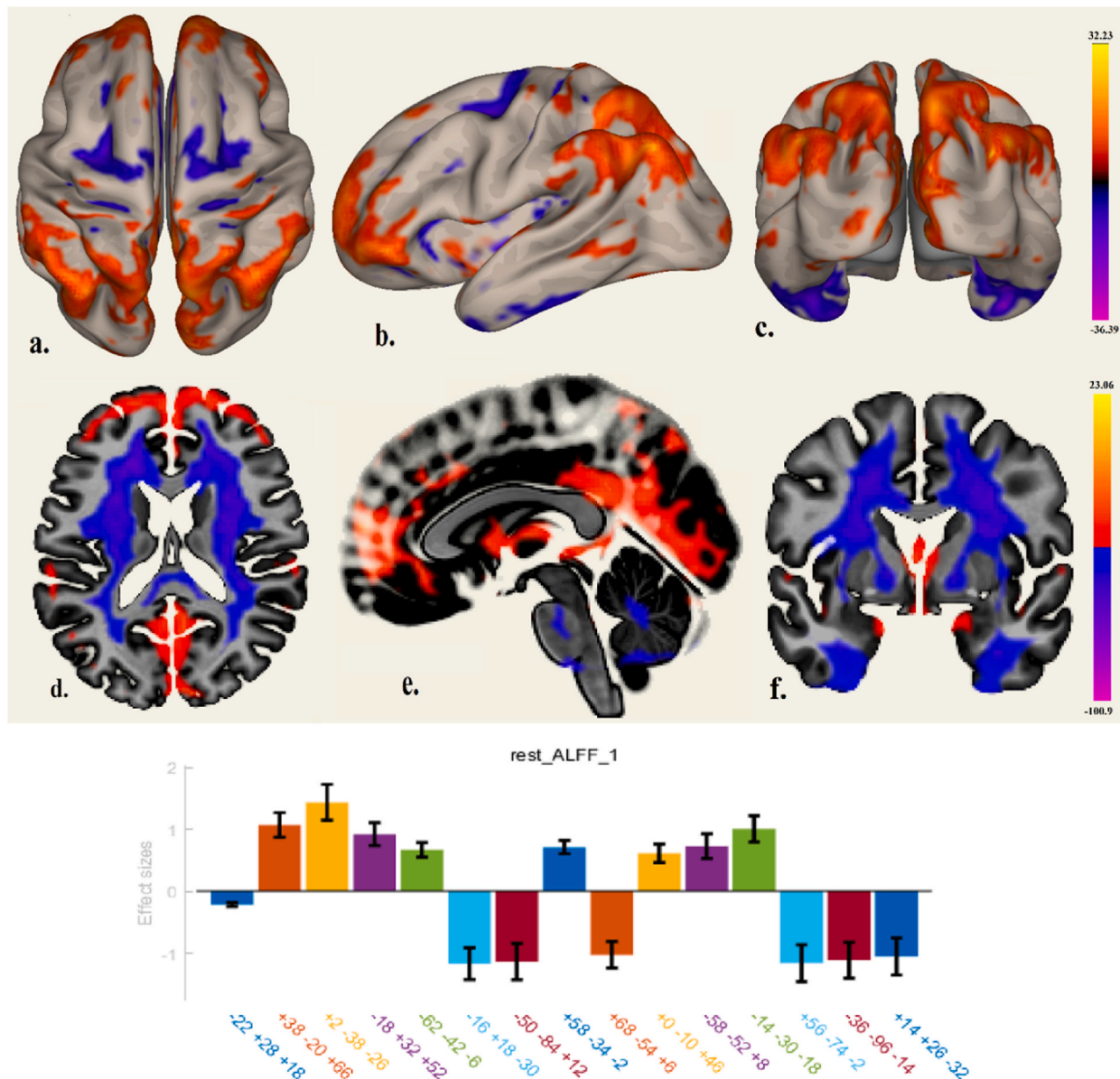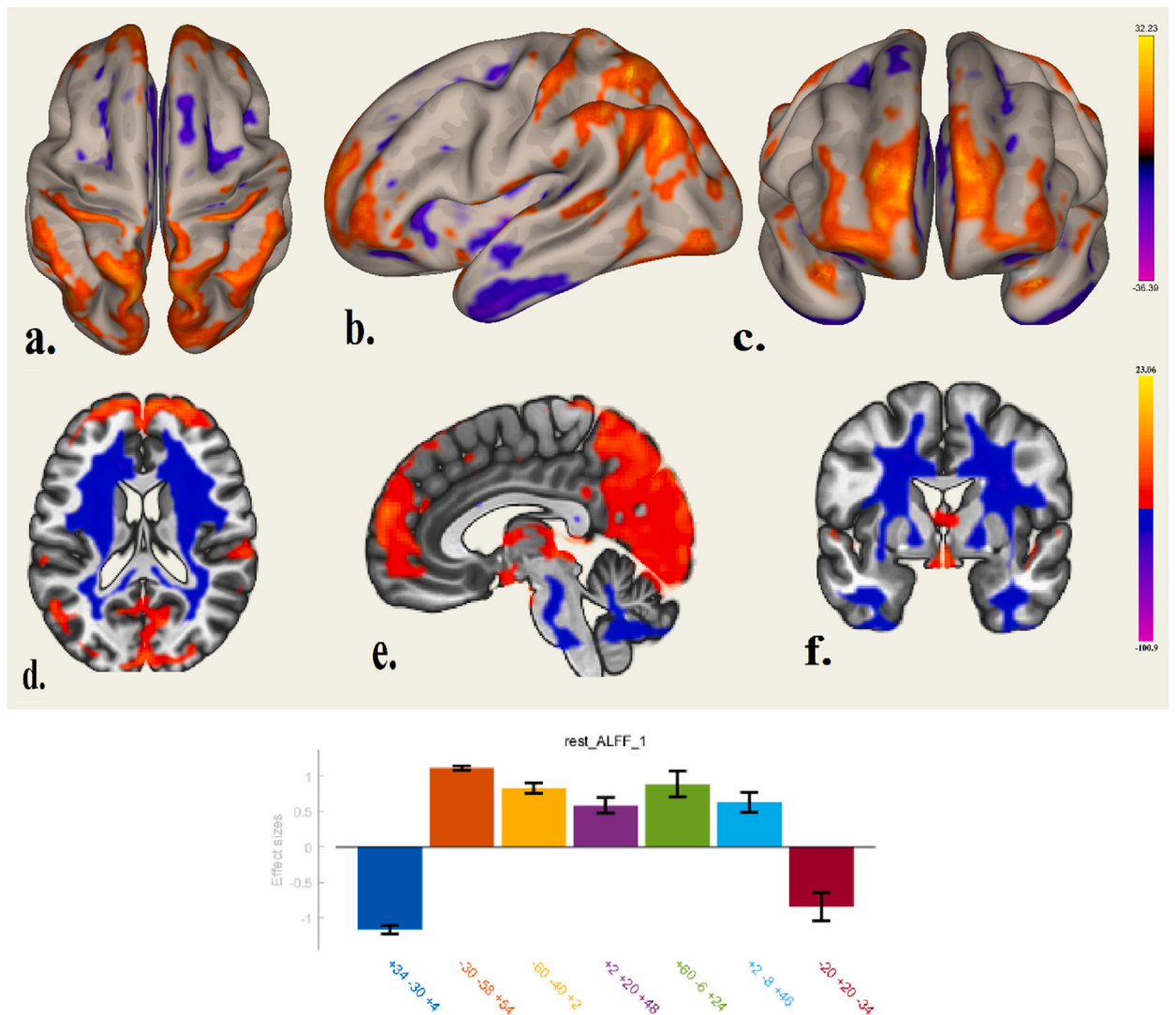
Fig. .4. ALFF analysis in the placebo session. Orange indicates a significant increase and blue indicates a significant decrease in the activity of brain regions associated with pain processing compared to baseline. The bar graphs show the signal intensity values in brain activated regions.

regions associated with the pain perception and processing in healthy subjects. To the best of our knowledge, we are the first who conduct a comprehensive study and analysis based on fMRI biomarkers to investigate the LSD effect on the pain neuro circuitry.

For this purpose, functional connectivity, ALFF, ICA, and DCM analysis were used and the findings provide detailed information on the brain activity and neural connectivity patterns, improving our understanding of LSD effects on architecture and underlying neuronal processes of pain in healthy participants.

Like other sensory modalities, signals from painful stimuli are transmitted to the thalamus via the spinal cord and brainstem and are eventually distributed to the various regions of the brain, including the cortex, sub cortex, and limbic system. The communication between brain regions is first revealed by changes in neural activity and functional networks, such as salience network (SN), default mode network (DMN), and central executive network (CEN) It is then followed by processing other aspects of pain including: sensory signals, sensory perception, cognitive and emotional reactions. As a result of stimulating these different pain pathways in the brain, an appropriate response to the pain stimulus is produced [20].

Previous studies have shown how multiple brain regions are involved in pain processing, connectivity and functional changes. However, there are still many uncertainties about the brain functions in processing and controlling pain, and their identification requires further studies. A number of studies have analyzed PNN, analgesic methods and their mechanism of action and reported that
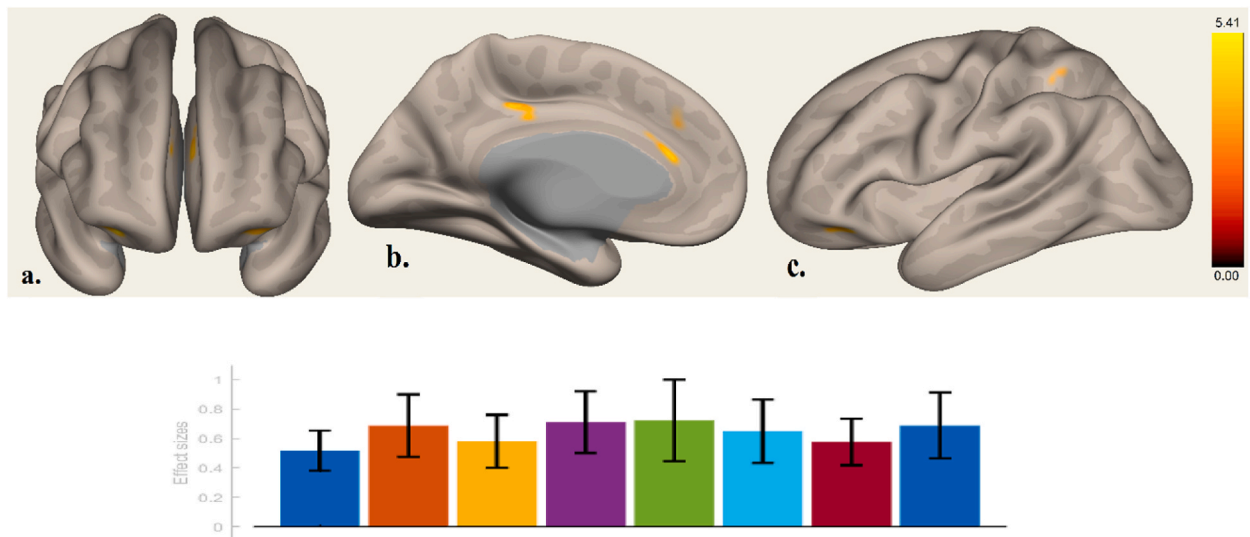
Fig. .5. ALFF analysis in the LSD session. Orange indicates a significant increase and blue indicates a significant decrease in the activity of brain regions associated with pain processing compared to baseline. The bar graphs show the signal intensity values in brain activated regions.

pain leads to patient disability by reducing the volume and activity of related areas in the brain. It has also been demonstrated that the use of analgesic drugs with simultaneous psychoactive and sedative effects (such as Morphine) reverses the changes caused by pain and can improve the performance of patients [21,22]. fMRI results showed that the thalamus is involved in processing sensory stimuli by sending pain signals to different areas of the brain. The ACC and dlPFC regions were found to play a significant role in emotional and cognitive processing of sensory stimuli, integrating this information and generating appropriate responses. They also affect the endogenous analgesic pathway. On the other hand, the results confirmed the more important role of the insula and PO region in processing emotional reactions to pain stimuli due to their close communication with each other. In addition, the FP region is mainly involved in modulating attention to different stimuli and targets and comparing them with each other, and selecting the appropriate responses [5,6,20,23–25].

In the present study, it was found that in both LSD and placebo sessions, spontaneous activity increases in all brain regions which is consistent with other similar studies of analgesic methods [26,27]. This increase in spontaneous activity may not be detected due to the lower spatial resolution of the EEG modality, whereas it is observed in fMRI imaging, although further studies are still needed to prove this issue.

However, ICA analysis indicated that in the left IC and left FP, the increase in activity in the LSD session was greater than in the placebo session, and in other brain regions, in the placebo session was greater than the LSD session [28]. Considering the role of the mentioned regions in information processing, it seems that LSD has a more effective performance in controlling emotions caused by pain and improving the individual's attention to different targets, as well as selecting appropriate response and making better decisions. Furthermore, given the role of the ACC and IC regions in the analgesic effects of the endogenous path through PFC to PAG, it is

Fig. .6. ALFF analysis in the placebo > LSD state. Orange indicates a significant increase and blue indicates a significant decrease in the activity of brain regions associated with pain processing compared to baseline. The bar graphs show the signal intensity values in brain activated regions.

Table 3
Number of activated voxels in the brain regions associated with pain processing.

Region	Placebo number of voxels (0.001)	LSD number of voxels (0.001)
FP r	4297	3541
FP l	2817	2672
ACC	682	392
Thalamus r	651	388
Thalamus l	547	419
IC r	289	202
IC l	235	114
PO r	196	145
PO l	116	64

concluded that increased activity of these brain regions due to the effect of LSD and placebo can reduce pain by enhancing the effects of endogenous analgesia. These findings are consistent with studies done in pain processing of chronic arthritis [26].

Increased neural activity in the thalamus due to the effects of LSD and placebo can also promote the information transfer rate between brain regions and improve its activity and function.

Some studies have shown that the increased PO activity along with decreased connectivity with the insula can relieve neuro-behavioral excitability and pain-induced discomfort [23]. Therefore, the increased activity observed in the PO region affected by LSD and placebo may be effective in preventing such reaction. Functional connectivity analysis of active brain regions in the placebo session has shown a connection between the left PO and the left IC.

Due to the greater activity of the left PO and the lower activity of the left IC in the placebo session compared to the LSD session, this connection seems to be negative, making both the left PO and the left IC less capable in processing and generating emotional responses. Therefore, in line with other studies, it can probably lead to a reduction in pain sensation [29].

Other studies have reported that attention to pain increases the perception of pain, and thus its lack of attention is important in assessing the placebo effect [6]. In the present study, due to the connections between left PO and left and right FPs observed in the placebo session, the placebo analgesic effect is likely to be associated with changes in attention. However, given that placebo is more effective on increasing the activity of left PO and right FP and less effective on the activity of left FP, it seems that increased activity in left PO is directly related to right FP and inversely related to left FP, and therefore right and left FPs play different roles in distributing attention to different stimuli and targets. Of course, these issues need to be further explored. It has also been shown that under the better effect of placebo on the activity of both left and right POs, the left thalamus has a direct connection with the right PO and therefore pain sensation reduces in the placebo session.

In addition, functional connectivity analysis in the LSD session showed connections between the left PO and both the left and right FPs. Compared with placebo, LSD does not significantly increase left PO and right FP activities, while increasing left FP activity. Consequently, less activity of left PO is inversely related to left FP and has an inhibitory effect, whereas its effect on right FP is probably stimulatory. These communications can also indicate the different roles of left and right FPs in controlling and distributing attention to different targets.
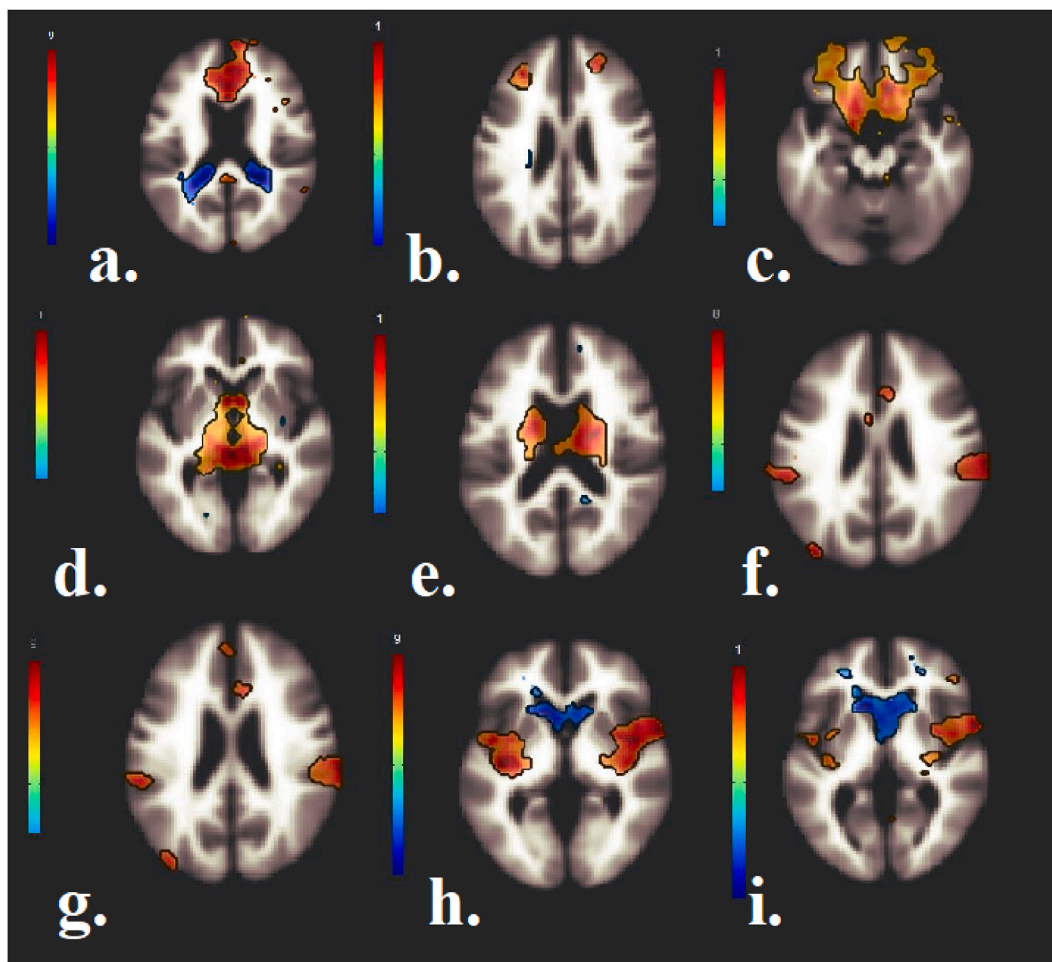
Fig. 7. Each frame shows activation maps in one of the target brain regions in the placebo session. The red color spectrum indicates an increase in activity and the blue color spectrum indicates a decrease in activity compared to the baseline state. a) ACC, b) FP l, c) FP r, d) THL-left, e) THL-right, f) Po l, g) Po r, h) IC l and I) IC r.

Table 4
Results of ICA analysis in brain regions associated with pain processing.

| Region	Coordinate	Session	Voxel	State	P
ACC	−12, 44, 8	Placebo	909	7.33917	0.000044
		LSD	92	5.65848	0.0000310
Thalamus left	−12, −22, 0	Placebo	1220	6.92694	0.000069
		LSD	897	7.78028	0.000028
Thalamus right	8, −4, 0	Placebo	1097	7.13566	0.000055
		LSD	933	5.37223	0.000449
IC left	−30, 24, 0	Placebo	428	9.555368	0.000005
		LSD	448	−10.0777	0.000003
IC r	36, 12, −2	Placebo	600	8.37917	0.000015
		LSD	439	−7.02365	0.000062
PO l	−50, −38, 26	Placebo	203	6.77817	0.000081
		LSD	150	−5.39501	0.000436
PO r	42, −32, 24	Placebo	203	5.67743	0.000303
		LSD	43	8.29944	0.000016
FP l	−18, 42, −12	Placebo	100	6.07597	0.000185
		LSD	257	5.71824	0.000288
FP r	26, 40, −16	Placebo	810	6.00687	0.000201
		LSD	821	5.27491	0.000511
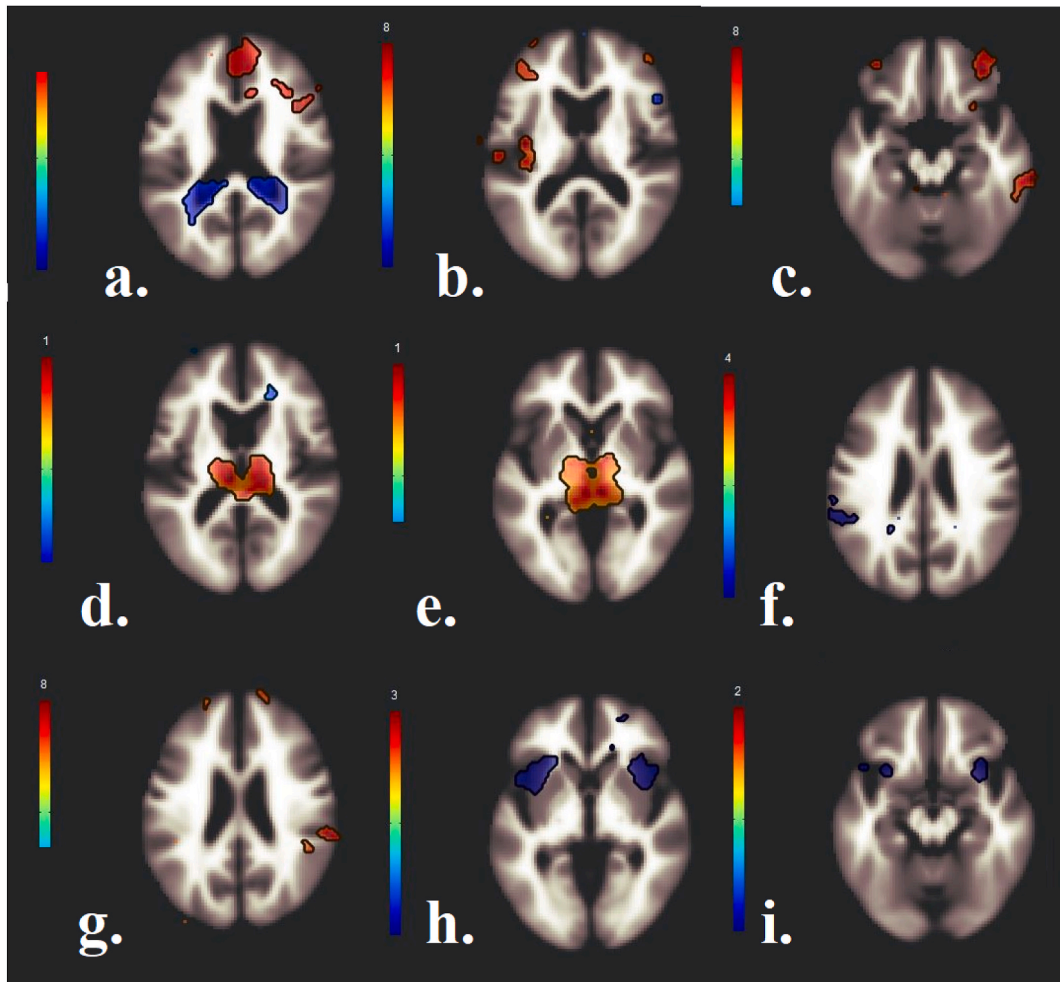
Fig. .8. Each frame shows activation maps in one of the target brain regions in the LSD session. The red color spectrum indicates an increase in activity and the blue color spectrum indicates a decrease in activity compared to the baseline state. a) ACC, b) FP l, c) FP r, d) THL-left, e) THL-right, f) Po l, g) Po r, h) IC l and I) IC r.

The results of DCM analysis showed that placebo significantly increased the lAIC-lAI effective connectivity compared to LSD. On the other hand, placebo significantly reduced the effective connectivity of lAIC-dlPFC and SII–SII compared to LSD. Since the insula is involved in the development of emotional responses, this result may indicate that LSD is not very effective in controlling emotional effects.

There was no change in effective connectivity of lAIC-dlPFC in the LSD session, whereas a negative connectivity was observed in the placebo session, indicating a reduction in the transmission of emotional information from the insula to the dlPFC as well as a reduction in stimulus-induced emotional processing in subjects. On the other hand, the negative effective connectivity of SII-dlPFC obtained in the placebo session could indicate a decrease in both information transfer from SII to dlPFC and processing of sensory stimuli. Also in the LSD session, a positive effective connectivity was found between SII and dlPFC, probably indicating an improvement in information processing of the nature of the stimulus under the influence of LSD.

In comparison with other psychedelics such as psilocybin and MDMA (3, 4-methylenedioxymethamphetamine), studies have shown both psilocybin and LSD impact brain connectivity and dynamics, with psilocybin showing significant increases in network global signal complexity and persistent alterations in hippocampal-DMN connectivity, while LSD induces changes in visual cortex activity, resting-state connectivity, and brain state dynamics associated with visual hallucinations and ego-dissolution [30,31]. Overall, both psilocybin and LSD exhibit unique but overlapping effects on brain function and connectivity, contributing to altered states of consciousness and perception [32,32].

In contrast, MDMA enhances emotional empathy and prosocial behavior, impacting brain regions associated with empathy and emotional processing [33].Comparing the neurological changes during LSD and MDMA use, LSD induces marked alterations in waking consciousness, leading to changes in thalamocortical connectivity, visual cortex activity, and network dynamics associated with visual hallucinations and ego-dissolution. While both LSD and MDMA have psychoactive effects, their specific neurological changes and
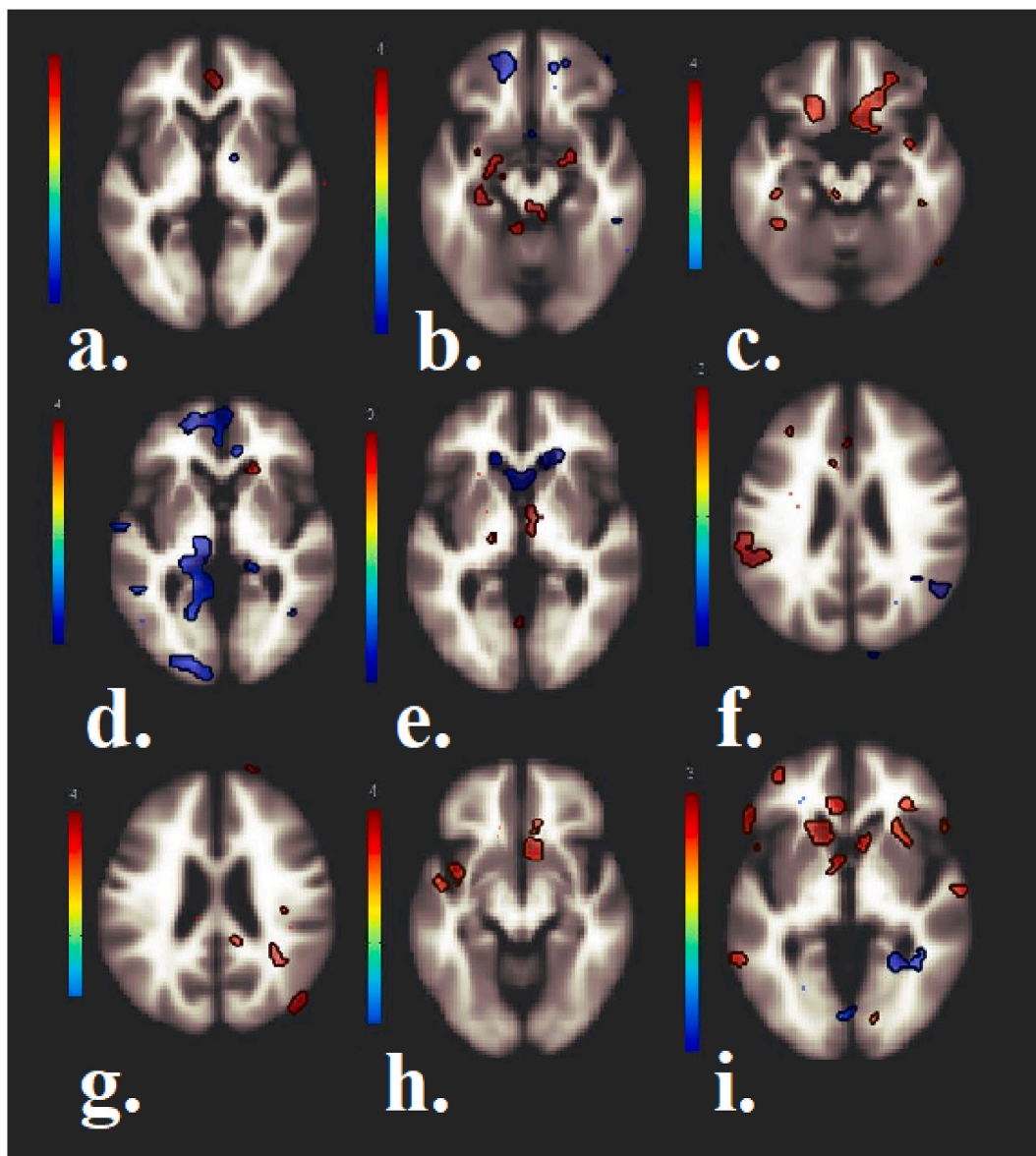
Fig. 9. Each frame shows activation maps in one of the target brain regions in the placebo session compared to the LSD session. The red color spectrum indicates an increase in activity and the blue color spectrum indicates a decrease in activity compared to the baseline state. a) ACC, b) FP l, c) FP r, d) THL-left, e) THL-right, f) Po l, g) Po r, h) IC l and I) IC r.

mechanisms of action differ, with LSD primarily affecting brain connectivity and visual processing, while MDMA influences emotional processing and empathy-related brain regions [7,32,34]. In terms of therapeutic potential, psilocybin has shown promise in treating depression and anxiety in cancer patients. LSD and MDMA have also demonstrated therapeutic effects for various mood disorders [35]. However, more research is needed to fully understand the therapeutic mechanisms and compare the efficacy of different psychedelics.

In the present study, it was shown that both LSD and placebo increase the activity of different areas of the brain, and help to relieve pain by enhancing the analgesic effects of endogenous pathways The difference in the effect of LSD and placebo on both the activity and connectivity of different brain regions shows that LSD is more effective in improving behaviors and cognitive responses to stimuli by better controlling pain-related emotions. Moreover, LSD compared to placebo, can improve the performance of healthy subjects.

5. Conclusion

In some recent studies, fMRI technique has been used to evaluate the effects of LSD on regional brain activity in healthy subjects. In this research, ALFF, ICA, DCM and functional connectivity analysis were used to assess the effects of LSD on healthy subjects to provide a powerful tool for future clinical applications by identifying neural connectivity patterns in the activated pain network of cerebral
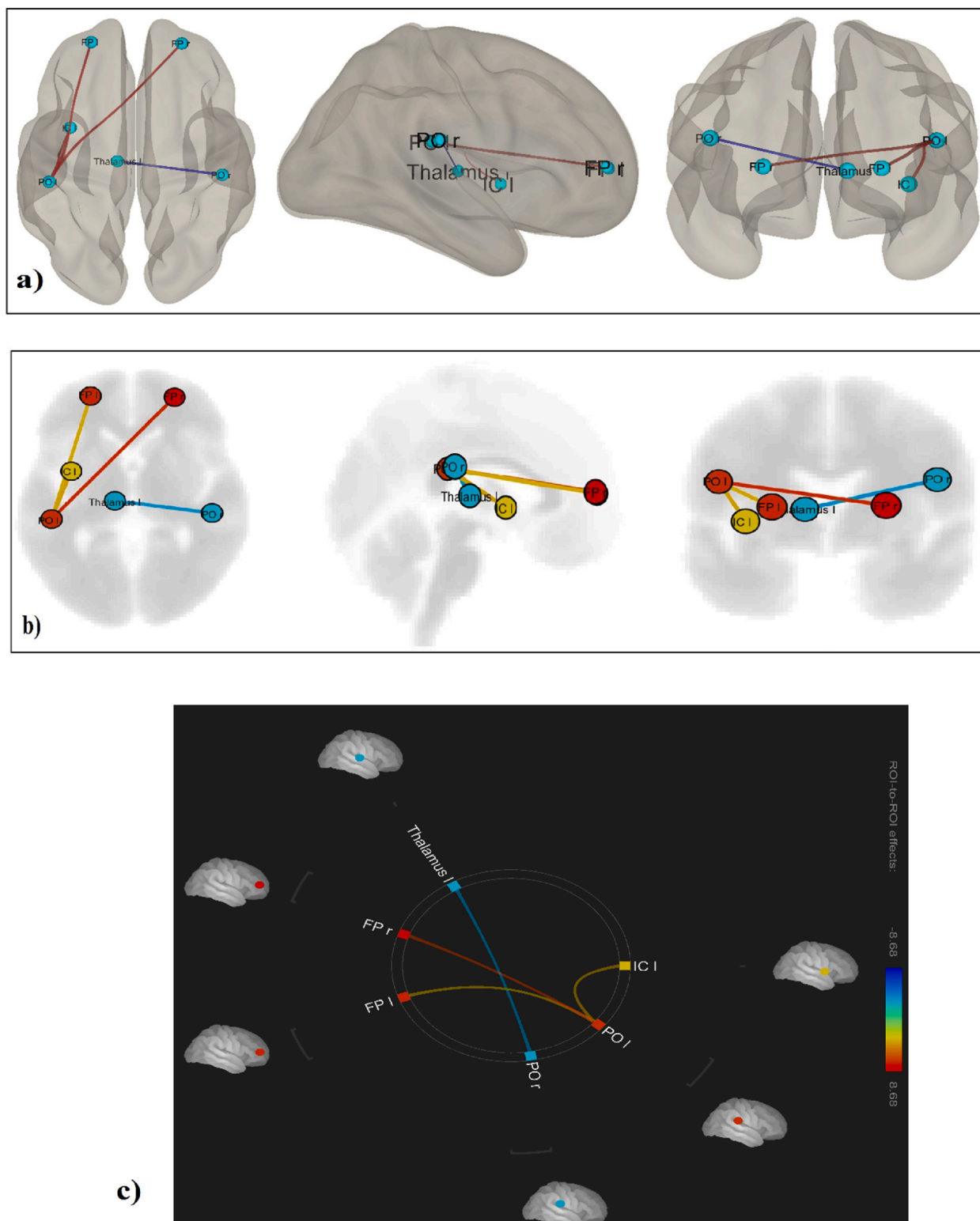
Fig. 10. Anatomical presentation of functional connectivity within the pain network in the placebo session (a) Three-dimensional presentation of functional connectivity in three anatomical views, (b) Two-dimensional presentation of functional connectivity in three anatomical views and (c) Presentation of ROI-to-ROI functional connectivity patterns within the pain network.
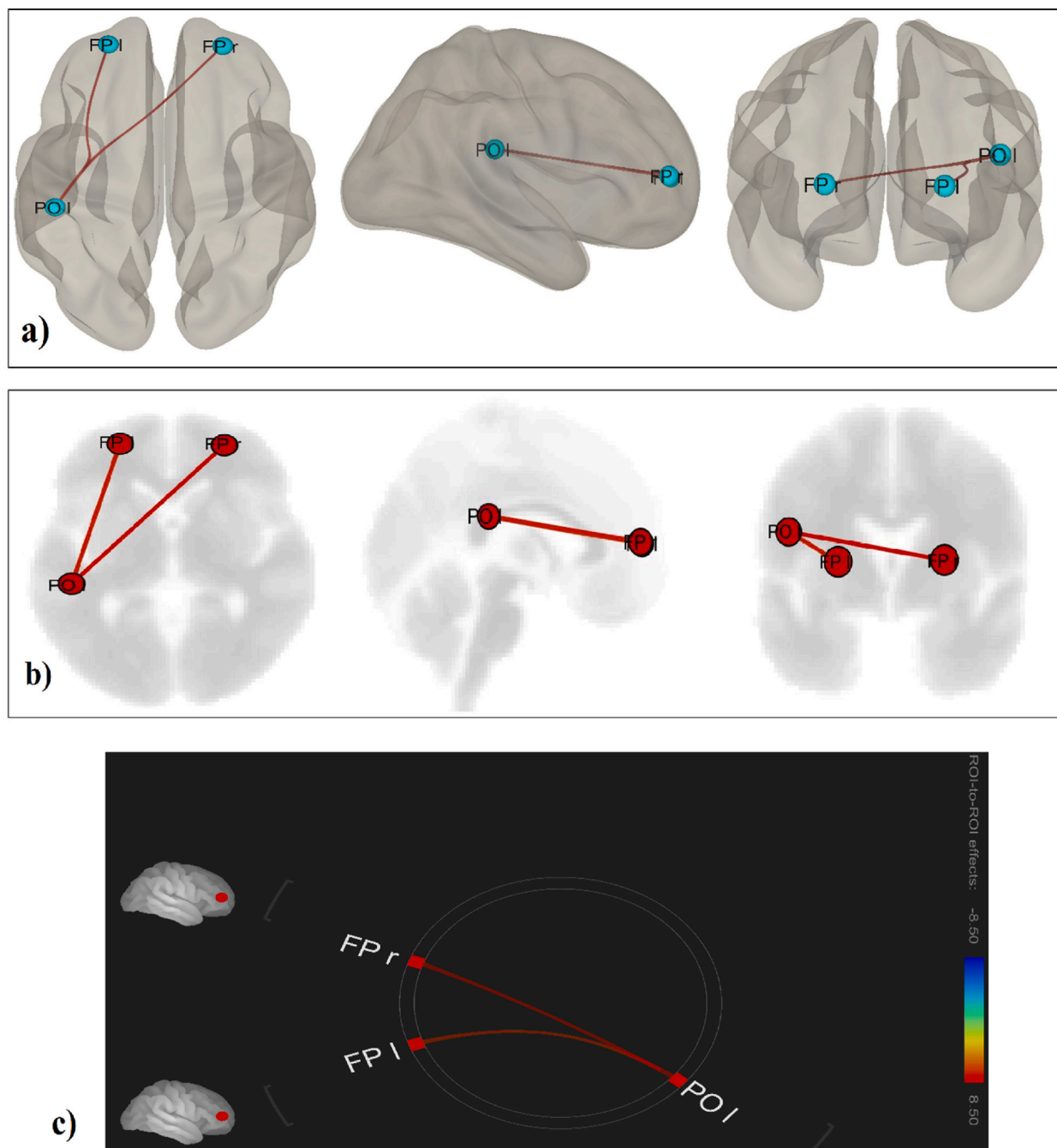
Fig. 11. Anatomical presentation of functional connectivity within the pain network in the LSD session (a) Three-dimensional presentation of functional connectivity in three anatomical views, (b) Two-dimensional presentation of functional connectivity in three anatomical views and (c) Presentation of ROI-to-ROI functional connectivity patterns within the pain network.

cortex. Some suggestions with a more precise approach for investigation the effects of LSD are as follows: (i) due to the large volume of computations on the fMRI dataset, increasing the number of computational nodes can lead to more accurate functional connectivity patterns in experimental sessions (ii) Fuzzy techniques play a significant role in reducing noise and artifacts in fMRI data, leading to more reliable and interpretable results in neuroimaging research Incorporating fuzzy techniques into fMRI analysis promises to preserve the quality of fMRI images by effectively reducing noise and artifacts while maintaining the integrity of anatomical details and functional information [36,37]. (iii) previous measurements were often made of brain areas related to pain sensation and processing. Getting fMRI information from other regions provides a more complete understanding of how pain is processed in the brain (iv) prolonged bed rest during fMRI scanning may make patients uncomfortable and increase clinical pain intensity and patient's

Table 5
Functional connectivity values for placebo and LSD sessions.

Session	Functional connectivity	T (9)	p.uncorrected	p.FDR
Placebo	PO l -FP l	8.68	0.0000	0.0001
	PO l -FP r	7.33	0.0000	0.0002
	PO l –IC l	3.91	0.0035	0.0095
	Thalamus l-PO r	−3.83	0.0040	0.0323
LSD	PO l -FP r	8.50	0.0000	0.0001
	PO l -FP l	7.49	0.0000	0.0002
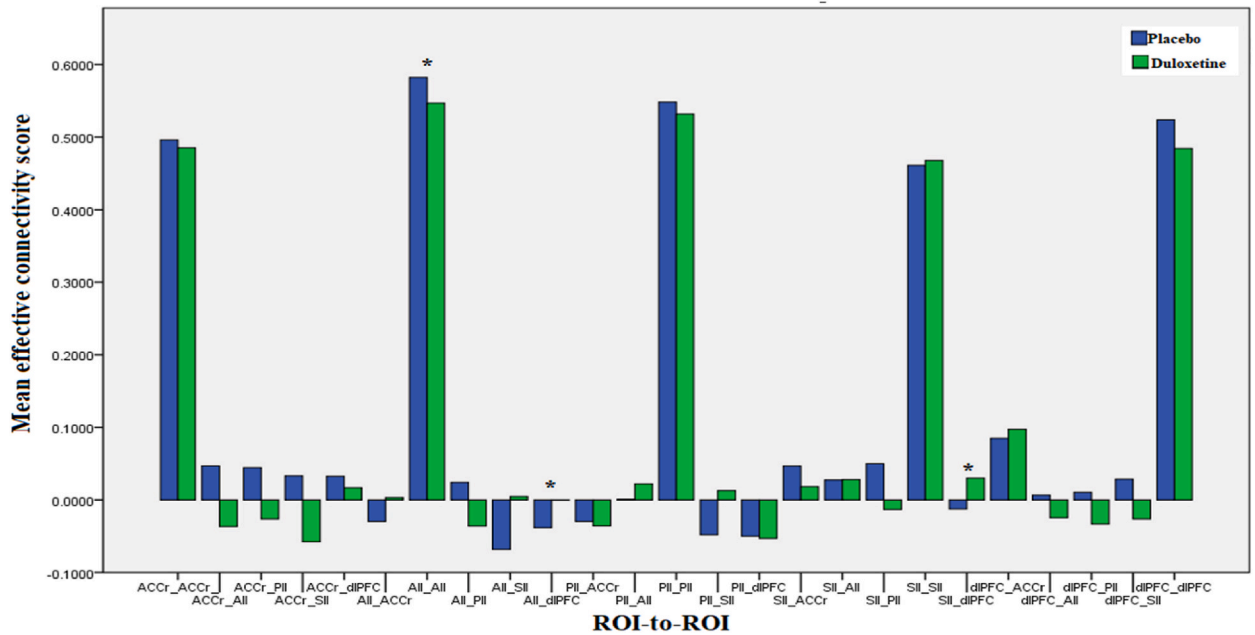
Fig. 12. The bar graphs show the effective connectivity strength of the placebo and LSD sessions compared to each other: *: indicates the significance of the mean comparison test.

Table 6
Statistical comparison of effective connectivity strength between placebo and LSD sessions.

Brain Connection	Statistics	df	Sig.(2-tailed)
lAIC- lAIC	2.748	19	0.011*
lAIC -dlPFC	2.740	19	0.037*
SII-dlPFC	2.745	19	0.014*

* Significant at p < 0.05.

reactions. As in this study, according to the radiologist, the scan time was reduced. Also, if methods can be provided to reduce noise during imaging, the accuracy of the study can be increased. In this regard, the present study offers better approaches for physicians and policymakers to use fMRI techniques in psychological trials to reduce costs, increase effectiveness, and improve the neurobiological-behavioral aspects on the personal level.

By providing the opportunity to identify brain markers predictive of response to LSD, especially in addicted individuals along with clinical trials and identifying individual differences in brain function, unnecessary exposure of addicted persons to ineffective therapies, as well as the duration and severity of pain are dramatically decreased.

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Ethical approval

The protocol of the human study was approved by the local ethical committee, Kermanshah University of Medical Sciences (KUMS), Kermanshah, Iran (Approval number: IR.KUMS.REC.1399.294)

CRediT authorship contribution statement

A. Faramarzi: Writing – original draft, Software, Resources, Methodology, Formal analysis. **M. Fooladi:** Writing – review & editing, Resources, Methodology, Conceptualization. **M. Yousef Pour:** Supervision, Project administration, Data curation, Conceptualization. **E. Khodamoradi:** Writing – review & editing, Visualization, Validation, Supervision. **A. Chehreh:** Resources, Formal analysis, Conceptualization. **S. Amiri:** Writing – review & editing, Project administration, Data curation, Conceptualization. **M. shavandi:** Validation, Resources, Methodology, Conceptualization. **H. Sharini:** Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] C.Y.J. Wenham, A.J. Grainger, P.G. Conaghan, The role of imaging modalities in the diagnosis, differential diagnosis and clinical assessment of peripheral joint osteoarthritis, Osteoarthritis Cartilage 22 (2014) 1692–1702.

[2] D. De Gregorio, S. Comai, L. Posa, G. Gobbi, d-Lysergic acid diethylamide (LSD) as a model of psychosis: mechanism of action and pharmacology, Int. J. Mol. Sci. 17 (2016) 1953.

[3] C. Montani, C. Canella, A.J. Schwarz, J. Li, G. Gilmour, A. Galbusera, K. Wafford, D. Gutierrez-Barragan, A. McCarthy, D. Shaw, The M1/M4 preferring muscarinic agonist xanomeline modulates functional connectivity and NMDAR antagonist-induced changes in the mouse brain, Neuropsychopharmacology 46 (2021) 1194–1206.

[4] H. Sharini, M. Fooladi, S. Masjoodi, M. Jalalvandi, M.Y. Pour, Identification of the pain process by cold stimulation: using dynamic causal modeling of effective connectivity in functional near-infrared spectroscopy (fNIRS), IRBM 40 (2019) 86–94.

[5] W.-Y. Ong, C.S. Stohler, D.R. Herr, Role of the prefrontal cortex in pain processing, Mol. Neurobiol. 56 (2019) 1137–1166.

[6] M. Ploner, M.C. Lee, K. Wiech, U. Bingel, I. Tracey, Flexible cerebral connectivity patterns subserve contextual modulations of pain, Cerebr. Cortex 21 (2011) 719–726.

[7] R.L. Carhart-Harris, S. Muthukumaraswamy, L. Roseman, M. Kaelen, W. Droog, K. Murphy, E. Tagliazucchi, E.E. Schenberg, T. Nest, C. Orban, R. Leech, L. T. Williams, T.M. Williams, M. Bolstridge, B. Sessa, J. McGonigle, M.I. Sereno, D. Nichols, P.J. Hellyer, P. Hobden, J. Evans, K.D. Singh, R.G. Wise, H.V. Curran, A. Feilding, D.J. Nutt, Neural correlates of the LSD experience revealed by multimodal neuroimaging, Proc. Natl. Acad. Sci. U.S.A. 113 (2016) 4853–4858, https://doi.org/10.1073/pnas.1518377113.

[8] M. Tahmasian, L.M. Bettray, T. van Eimeren, A. Drzezga, L. Timmermann, C.R. Eickhoff, S.B. Eickhoff, C. Eggers, A systematic review on the applications of resting-state fMRI in Parkinson's disease: does dopamine replacement therapy play a role? Cortex 73 (2015) 80–105.

[9] W.-S. Tae, S. Lee, S. Choi, S.-B. Pyun, Effects of aging on brain networks during swallowing: general linear model and independent component analyses, Sci. Rep. 11 (2021) 1–9.

[10] K.J. Friston, L. Harrison, W. Penny, Dynamic causal modelling, Neuroimage 19 (2003) 1273–1302.

[11] K.E. Stephan, W.D. Penny, R.J. Moran, H.E.M. den Ouden, J. Daunizeau, K.J. Friston, Ten simple rules for dynamic causal modeling, Neuroimage 49 (2010) 3099–3109.

[12] S.J. Kiebel, S. Klöppel, N. Weiskopf, K.J. Friston, Dynamic causal modeling: a generative model of slice timing in fMRI, Neuroimage 34 (2007) 1487–1496.

[13] K.J. Friston, Bayesian estimation of dynamical systems: an application to fMRI, Neuroimage 16 (2002) 513–530.

[14] J. Huang, J.-Y. Jung, C.S. Nam, Estimating effective connectivity in Alzheimer's disease progression: a dynamic causal modeling study, Front. Hum. Neurosci. 16 (2022) 1060936.

[15] B. Schuyler, J.M. Ollinger, T.R. Oakes, T. Johnstone, R.J. Davidson, Dynamic causal modeling applied to fMRI data shows high reliability, Neuroimage 49 (2010) 603–611.

[16] K.B. Jensen, C. Regenbogen, M.C. Ohse, J. Frasnelli, J. Freiherr, J.N. Lundström, Brain activations during pain: a neuroimaging meta-analysis of patients with pain and healthy controls, Pain 157 (2016) 1279–1286.

[17] A.V. Apkarian, M.C. Bushnell, R.-D. Treede, J.-K. Zubieta, Human brain mechanisms of pain perception and regulation in health and disease, Eur. J. Pain 9 (2005) 463–484.

[18] K.E. Stephan, A. Roebroeck, A short history of causal modeling of fMRI data, Neuroimage 62 (2012) 856–863.

[19] H. Almgren, F. Van de Steen, A. Razi, K. Friston, D. Marinazzo, The effect of global signal regression on DCM estimates of noise and effective connectivity from resting state fMRI, Neuroimage 208 (2020) 116435.

[20] R.A. Moss, Psychotherapy in pain management: new viewpoints and treatment targets based on a brain theory, AIMS Neurosci 7 (2020) 194.

[21] J. Chen, S. Wang, Z. Li, Y. Li, P. Huang, J. Zhu, F. Wang, Y. Li, W. Liu, J. Xue, The effect of long-term methadone maintenance treatment on coupling among three large-scale brain networks in male heroin-dependent individuals: a resting-state fMRI study, Drug Alcohol Depend. (2022) 109549.

[22] K. Peng, M.A. Yücel, S.C. Steele, E.A. Bittner, C.M. Aasted, M.A. Hoeft, A. Lee, E.E. George, D.A. Boas, L. Becerra, Morphine attenuates fNIRS signal associated with painful stimuli in the medial frontopolar cortex (medial BA 10), Front. Hum. Neurosci. 12 (2018) 394.

[23] S. Kamping, J. Andoh, I.C. Bomba, M. Diers, E. Diesch, H. Flor, Contextual modulation of pain in masochists: involvement of the parietal operculum and insula, Pain 157 (2016) 445.

[24] F.A. Mansouri, M.J. Buckley, M. Mahboubi, K. Tanaka, Behavioral consequences of selective damage to frontal pole and posterior cingulate cortices, Proc. Natl. Acad. Sci. USA 112 (2015) E3940–E3949.

[25] B. Horing, C. Sprenger, C. Büchel, The parietal operculum preferentially encodes heat pain and not salience, PLoS Biol. 17 (2019) e3000205.

[26] A. Inserra, D. De Gregorio, T. Rezai, M.G. Lopez-Canul, S. Comai, G. Gobbi, Lysergic acid diethylamide differentially modulates the reticular thalamus, mediodorsal thalamus, and infralimbic prefrontal cortex: an in vivo electrophysiology study in male mice, J. Psychopharmacol. 35 (2021) 469–482.

[27] Č. Vejmola, F. Tylš, V. Piorecká, V. Koudelka, L. Kadeřábek, T. Novák, T. Páleníček, Psilocin, LSD, mescaline, and DOB all induce broadband desynchronization of EEG and disconnection in rats with robust translational validity, Transl. Psychiatry 11 (2021) 1–8.

[28] R.G. Dos Santos, F.L. Osorio, J.A.S. Crippa, J.E.C. Hallak, Classical hallucinogens and neuroimaging: a systematic review of human studies: hallucinogens and neuroimaging, Neurosci. Biobehav. Rev. 71 (2016) 715–728.

[29] A. Alahmari, Neuroimaging Documentation of Psychedelic Drugs Effect on the Brain: DMT, LSD, Psilocybin, and Ibogaine as Examples: A Mini Review, (n.d.).

[30] S.P. Singleton, A.I. Luppi, R.L. Carhart-Harris, J. Cruzat, L. Roseman, D.J. Nutt, G. Deco, M.L. Kringelbach, E.A. Stamatakis, A. Kuceyeski, Receptor-informed network control theory links LSD and psilocybin to a flattening of the brain's control energy landscape, Nat. Commun. 13 (2022) 5812.

[31] J.S. Siegel, S. Subramanian, D. Perry, B. Kay, E. Gordon, T. Laumann, R. Reneau, C. Gratton, C. Horan, N. Metcalf, Psilocybin desynchronizes brain networks, medRxiv (2023).

[32] S.P. Singleton, A.I. Luppi, R.L. Carhart-Harris, J. Cruzat, L. Roseman, D.J. Nutt, G. Deco, M.L. Kringelbach, E.A. Stamatakis, A. Kuceyeski, LSD and psilocybin flatten the brain's energy landscape: insights from receptor-informed network control theory, bioRxiv (2021) 2005–2021.

[33] F. Holze, P. Vizeli, F. Müller, L. Ley, R. Duerig, N. Varghese, A. Eckert, S. Borgwardt, M.E. Liechti, Distinct acute effects of LSD, MDMA, and D-amphetamine in healthy subjects, Neuropsychopharmacology 45 (2020) 462–471.

[34] M. Avram, F. Müller, H. Rogg, A. Korda, C. Andreou, F. Holze, P. Vizeli, L. Ley, M.E. Liechti, S. Borgwardt, Characterizing thalamocortical (dys) connectivity following d-amphetamine, LSD, and MDMA administration, Biol, Psychiatry Cogn. Neurosci. Neuroimaging. 7 (2022) 885–894.

[35] D. De Gregorio, A. Aguilar-Valles, K.H. Preller, B.D. Heifets, M. Hibicke, J. Mitchell, G. Gobbi, Hallucinogens in mental health: preclinical and clinical studies on LSD, psilocybin, MDMA, and ketamine, J. Neurosci. 41 (2021) 891–900.

[36] F.C. Morabito, M. Versaci, G. Pautasso, C. Tichmann, A.U. Team, Fuzzy-neural approaches to the prediction of disruptions in ASDEX upgrade, Nucl. Fusion 41 (2001) 1715.

[37] Y. Kong, J. Wu, G. Yang, Y. Zuo, Y. Chen, H. Shu, J.L. Coatrieux, Iterative spatial fuzzy clustering for 3D brain magnetic resonance image supervoxel segmentation, J. Neurosci. Methods 311 (2019) 17–27.