Nicolas Viulet, 1007696744
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Acute Functional Brain Changes Associated with Psilocybin in Depression: a protocol for a systematic review and meta-analysis

Nicolas V. Viulet

Abstract

Major Depressive Disorder (MDD) is associated with disrupted functional connectivity in emotion regulation networks. Psilocybin, a serotonergic psychedelic, shows promise for MDD treatment, yet its neural mechanisms remain unclear. This systematic review employs activation likelihood estimation (ALE) to synthesize neuroimaging data on psilocybin's effects in MDD patients and healthy controls (HCs). Psilocybin administration in MDD patients was linked to increased activation in the left insula, a key region for salience detection and emotional regulation, suggesting potential mechanisms for therapeutic effects. In HCs, activation was observed in the right superior temporal gyrus (STG), involved in self-awareness and sensory integration. Despite significant clusters in both groups, contrast analyses revealed no group-specific differences, indicating that psilocybin's effects may involve shared network-level changes rather than pathology-specific alterations. However, the lack of significant findings was more likely due to the low number of foci included in the contrast analysis. These findings highlight psilocybin's role in modulating mood-related brain networks, promoting emotional flexibility and cognitive adaptability.

Introduction

Major Depressive Disorder (MDD) is one of the most prevalent and disabling neuropsychiatric conditions in the world[1,2]; MDD is associated with a host of symptoms such as

mood disturbances, suicidal ideation, and anhedonia which all contribute to the higher mortality in MDD patients versus the general population[3]. The heterogeneity of symptom presentation in MDD can be linked to the disruption of connectivity within and between mood-related brain networks such as the default mode (DMN), salience (SN), limbic, and cognitive control (CCN) networks, often seen in neuroimaging studies with MDD patients[1,4].

The distributed impact of MDD across brain networks and the resulting symptoms make MDD particularly difficult to treat effectively[1,5,6]. Approximately one-third of patients with MDD have treatment resistant depression (TRD), meaning that they do not achieve remission after two or more treatment trials with first-line traditional antidepressants[5] (e.g. SSRIs and SNRIs). Therefore, research investigating alternatives to traditional antidepressants is worthwhile as a significant portion of MDD patients could benefit from the development of alternative interventions.

Psilocybin, the psychoactive component of "magic mushrooms", has recently surfaced as a potential alternative to traditional antidepressants for treating MDD; the therapeutic value and prevalence of psilocybin for treating MDD cannot be overlooked[7,8]. There is evidence to suggest that psilocybin can be effective in alleviating disruptions in brain activity and functional connectivity induced by depression[7,8,11-13]. However, the neural mechanisms underlying mood-related outcomes in MDD patients are not fully understood. Furthermore, insufficient consistency of neuroimaging results across MDD studies in which psilocybin is administered must be addressed to legitimize psilocybin as an alternative therapeutic for treating MDD.

Neuroimaging studies have revealed the effects of MDD on brain network connectivity and changes in activation across different regions of the brain. Lower resting state functional connectivity (rsFC) of the dorsolateral prefrontal cortex (dl PFC) has been shown in MDD patients;

this finding maps to the lack of inhibitory cognitive control of emotional regulation seen in MDD patients[9]. Furthermore, activity in the subgenual anterior cingulate cortex (sgACC), a limbic network structure, is elevated in MDD patients[10]. In MDD this is thought to mainly manifest as elevated autonomic responses to emotional experiences and stressful stimuli[10]. There are also network wide effects associated with MDD symptoms. For example, hyperconnectivity within the DMN is often associated with negative self-referential thought and rumination in MDD[4]. Studying both regions of interest (ROIs) and functionally connected brain networks is important to fully capturing the variability within MDD experience but also provides an opportunity to observe consistency in neuroimaging data regarding focal and global activation that can serve to provide neural basis for targeted therapeutic interventions. This systematic review (SR) aims to take this approach to understand how psilocybin affects both MDD patients and healthy controls.

Activation likelihood estimation (ALE) is a robust tool for meta-analysis of psilocybin clinical trials and neuroimaging research. The GingerALE software[14] is used in this study to investigate and summarize post-psilocybin administration connectivity changes within and between mood-related brain networks in both MDD patients and healthy controls. There is a lack of research fully exploring how psilocybin affects mood-related brain networks implicated in MDD, as most research focuses on a limited number of predefined ROIs. This SR aimed to fill this gap in the literature and supplement the EMBRACE clinical trial[3] by understanding the impact of psilocybin on a-priori ROIs and mood-related brain networks implicated in MDD.

Materials and methods

Search Strategy

The SR was registered to the international prospective register of systematic reviews (PROSPERO) before the start of the literature search and was conducted as per the Preferred

Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[16]. Literature searches were conducted using the OvidSP platform through which the CENTRAL, EMBASE, MEDLINE, and PsycINFO databases were accessed. The databases were searched for English-language articles including terms related to psilocybin and neuroimaging, with the exact search terms included in the supplementary information. Outcome measures were not included in the search strategy but were incorporated in the inclusion/exclusion criteria for study selection.

Selection Criteria

All records identified through the literature searches were exported to Covidence systematic review software; after excluding duplicates, citations were exported to Microsoft (MS) Excel®. Two independent reviewers screened the titles and abstracts, and selected studies based on pre-determined selection criteria following the Patients, Intervention, Comparator, Outcomes, and Study (PICOS) design format[17]. The criteria for study selection were: (1) neuroimaging studies involving adults (≥18 years) diagnosed with major or clinical depression based on standardized DSM- or ICD-aligned interviews. Studies using validated self-report measures or rating scales with established cutoffs were also considered; (2) neuroimaging studies on psilocybin in healthy adults (≥18 years); (3) studies involving psilocybin administration; (4) studies reporting of MNI or Talairach coordinates in standard space; (5) randomized clinical trials (RCTs), open-label trials, observational studies; (6) Studies including neuroimaging outcomes (e.g., BOLD-fMRI, ASL-fMRI, PET SPECT, EEG) reporting changes in regional brain activity or network functional connectivity. Non-human, and preclinical studies; articles without neuroimaging data; literature reviews and meta-analyses; and studies where psilocybin was combined with other psychedelics without separate analyses were excluded. Full-text studies retained after title/abstract review were again reviewed by two independent reviewers, and those lacking relevance were excluded. In case

of disagreement among reviewers, consensus was reached either by reconciliation or through arbitration by a third independent reviewer.

Data Extraction

Data extraction was performed by three reviewers and all three reviewers cross-verified data extraction. Data were extracted from selected full-text studies using a standardized data-extraction template (in MS Excel®). From each selected record, sample size, psilocybin dose, image modality, psilocybin dosage timeline, group comparison type, neuroimaging methodology, the inclusion or exclusion of a task condition, peak activation foci, anatomical regions associated with peak activation foci, and directional change of peak activation were extracted and tabulated. No formal quality assessment of included studies was carried out and not all data types included in the MS Excel® template were extracted from every study.

Activation Likelihood Estimation Workflow and ROIs

Activation foci were manually extracted from peak coordinates, in Montreal Neurological Institute (MNI) space, from included MDD and healthy control neuroimaging studies. All foci were compiled into text files formatted for GingerALE 3.0.2[14,15], ensuring proper study-level weighting. A single-dataset analysis was conducted separately for MDD and HC groups using activation likelihood estimation (ALE) with cluster-level family-wise error (FWE) correction ($p < 0.05$). To compare group differences, a contrast analysis was performed in GingerALE[14,15], subtracting HC activation patterns from MDD (and vice versa). The resulting thresholded ALE and contrast maps were visualized using Mango. The Colin27 high-resolution MNI template was used as anatomical reference. ALE maps were overlaid onto the template, and color scale adjustments were applied to highlight significant clusters. Final visualizations included axial, sagittal, and coronal slices to comprehensively represent spatial activation patterns. Parameters

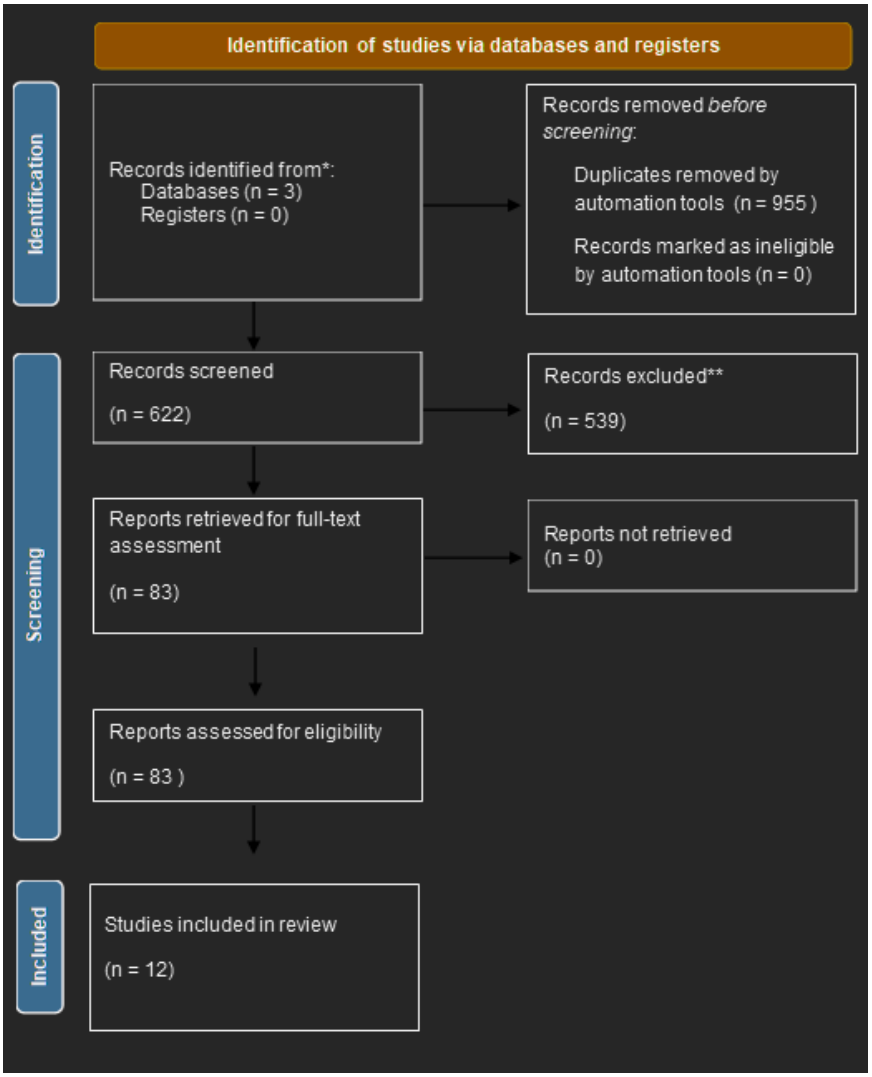
and the MNI template used for analysis are included in the Supplementary information. The regions of interest for this SR consist of regions of the DMN (PCC, mPFC, IPL), the salience network (insula and ACC, amygdala), central executive network (dlPFC, PPC), limbic network (hippocampus, sgACC, hypothalamus, thalamus).

Results

Literature Search

The search identified 1577 records from the four databases. After removing 955 duplicates, 622 records were screen for title and abstract eligibility, Among the 622 records screen, 83 records were selected for full text review and 539 records were excluded for being out of scope. Among the 83 records selected for full text review, 12 studies were found to be relevant and selected for data extraction, while 71 records were excluded for being out of scope. All 12 studies were included in data extraction and analysis.

Fig. 1: PRISMA flowchart for identification, screening, and selection of studies for SR.



ALE Analysis of Psilocybin Administration for MDD Patients

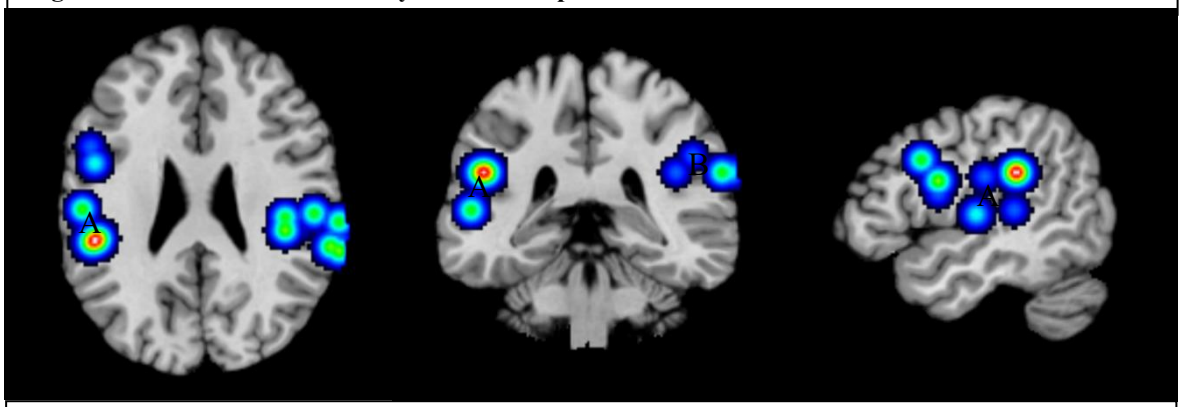
Three open-label clinical trials of psilocybin administration for MDD patients published between 2017 and 2023 with sample sizes ranging from 16-19 subjects were included in this report[18-20]. All studies used two doses of psilocybin — 10mg followed by 25mg — and assessed the effects of these doses on the brain using fMRI and within-group comparison of the corresponding imaging data. However, there was variation across studies in the timing of psilocybin doses, methods of neuroimaging analysis (e.g. DCM or ICA), use of a task or rest conditions, and ROIs identified. Two significant clusters were identified from the single dataset ALE cluster-level FWE analysis (Table 1). However, only cluster one, centered at MNI coordinate -50, -34, 24, shows moderate activation likelihood (ALE = 0.0134) while cluster two showed very low activation likelihood (ALE = 0.0010). Figure 2 provides a visualization of the outcomes from MDD studies mapped onto a standard MNI152 template; cluster one is clearly localized around the left insula and visible in all three brain slices (Fig. 2).

ALE Analysis of Psilocybin Administration for Healthy Controls

Nine studies of psilocybin administration for healthy controls published between 2017 and 2023 with sample sizes ranging from 7-60 subjects were included in this report[18,21-28]. All studies assessed the effects of these doses on the brain using fMRI. However, there was variation across studies in the timing, number, and quantity of psilocybin doses; methods of neuroimaging analysis (e.g. DCM, ICA, voxel-wise analysis); use of a task or rest conditions; type of group comparisons (e.g. placebo vs active, within group, etc.,); and ROIs identified. One significant cluster was identified from the single dataset ALE cluster-level FWE analysis (Table 2). However, this cluster, centered at MNI coordinate 52, -52, 32, shows weak activation likelihood (ALE = 0.0097). Figure 3 provides a visualization of the outcomes from healthy control studies mapped onto a standard

MNI152 template; the axial slice shows that cluster one is clearly localized around the right superior temporal gyrus. Contrast analysis[14,15] of the MDD and healthy control datasets did not yield any meaningful comparisons as only one cluster, centered at MNI coordinate 55.7, -30, 24.9 in the right inferior parietal lobe, with no activation likelihood (ALE = 0) was found (Supplementary Table 1).

Fig. 2: Activation likelihood analysis for MDD patients



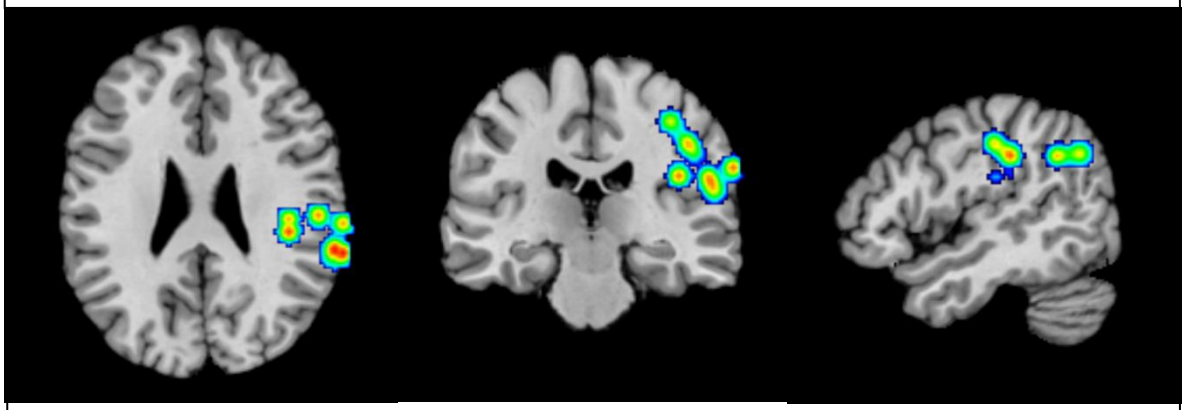
Cluster-level FWE (threshold permutations = 1000, $p < .05$) analysis of peak coordinates of psilocybin activation done with GingerALE[14,15]. Areas with warm color suggest strong convergence of activation for MDD patients. Cluster one (A) had a peak MNI coordinate (-50, -34, 24) in the left insula that showed moderate activation likelihood across 29 foci collected from 54 MDD patients. Cluster two (B) had no meaningful activation likelihood in any of the cluster areas.

Cluster Number	**Cluster Volume (mm^3)**	**Peak MNI Coordinate**	**Anatomical Location of Peak Coordinate**	**Peak ALE Value**	**z-score**	**p-value**
1	28880	(-50, -34, 24)	Left Insula	0.0134	4.87	5.706^{-7}
2	18464	(55.7, -30, 24.9)	Right Inferior Parietal Lobe	0.0010	1.91	0.028

	Anatomical Composition of Cluster (% out of 100%)
1	24.4% Superior Temporal Gyrus, 23.9% Precentral Gyrus, 12.7% Inferior Frontal Gyrus, 11.4% Insula, 7.4% Postcentral Gyrus, 7.1% Middle Temporal Gyrus, 4.7% Inferior Parietal Lobule, 4.4% Transverse Temporal Gyrus, 4.1% Middle Frontal Gyrus
2	32.9% Inferior Parietal Lobule, 30.4% Insula, 16.1% Postcentral Gyrus, 14.3% Superior Temporal Gyrus, 6.2% Supramarginal Gyrus

Table 1: Activation clusters from MDD ALE meta-analysis.
Two significant (threshold permutations = 1000, $p < .05$), FWE-corrected, activation clusters were found following GingerALE[14,15] single dataset analysis. Peak MNI coordinates (x, y, z) indicate the voxel with the highest activation likelihood within each cluster. The anatomical location corresponds to the peak coordinate based on the MNI atlas. The ALE score represents activation convergence across studies, with higher values indicating greater consistency. The z-score and p-value denote statistical significance at the peak coordinate. The anatomical composition of each cluster is expressed as a percentage of the total cluster volume, with only regions contributing ≥1% reported.

Fig. 3: Activation likelihood analysis for healthy controls



Cluster-level FWE (threshold permutations = 1000, $p < .05$) analysis of peak coordinates of psilocybin activation done with GingerALE[14,15]. Areas with warm color suggest strong convergence of activation for healthy controls. Cluster one had a peak MNI coordinate (52, -52, 32) in the right superior temporal gyrus that showed moderate activation likelihood across 101 foci collected from 195 healthy controls.

Cluster Number	Cluster Volume (mm^3)	Peak MNI Coordinate	Anatomical Location of Peak Coordinate	Peak ALE Value	z-score	p-value
1	13824	(52, -52, 32)	Right Superior Temporal Gyrus	0.0097	3.49	2.383^{-4}
Anatomical Composition of Cluster (% out of 100%)						
1	28.1% Inferior Parietal Lobule, 19.8% Postcentral Gyrus, 18.3% Insula, 16.5% Superior Temporal Gyrus, 10.8% Supramarginal Gyrus, 4.2% Middle Temporal Gyrus, 1.3% Transverse Temporal Gyrus, 1% Precentral Gyrus					

Table 2 Activation clusters from healthy controls ALE meta-analysis.
One significant (threshold permutations = 1000, $p < .05$), FWE-corrected, activation cluster was found following GingerALE[14,15] single dataset analysis. Peak MNI coordinates (x, y, z) indicate the voxel with the highest activation likelihood within the cluster. The anatomical location corresponds to the peak coordinate as defined by the MNI atlas. The ALE score quantifies activation convergence across studies, with higher values indicating greater consistency. The z-score and p-value denote statistical significance at the peak coordinate. The anatomical composition of the cluster is expressed as a percentage of the total cluster volume, with only regions contributing ≥1% reported.

Discussion

Relevance of psilocybin administration for brain connectivity and

This meta-analysis used activation likelihood estimation (ALE) to synthesize neuroimaging findings on psilocybin's effects in MDD patients and healthy controls (HCs). The results highlight regionally specific alterations in brain activity that may underlie the therapeutic benefits of psilocybin. Two significant activation clusters were identified. Cluster one, centered around the left insula, involved in emotion regulation and salience detection, showed the strongest

activation likelihood across MDD foci. Like the DMN, dysfunction in the insula is linked to negative self-referential processing in MDD[29]. Psilocybin may facilitate reorganization of salience network activity, promoting emotional flexibility. Cluster two, centered around the inferior parietal lobule, was significant but did not show any meaningful activation likelihood.

In contrast to MDD patients, HCs exhibited activation in the right superior temporal gyrus (STG), a region involved in auditory and emotional processing, social cognition, and self-perception[23]. This aligns with subjective reports of enhanced emotional and sensory processing following psilocybin[23,29]. However, weaker ALE values in HCs suggest that effects may be more variable, potentially influenced by individual differences in baseline connectivity, prior psychedelic experience, and psychological traits. Future studies could examine dose-dependent effects to clarify response variability. The contrast analysis did not reveal significant differences between MDD and HC groups, this could indicate that psilocybin's therapeutic effects arise from network-level reorganization, rather than a pathology-specific mechanism. However, the lack of meaningful data from contrast analysis is more likely due to the low number of foci included in the analysis. Furthermore, none of the clusters in either MDD patients or healthy controls implicated the sgACC or dLPFC; however, this does not mean these regions are not relevant to MDD.

These findings suggest that psilocybin's therapeutic potential in MDD is linked to modulation of emotional regulation and self-referential processing networks. The insula and STG findings highlight its role in affective processing, self-awareness, and cognitive flexibility. However, the absence of significant contrast findings suggests that more refined methods, such as connectivity-based analyses, may be necessary to capture disease-specific effects. Future research should focus on standardizing neuroimaging protocols and exploring long-term neuroplastic

changes to establish treatment durability. This SR provides evidence that psilocybin modulates mood-related brain networks in MDD and HCs. Insula and STG activation changes suggest mechanisms underlying emotional processing and cognitive flexibility. Further research is needed to refine our understanding of individual variability, long-term effects, and optimal treatment parameters for clinical application.

Limitations of current methodology and preliminary results

The results included in this report represent findings from a relatively small number of clinical trials and neuroimaging studies on psilocybin. Ideally the findings presented in this SR would include a minimum of ten studies from both MDD patients and healthy controls assessing the impact of psilocybin on mood-related brain networks. Given the number of studies currently included in data extraction, more impactful results could be generated by running the GingerALE protocol with pooled foci from both MDD patients and healthy controls.

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