LSD flattens the functional hierarchy of the human brain

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Abstract

LSD is a potent serotonergic psychedelic compound. Findings indicate that psychotherapeutic applications of LSD and related psychedelic compounds have value in the treatment of mental health disorders. Deepening our understanding of LSD brain action may shed light on the mechanisms underlying the effectiveness of psychedelic therapy. A recent model hypothesized reduced whole-brain hierarchical organization as a key mechanism underlying the psychedelic state, but this has yet to be directly tested. Here, we applied an unsupervised manifold learning technique that is sensitive to cortical hierarchy to pharmacological resting-state fMRI data to assess cortical organization in the LSD state. Results directly supported our primary hypothesis: The Principal Gradient, describing a hierarchical transition from unimodal to transmodal cortex, was significantly contracted under LSD relative to placebo. Between-condition contrasts revealed that this was primarily driven by a reduction of functional differentiation at both hierarchical extremes – default and frontoparietal networks at the upper end, and somatomotor cortex at the lower. In addition, results pertaining to the visual-somatomotor Second Gradient revealed that LSD reduced the differentiation of visual from auditory/somatomotor and association regions. Significantly, gradient changes tracked state-specific changes in specific dimensions of self-reported LSD experience. These findings support a recent mechanistic model of the psychedelic state that has relevance to therapeutic applications of psychedelics. More fundamentally, these findings provide the first evidence that cortical hierarchical organization can be modulated in a state-dependent manner, highlighting an important relationship between the features of neural topography and ongoing conscious experience.

Introduction

Recent years have seen a resurgence of scientific interest in lysergic acid diethylamide (LSD) and related serotonergic psychedelic compounds such as psilocybin (the active compound in 'magic mushrooms') and DMT/ayahuasca (Johnson, Hendricks, Barrett, & Griffiths, 2019; Nichols, 2016). This interest has been motivated by preliminary clinical trials suggesting their effectiveness in the treatment of multiple mental health conditions (Carhart-Harris, Bolstridge, et al., 2016; Gasser, Kirchner, & Passie, 2014; Griffiths et al., 2016; Johnson et al., 2019; Krebs & Johansen, 2012). While mechanisms underlying the potential effectiveness of psychedelic therapy remain poorly understood, investigating alterations in brain function related to the acute psychedelic state may provide relevant insights.

Functional neuroimaging investigations of the acute LSD state have provided evidence of significant alterations to whole-brain functional organization and dynamics (Carhart-Harris et al., 2012; Carhart-Harris & Friston, 2019; Carhart-Harris, Muthukumaraswamy, et al., 2016; Preller et al., 2018; Tagliazucchi et al., 2016). Findings suggest that LSD administration shifts the brain towards greater global functional integration, as reflected by the reduced functional segregation of large-scale brain networks and increased regional-global functional connectivity (FC; Carhart-Harris, Muthukumaraswamy, et al., 2016; Preller et al., 2018; Tagliazucchi et al., 2016). In addition, population-level activity becomes more 'entropic' - exhibiting greater dynamic complexity in the psychedelic state (Carhart-Harris, 2018; Carhart-Harris et al., 2014; Lebedev et al., 2016; Lord et al., 2019; Schartner, Carhart-Harris, Barrett, Seth, & Muthukumaraswamy, 2017; Varley, Carhart-Harris, Roseman, Menon, & Stamatakis, 2019).

The recently proposed RElaxed Beliefs Under Psychedelics (REBUS) model (Carhart-Harris & Friston, 2019) links past psychological and neural findings with psychedelics into a common framework based on hierarchical predictive coding and Free Energy Principle accounts of brain function (Friston, 2010). The model proposes that psychedelics elicit their characteristic subjective effects by decreasing the precision-weighting of high-level priors (e.g., beliefs or assumptions) which are encoded by transmodal cortex, thereby increasing the susceptibility of these priors to revision by bottom-up sensory or limbic inputs (Carhart-Harris & Friston, 2019). Acute reduction in the hierarchical differentiation of transmodal versus unimodal cortex would directly support this hypothesized effect (Carhart-Harris & Friston, 2019) but the impact of psychedelics on neural hierarchical organization has yet to be directly tested. The present study addresses this knowledge gap.

Recent years have seen increasing interest in the application of 'gradient mapping' approaches to the characterization of cortical organization (de Wael et al., 2020; Haak, Marquand, & Beckmann, 2018; Huntenburg, Bazin, & Margulies, 2018). In contrast to parcellation-type approaches which seek to decompose the brain into discrete regions or networks, these approaches model the brain

as the superposition of multiple gradients along particular axes of differentiation (Huntenburg et al., 2018). One such approach that has garnered significant attention is the application of a 'diffusion map embedding' technique (Coifman et al., 2005; Margulies et al., 2016). This unsupervised non-linear manifold learning technique, as applied to resting-state fMRI data, decomposes the FC similarity structure of the data into a low dimensional set of embedding components, each of which represents a gradient of functional differentiation along the cortical mantle. Notably, this approach as applied in humans has consistently revealed a principal gradient of FC similarity which spans from unimodal sensorimotor regions to transmodal association regions centered on the default network (Bethlehem et al., 2020; Hong et al., 2019; Margulies et al., 2016). This gradient is consistent with long-standing tract-tracing work identifying hierarchical cortical organization in human and non-human primates (Mesulam, 1998), corresponds to cortical myeloarchitectonic and transcriptomic transitions (Burt et al., 2018; Huntenburg et al., 2017; Paquola, De Wael, et al., 2019), and appears to represent a functional hierarchy from low-level sensorimotor processing to abstract cognition (Huntenburg et al., 2018; Margulies et al., 2016; Murphy et al., 2018). Gradient-mapping approaches have now been used to characterize functional neural hierarchy in diverse contexts, including autism (Hong et al., 2019), neonatal development (Larivière et al., 2019), schizophrenia (Dong et al., 2020), and lifespan development (Bethlehem et al., 2020; Paquola, Bethlehem, et al., 2019).

The present study applies diffusion map embedding to characterize alterations of macroscale cortical hierarchy in the LSD state. We apply a combination of cortex-wide and network-specific analyses to characterize the spatial distribution of LSD-dependent changes in cortical gradients. Primary analyses focus on characterizing LSD-dependent changes in the Principal Gradient spanning from unimodal to transmodal cortices. We predicted that LSD will be associated with a contraction of this gradient, indicating reduced differentiation between these typically segregated functional cortical zones. We also investigated LSD-induced changes to the Second Gradient, which differentiates visual from somatomotor and auditory cortex (Hong et al., 2019; Margulies et al., 2016). Previous work has demonstrated markedly increased whole-brain FC of visual cortex in the LSD state (Carhart-Harris, Muthukumaraswamy, et al., 2016; Preller et al., 2018). Here we predicted alterations in the Second Gradient consistent with reduced differentiation of visual cortex from the rest of the brain. Finally, we examined whether changes in cortical organization, as reflected by LSD-induced alterations of cortical gradients, are associated with the drug's characteristic subjective effects.

Methods

Participants

Twenty healthy participants were recruited via word of mouth and provided written informed consent to participate after study briefing and screening for physical and mental health. The

screening for physical health included electrocardiogram (ECG), routine blood tests, and urine test for recent drug use and pregnancy. A psychiatric interview was conducted, and participants provided full disclosure of their drug use history. Key exclusion criteria included: < 21 years of age, personal history of diagnosed psychiatric illness, immediate family history of a psychotic disorder, an absence of previous experience with a classic psychedelic drug (e.g. LSD, mescaline, psilocybin/magic mushrooms or DMT/ayahuasca), any psychedelic drug use within 6 weeks of the first scanning day, pregnancy, problematic alcohol use (i.e. > 40 units consumed per week), or a medically significant condition rendering the volunteer unsuitable for the study.

Neuroimaging Data Acquisition

Neuroimaging data from an already published dataset (Carhart-Harris, Muthukumaraswamy, et al., 2016) was used for the present analyses. The data acquisition and preprocessing details have been described in detail elsewhere (Carhart-Harris, Muthukumaraswamy, et al., 2016); we outline them in brief here. Subjects participated in two scanning days that were separated by 14 days and which each featured three 7-minute resting-state fMRI scans. On a given scanning day, subjects received either a placebo (10ml saline) or LSD (75 µg) via a bolus intravenous injection. The low-moderate LSD dosage was selected to minimize the potential for intra-scanner anxiety while ensuring drug effects (Johnson, Richards, & Griffiths, 2008). The order of the conditions was balanced across participants; participants were blind to this order, but the researchers and those analyzing the data were not. The scans on each of the days were as follows: (1) resting-state eyes-closed with no music, (2) resting-state eyes-closed with music, (3) resting-state eyes-closed with no music. Scans featuring no music (scans 1 and 3) were used in the present analyses.

Resting-state BOLD fMRI data were acquired using a gradient echo planar imaging sequence, TR/TE = 2000/35ms, FoV = 220mm, 64×64 acquisition matrix, parallel acceleration factor = 2, 90◦ flip angle. Thirty-five oblique axial slices were acquired in an interleaved fashion, each 3.4mm thick with zero slice gap (3.4mm isotropic voxels). Structural T1w images were acquired on a 3T GE HDx system. These were 3D fast spoiled gradient echo scans in an axial orientation, with field of view = 256 × 256 × 192 and matrix = 256 × 256 × 20 192 to yield 1mm isotropic voxel resolution. TR/TE = 7.9/3.0ms; inversion time = 450ms; flip angle = 20°.

Ethics Statement

This study was approved by the National Research Ethics Service committee London-West London and was conducted in accordance with the revised declaration of Helsinki (2000), the International Committee on Harmonization Good Clinical Practice guidelines, and National Health Service Research Governance Framework. Imperial College London sponsored the research, which was conducted under a Home Office license for research with schedule 1 drugs.

Neuroimaging Data Pre-Processing

Of the 20 subjects that underwent scanning, 15 were used in the current analyses. One participant was unable to complete the scanning due to anxiety, and four were discarded from analyses due to excessive head motion as measured by framewise displacement (subjects were rejected based on having >15% volumes with FD >= 0.5). This excessive head motion was found in scans conducted during the LSD condition. The following pre-processing steps were performed on the BOLD resting-state fMRI data: removal of the first three volumes, de-spiking (3dDespike, AFNI), slice time correction (3dTshift, AFNI), motion correction (3dvolreg, AFNI) by registering each volume to the volume most similar to all others, brain extraction (BET, FSL); rigid body registration to anatomical scans, non-linear registration to a 2mm MNI brain (Symmetric Normalization (SyN), ANTS), scrubbing (FD = 0.4), spatial smoothing (FWHM) of 6mm, band-pass filtering between [0.01 to 0.08] Hz, linear and quadratic de-trending (3dDetrend, AFNI), regression of 6 motion parameters, and regression of 3 anatomical nuisance regressors (ventricles, draining veins, and local white matter). Global signal regression was not performed. Quality control tests confirmed the lack of distance-dependent motion confounds in the denoised data (Carhart-Harris, Muthukumaraswamy, et al., 2016).

Structural T1w images were processed using Freesurfer v5.3 (http://surfer.nmr.mgh.harvard.edu/). Structural processing included bias field correction, registration to stereotaxic space, intensity normalization, skull-stripping, and white matter segmentation. A deformable mesh model was fit on the white matter volume via a triangular surface tessellation. This resulted in >160,000 vertices which differentiate gray matter, white matter, and pial surfaces. Individual subject surfaces were fit to the fsaverage5 spherical surface template, which enables stronger inter-subject correspondence in gyral and sulcal folding patterns.

Subjective Measures

Subjects completed a number of intra-scanner visual analogue scale (VAS) ratings at the end of each scan, reporting on different facets of the LSD experience (Carhart-Harris, Muthukumaraswamy, et al., 2016). In addition, subjects completed the 11-factor altered states of consciousness (ASC) questionnaire (Dittrich, 1998; Studerus, Gamma, & Vollenweider, 2010) at the end of each scan day. In the present study, we conducted brain-behaviour analysis with two self-report measures which relate to core components of the psychedelic experience: ego dissolution ("I experienced a disintegration of my 'self' or 'ego") and complex imagery (a composite of "I could see images from my memory or imagination with exceeding clarity", "I saw whole scenes in complete darkness or with closed eyes", and "My imagination was extremely vivid"). The former measure was an intra-scanner VAS rating, while the latter measures were ASC measures conducted at the end of the scan day.

Gradients Analysis

Cortical gradients were computed using the BrainSpace toolbox (https://github.com/MICA-MNI/BrainSpace; (de Wael et al., 2020)) as implemented in MATLAB. Surfaces were first downsampled from fsaverage 5 space (20,484 vertices) to 10,000 vertices for computational efficiency. For each subject, a 10,000 x 10,000 connectivity matrix was then computed by calculating the pairwise Pearson's correlation across all vertices. As has been done previously (e.g., Hong et al., 2019; Margulies et al., 2016), this matrix was z-transformed and thresholded row-wise at 10% sparsity in order to retain only the strongest connections. Cosine similarity was then computed on the thresholded z-matrix in order to generate a similarity matrix which captures the similarity in whole-brain connectivity patterns between vertices. This similarity matrix is required as input to the diffusion map embedding algorithm we employed here. The use of cosine similarity as the similarity metric of choice is consistent with past work using this approach (Hong et al., 2019; Margulies et al., 2016; Paquola et al., 2020).

Diffusion map embedding (Margulies et al., 2016), a non-linear manifold learning technique from the family of graph Laplacians, was applied to similarity matrices in order to identify gradient components at the individual subject level. The technique estimates a low-dimensional set of embedding components (gradients) from a high-dimensional similarity matrix, where each embedding can intuitively be viewed as a dimension of FC pattern similarity covariance. In the embedding space, vertices that are strongly connected (as weighted by FC pattern similarity) by many connections or a few very strong connections are closer together, whereas vertices with little or no connections are farther apart. Euclidean distance between two points in the embedding space is equivalent to the diffusion distance between probability distributions centered at those points (hence the name of the algorithm), each of which are equivalent to 'difference in gradient value' as referred to in the main text. The algorithm is controlled by a single parameter α, which controls the influence of density of sampling points on the manifold ($\alpha = 0$, maximal influence; $\alpha = 1$, no influence). For the current study, we set $\alpha = 0.5$ as had been done in previous work (Hong et al., 2019; Margulies et al., 2016). This allows the influence of both global and local relationships between data points in the embedding space. To enable comparisons across subjects, Procrustes rotation was performed to align individual-subject embedding (gradient) components to a group average template. Group contrasts and behavioural associations were conducted using surface-based linear models, as implemented in the SurfStat toolbox (http://www.math.mcgill.ca/keith/surfstat; Worsley et al., 2009). Additional methods can be found in the Supplementary Information.

Data and code availability

All analyses were conducted in MATLAB using custom scripts based on functions within the BrainSpace and SurfStat toolboxes described in the preceding section. Scripts are available upon request from Manesh Girn (manesh.girn@mail.mcgill.ca). Data is available upon request from Robin L. Carhart-Harris (r.carhart-harris@imperial.ac.uk).

Results

We applied diffusion map embedding to characterize differences in macroscale cortical gradients in LSD and placebo states. Compared to other nonlinear manifold learning techniques, the algorithm is robust to noise, computationally inexpensive, and governed by a single parameter controlling the influence of the sampling density on the manifold (see *Methods* for details). Applying this algorithm with standard settings revealed 98 mutually orthogonal gradient components per subject, listed in descending order based on the amount of FC variance explained (see Supplementary Figure 1 for scree plots). We focus on the first two gradients revealed by this approach, the Principal and Second Gradient, which reflect global hierarchical organization and visual cortex functional differentiation, respectively.

Principal gradient

The principal gradient of cortical connectivity revealed in the present dataset replicates past findings (Hong et al., 2019; Margulies et al., 2016) of a putatively hierarchical axis of FC similarity variance spanning from unimodal regions centered in somatomotor cortex on one end to transmodal regions centered on the default network and superior frontal gyrus on the other (Figure 1A). This gradient explained the greatest amount of variance in both the placebo (mean 15% variance explained) and LSD (mean 13% variance explained) conditions.

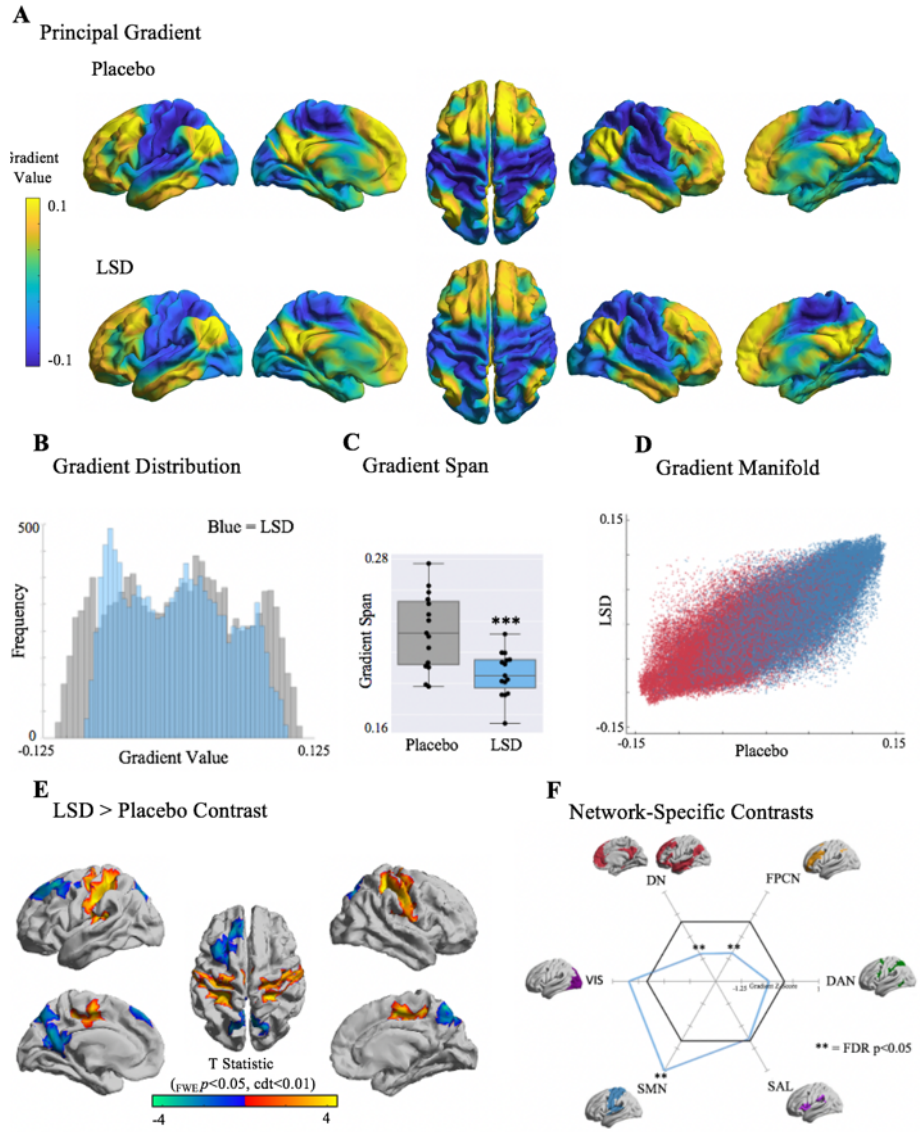

Figure 1. (A) The mean Principal Gradient in each of LSD and placebo conditions, representing a hierarchical axis of FC similarity variance that spans from unimodal to transmodal cortex. (B) Histogram showing the distribution of Principal Gradient values. Gray indicates placebo. (C) Principal Gradient span calculated as the difference between each subject's maximum and minimum gradient value. Between-condition comparisons indicate a significant contraction in the LSD state (t^{28} = -3.5, p<0.001). (D) Scatter plot representing the Principal Gradient manifold for both LSD (y-axis) and placebo (x-axis) conditions, color coded for overall trends in between-condition differences. Red colors indicate increased values in the LSD state, while blue indicated decreased values. (E) LSD > Placebo between-condition contrast revealing statistically significant clusters at $_{FWE}p$<0.05. (F) Spider plot displaying mean intra-network principal gradient scores for each of six functional networks, following the (Yeo et al., 2011) parcellation. Values are normalized to the placebo condition (black lines). Blue lines indicate the LSD condition. DN = default network; FPCN = frontoparietal control network; DAN = dorsal attention network; SAL = salience network; SMN = somatomotor network; VIS = visual network.

Principal Gradient histograms indicated a contraction on both sides of this gradient in the LSD condition relative to placebo, providing qualitative support for our hypothesis of reduced hierarchical differentiation in the LSD state (Figure 1B). To quantitatively assess this, we

calculated the difference between each subject's maximum and minimum Principal Gradient value and compared these differences across groups (Figure 1C). Results confirmed the presence of a significant contraction in the LSD state (t^{28} = -3.5, p<0.001), reflective of a 'flatter' hierarchy. In order to examine overall trends in LSD-dependent changes in hierarchy, we then visualized the Principal Gradient as a scatter plot, color coded for increases (red) and decreases (blue) in the LSD state (Figure 1D). This indicated that, as an overall pattern, unimodal-proximal regions tended to increase in hierarchical position, while transmodal-proximal regions exhibited decreases – again suggesting a contraction on both sides of the gradient.

Next, we quantitatively assessed between-condition differences in gradient score values at a cortex- (Figure 1E) and network-wise (Figure 1F) level. Cortex-wise contrasts revealed increased Principal Gradient scores (greater 'transmodalness') in the LSD condition in somatomotor cortex and decreased gradient scores (greater 'unimodalness') in the left posterior cingulate/retrosplenial cortex, bilateral precuneus, and left superior and middle frontal gyrus (Figure 1E). Network-wise differences were assessed according to the Yeo et al. (2011) network parcellation scheme. Results indicated a significant increase in transmodalness for the somatomotor network and decrease in transmodalness for the default network and frontoparietal control network (Figure F). These results offer further quantitative support for the qualitative trend seen in Figure 1D. Namely, that the LSD state is characterized by a pulling-together of both unimodal (visual and somatomotor) and transmodal (default, frontoparietal control, dorsal attention) networks in gradient space – reflective of relatively symmetrical reduced hierarchical differentiation.

Second gradient

Having found support for our hypothesis of a contraction in macroscale functional hierarchy in the LSD state, we next examined the Second Gradient of macroscale functional organization. The Second Gradient revealed in our dataset represents an axis of FC similarity variance that separates visual cortex on one end from the somatosensory/auditory on the other. This gradient explained the second most variance in both placebo (mean 10% variance explained) and LSD (mean 8% variance explained) conditions.

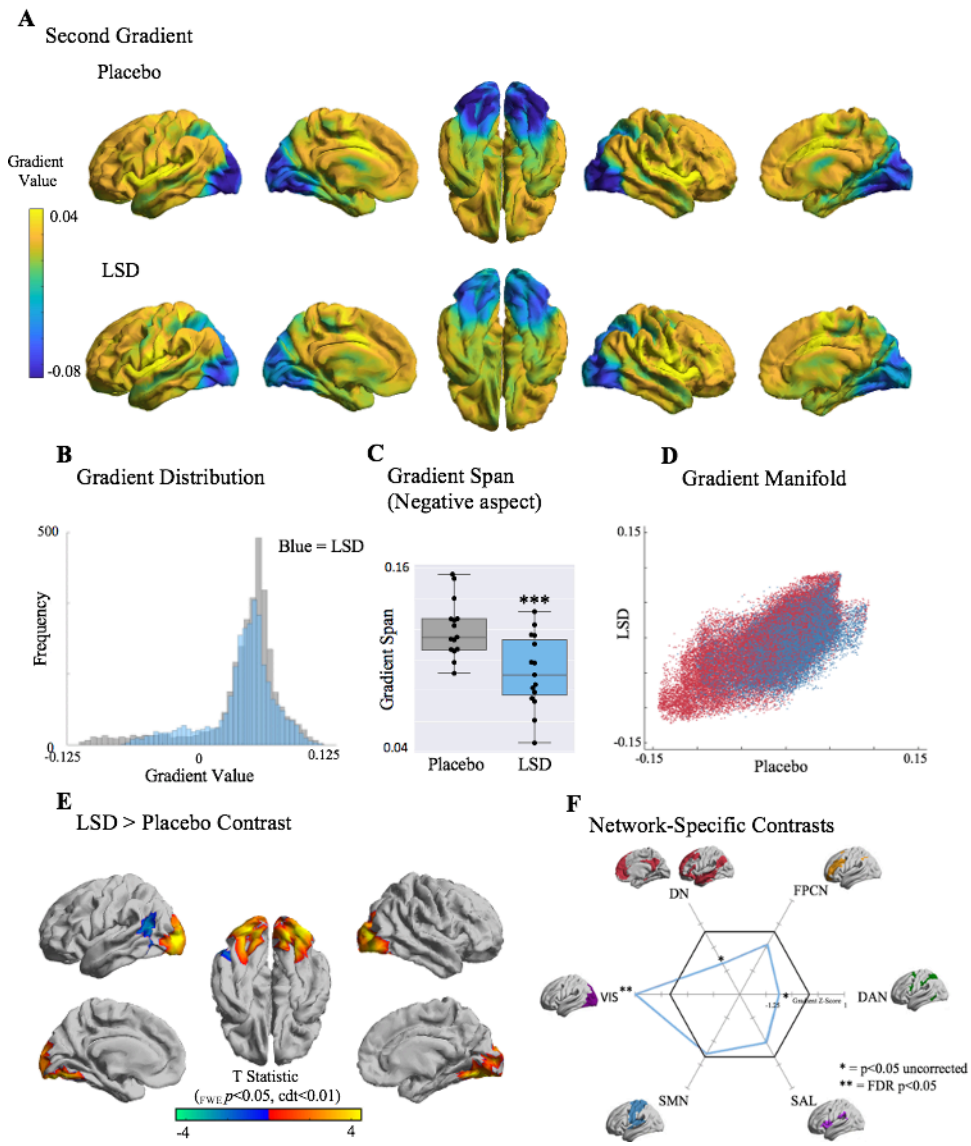

Figure 2. (A) The mean Second Gradient in each of LSD and placebo conditions, representing an axis of FC similarity variance that spans from visual to somatomotor/auditory cortex. (B) Histogram showing the distribution of Second Gradient values (C) Second Gradient span calculated as the difference between each subject's median and minimum Second Gradient value. Between-condition comparisons indicate a significant contraction on the negative (visual) aspect of the gradient in the LSD state (t^{28} = -3.8, p<0.001). (D) Scatter plot representing the Second Gradient manifold for both LSD (y-axis) and placebo (x-axis) conditions, color coded for overall trends in between-condition differences. Red colors indicate increased values in the LSD state, while blue indicated decreased values. (E) LSD > Placebo between-condition contrast revealing statistically significant clusters at $_{FWE}$ p<0.05. (F) Spider plot displaying mean intra-network Second Gradient values for each of six functional networks, following the (Yeo et al., 2011) parcellation. Values are normalized to the placebo condition (black lines). Blue lines indicate the LSD condition. DN = default network; FPCN = frontoparietal control network; DAN = dorsal attention network; SAL = salience network; SMN = somatomotor network; VIS = visual network.

Second Gradient histograms qualitatively indicated a contraction on the negative (visual) side of the gradient in the LSD condition relative to placebo, suggesting a reduced differentiation of visual

cortex from somatomotor/auditory cortex (Figure 2B). To quantitatively assess this effect, we calculated the difference between each subject's median and minimum Second Gradient value and compared across groups (Figure 2C). This confirmed the presence of a significant visual contraction in the LSD state (t^{28} = -3.8, p<0.001). We then visualized the Second Gradient as a scatter plot, color coded for increases (red) and decreases (blue) in the LSD state (Figure 2D). This indicated that there was a large overlap along the extent of the gradient in terms of decreases and increases, with the exception of the far negative (visual) portion which only exhibited increases.

Next, we quantitatively assessed between-condition differences in gradient score values at both a cortex-wide (Figure 2E) and network-wise (Figure 2F) level. Cortex-wide contrasts revealed increased Second Gradient values (less 'visualness') in the LSD condition in a large portion of visual cortex, especially on its lateral and ventral aspect, and decreased gradient values (greater 'visualness') in a left lateralized region encompassing parts of Wernicke's area and the angular gyrus (Figure 2E). Network-wise differences were assessed according to the Yeo et al. (2011) network parcellation scheme. Results revealed a significant increase in visual networks Second Gradient scores and, at a less stringent significance threshold, significant decreases in the DN and DAN (Figure 2F). Overall, these results are consistent with a reduction in the functional differentiation of visual cortex from both somatomotor/auditory cortex and higher-order association networks.

Principal-second gradient manifold

To visualize the relationship between the Principal and Second Gradient and how this differs across LSD and placebo conditions, we created combined Primary-Second Gradient manifold scatterplots (Figure 3). Qualitative comparisons across conditions display a clear contraction in the Principal Gradient in the LSD state, seen as a lower/less dense transmodal peak on one end and significantly higher (less negative) somatomotor peak on the other. In addition, the visual network notably appears less diffuse in its embedding space distribution in the LSD state, consistent with a subset of its constituent vertices becoming more differentiated from dorsal attention and somatomotor networks.

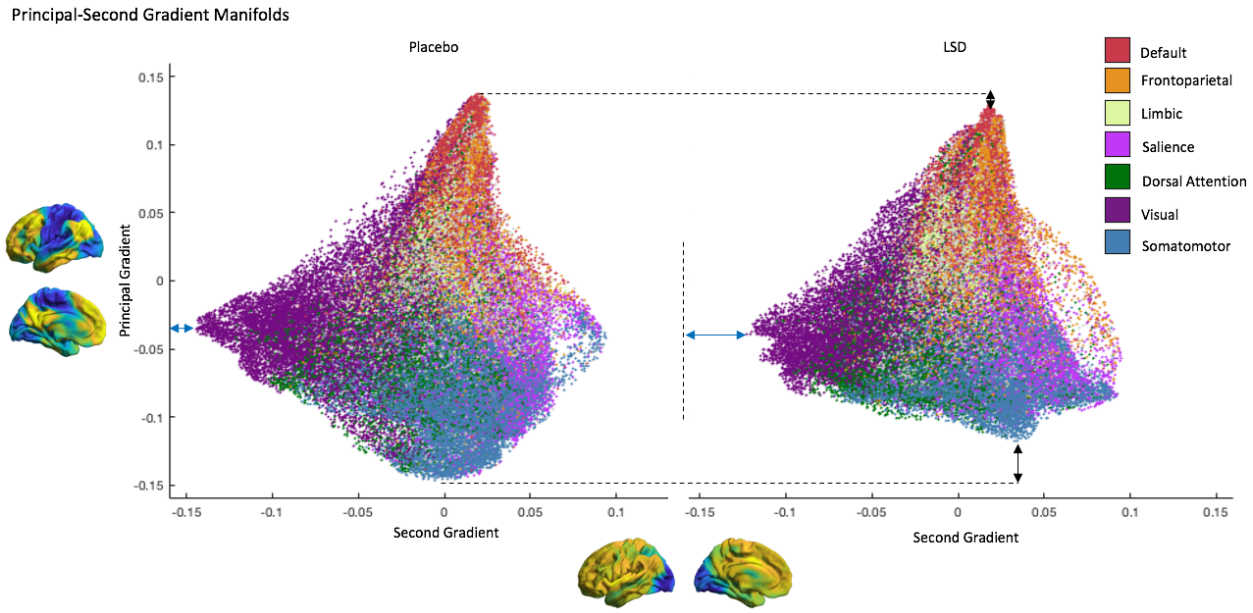

Figure 3. Scatterplots representing the Principal-Second gradient embedding space, for placebo (left) and LSD (right) conditions. Scatter plot colors indicate functional network as per the (Yeo et al., 2011) 7-network parcellation scheme (see inset for legend). Blue arrows indicate gradient contraction on the negative (visual) aspect of the Second Gradient. Black arrows indicate Principal Gradient contraction.

Relationship to subjective measures

Finally, to ascertain the subjective relevance of changes in macroscale gradients during the LSD state, we assessed relationships between LSD-placebo differences in Principal and Second Gradient scores and LSD-placebo differences in two self-report measures which index core components of the psychedelic experience: ego dissolution and complex imagery. Ego dissolution corresponds to the experience that one's sense of self and what is usually perceived as 'not self' (e.g. the outer world) becomes blurred or dissolved completely (Girn & Christoff, 2018; Nour & Carhart-Harris, 2017). Complex imagery corresponds to closed-eye visionary experiences of scenes, landscapes, and images, and is contrasted from simple imagery such as geometric patterns and shapes (Carhart-Harris, Muthukumaraswamy, et al., 2016). Two significant relationships were found: a positive association between the Principal Gradient and ego dissolution and negative association between the Second Gradient and complex imagery (both $_{FWE}$ $p<0.05$; Figure 4A and B). With regard to the Principal Gradient, results indicate that increases in the degree of 'transmodalness' of a large portion of right medial prefrontal cortex/anterior cingulate cortex extending laterally into the superior frontal gyrus was significantly associated with increases in ego dissolution scores. Results pertaining to the Second Gradient indicate that reductions in the degree of 'visualness' of clusters in superior-medial somatomotor cortex and the superior temporal gyrus were significantly associated with reductions in complex imagery scores.

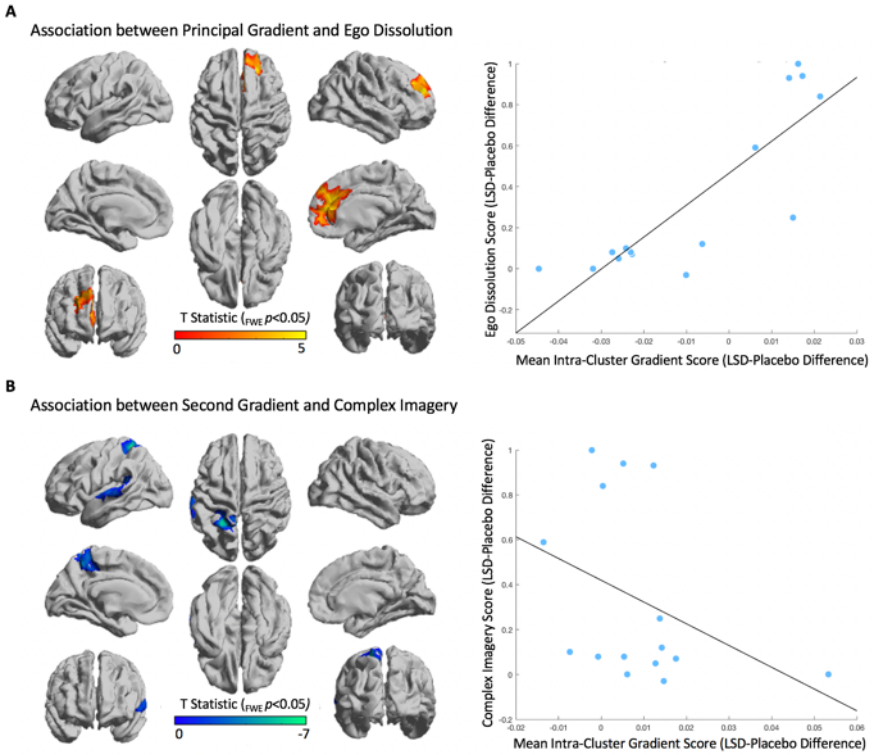

Figure 4. (A) A cluster displaying a significant positive association (r = 0.85) between Principal Gradient scores and self-report scores of ego dissolution (FWE p<0.05). (B) A cluster displaying a significant negative association (r = -0.35) between Second Gradient scores and self-report scores of complex imagery (FWE p<0.05).

Discussion

To investigate LSD-dependent changes in cortical functional organization and to test the hypothesis that LSD attenuates brain hierarchical organization, we applied diffusion map embedding to characterize macroscale cortical gradients in the LSD state. Our results for both LSD and placebo conditions identified the predicted Principle Gradient spanning a hierarchical axis from sensorimotor to transmodal cortex. Between-condition contrasts supported our primary hypothesis: relative to placebo, the Principal Gradient exhibited a significant contraction in the LSD state, reflective of a flatter or less differentiated hierarchical organization. This was primarily driven by increases in the 'transmodalness' of regions comprising the somatomotor network and increases in the 'unimodalness' of regions comprising the default network and frontoparietal control network. We also examined the Second Gradient, which pertains to an axis differentiating visual cortex from somatomotor/auditory cortex. These results revealed a significant contraction on the negative (visual) side of this gradient, driven by a reduction in the 'visualness' of a large portion of occipital cortex - indicating reduced differentiation of this region from the rest of cortex. Results with the Second Gradient also revealed a significant increase in 'visualness' of default and dorsal attention networks. These findings extend previous work suggesting greater whole-brain FC of visual cortices in the LSD-state (Carhart-Harris, Muthukumaraswamy, et al., 2016; Preller

et al., 2018). Finally, we observed significant associations between LSD-related changes in cortical gradients and reported psychedelic experiences. Principal Gradient scores were associated with ego-dissolution, while Second Gradient scores were associated with reports of complex imagery. Collectively, these results provide evidence of significant alterations to whole-brain functional organization in the LSD state, marked by attenuation of hierarchical differentiation between unimodal and transmodal cortices as well as reduced differentiation of visual cortex from somatomotor and association cortex.

The greatest LSD-dependent increases in the unimodal-transmodal hierarchical gradient were in regions at the lowest end of the cortical hierarchy – i.e. the somatomotor cortex, while the greatest decreases occurred within regions at the highest end i.e. regions pertaining to the default and frontoparietal control networks such as the ventral posterior cingulate/retrosplenial cortex, the precuneus, and the superior frontal gyrus. This finding provides evidence for a relatively symmetrical levelling of hierarchical organization under LSD. In other words, both ends of the continuum become more similar to another in their patterns of whole-brain FC. This provides a novel extension to past work. Past neuroimaging investigations with LSD have revealed that it elicits a complex mosaic of spatially-specific increases and decreases in FC (Carhart-Harris, Muthukumaraswamy, et al., 2016; Preller et al., 2018). Within this, a general trend has been found towards increased global integration of individual regions (Preller et al., 2018; Tagliazucchi et al., 2016), which is mirrored at the large-scale network level by reduced within- and increased between- network connectivity (Carhart-Harris, Muthukumaraswamy, et al., 2016). These results suggest that the brain – at both the regional and network level – exhibits a more globally distributed pattern of connectivity in the LSD state. Pertinent for the current context, globally distributed connectivity is a defining characteristic of transmodal cortex (Hong et al., 2019; Paquola et al., 2020; Sepulcre, Sabuncu, Yeo, Liu, & Johnson, 2012; van den Heuvel & Sporns, 2013). Thus, the unimodal-transmodal FC similarity axis derived here may be interpreted as an axis which differentiates the globally distributed FC of transmodal regions from the more local, modular FC of unimodal regions (Hong et al., 2019; Sepulcre et al., 2012). As such, the present results draw attention to a structured non-uniformity in the distributed FC changes elicited by LSD, wherein unimodal regions become more global while transmodal regions become more local.

Our findings also imply a reduction in the number of intervening processing steps between low-level sensorimotor cortex and high-level association cortex. Consistent with this interpretation, resting-state FC fMRI investigations of cortical organization have provided evidence that cortical signals propagate through the macroscale processing hierarchy in a step-wise fashion, moving from modular sensory processing, to multi-modal integration, to higher-order processing within a distributed network of transmodal hubs (Hong et al., 2019; Sepulcre et al., 2012; Vazquez-Rodriguez, Liu, Hagmann, & Misic, 2020). Further, both meta-analytic and task-based analyses have provided evidence that lower levels of the hierarchy pertain to behaviours that are coupled to immediate sensory input, while higher hierarchical zones pertain to perceptually-decoupled and

mnemonically based cognitive processes (Margulies et al., 2016; Murphy et al., 2018). Thus, in the LSD state, the observed levelling of hierarchy may correspond to decreased functional differentiation or, conversely, increased cross-talk, between higher and lower level cortical processing – directly consistent with the predictions of the REBUS model (Carhart-Harris & Friston, 2019).

This conception of dedifferentiation between concrete versus abstract processing is further consistent with the subjective effects of LSD, which can feature a blurring of the internal-external/subject-object distinction alongside an increased influence of internal mentation on perceptual processing (Fox, Girn, Parro, & Christoff, 2018; Girn & Christoff, 2018; Girn, Mills, Roseman, Carhart-Harris, & Christoff, 2020; Kraehenmann et al., 2017). Notably, we observed a significant positive association between Principle Gradient scores and self-reported ego-dissolution within right medial prefrontal/anterior cingulate cortex (mPFC/ACC). This indicates that a greater experience of blurred boundaries between self and world/other was associated with greater 'transmodalness' of this cluster's FC patterns in the LSD state. Interestingly, the mPFC/ACC is strongly implicated in self-referential processing as well as mentalizing about others (D'Argembeau et al., 2007; Dixon, Thiruchselvam, Todd, & Christoff, 2017). This association between hierarchical position and ego-dissolution is particularly noteworthy given past findings that ego-dissolution and related mystical-type phenomena are key mediators of positive outcomes in psychedelic treatment paradigms (Belser et al., 2017; Johnson et al., 2019; Roseman, Nutt, & Carhart-Harris, 2018; Watts, Day, Krzanowski, Nutt, & Carhart-Harris, 2017).

In addition to changes in cortical hierarchy, we examined changes in the Second Gradient of cortical connectivity given that this had been shown to represent the differentiation of visual from somatomotor/auditory cortex (Margulies et al., 2016). This is relevant to the LSD state as significant increases in V1 cerebral blood flow and whole-brain FC have been reported in past studies (Carhart-Harris, Muthukumaraswamy, et al., 2016; Preller et al., 2018). Further, synaesthesia-like experiences, which involve a mixing of sensory modalities, have long been linked to the LSD state (although, see (Terhune et al., 2016)). We identified a significant LSD-dependent reduction in the differentiation of visual cortex from somatomotor/auditory cortex. In addition, intermediary and high-level association networks (the default, frontoparietal control, dorsal attention, and salience networks) all exhibited trends towards increased 'visualness' in the LSD state. While speculative, this pattern of increased cross-talk between visual regions and regions subserving abstract cognitive processing may relate to the vivification of eyes-closed imagery that is characteristic of the psychedelic experience (Carhart-Harris, Muthukumaraswamy, et al., 2016; Kraehenmann et al., 2017; Roseman, 2018). Consistent with this idea, we observed a significant relationship between the 'visualness' of clusters in the superior temporal gyrus and superior-medial somatosensory cortex and complex imagery in the LSD state.

An important limitation to the present results is the relatively small sample size. For this reason, we encourage cautious interpretation of our findings, while also acknowledging that our sample is consistent with previously published functional neuroimaging research with psychedelics (e.g., Carhart-Harris et al., 2012; Carhart-Harris, Muthukumaraswamy, et al., 2016; Lebedev et al., 2016; Lord et al., 2019; Tagliazucchi et al., 2016; Varley et al., 2019). Despite consistent evidence suggesting their safety in controlled research settings (Johnson et al., 2008; Schmid et al., 2015), collecting large datasets with psychedelics is currently difficult due to hurdles pertaining to funding and ethics board approval (Nutt, King, & Nichols, 2013). Thus, our findings primarily serve as a foundational guide for future research in this nascent field and as motivation for replication in larger samples.

LSD is a potent psychedelic compound that elicits a wide variety of subjective effects via 5-HT2A receptor agonism and has been shown to have significant potential in the treatment of mental health conditions (Gasser et al., 2014; Johnson et al., 2019; Krebs & Johansen, 2012). Past investigations into the neural underpinnings of the LSD state revealed a number of spatially distributed changes in functional organization and dynamics, collectively suggestive of a more globally-integrated and 'entropic' mode of neural processing (Carhart-Harris, 2018; Carhart-Harris, Muthukumaraswamy, et al., 2016; Lebedev et al., 2016; Schartner et al., 2017; Tagliazucchi et al., 2016). Here, we extended these findings by revealing that the whole-brain effects of LSD can be conceptualized as a contraction or levelling of the brain's macroscale functional hierarchy – directly in line with a recently proposed unified model of psychedelic brain action (Carhart-Harris & Friston, 2019). Future work is needed to ascertain whether reductions in cortical hierarchy in the acute psychedelic experience relate to findings of rapid and sustained positive changes in relevant mental health parameters observed via psychotherapeutically-mediated experiences with these drugs (Barrett, Doss, Sepeda, Pekar, & Griffiths, 2020; Carhart-Harris, Bolstridge, et al., 2016; Griffiths et al., 2016; Johnson et al., 2019; Ross et al., 2016).

To our knowledge, this is the first investigation which has shown significant state-dependent alterations of macroscale cortical gradients. In particular, we have demonstrated that cortical gradients may be altered by acute pharmacological manipulations and, further, that these changes are closely paralleled by specific changes in conscious experience. These findings therefore shed light on the state-dependent malleability of the macroscale functional organization of the brain. Taken together, this study lends further weight to the view that psychedelics are exceptionally powerful scientific tools for probing mind-brain relationships.

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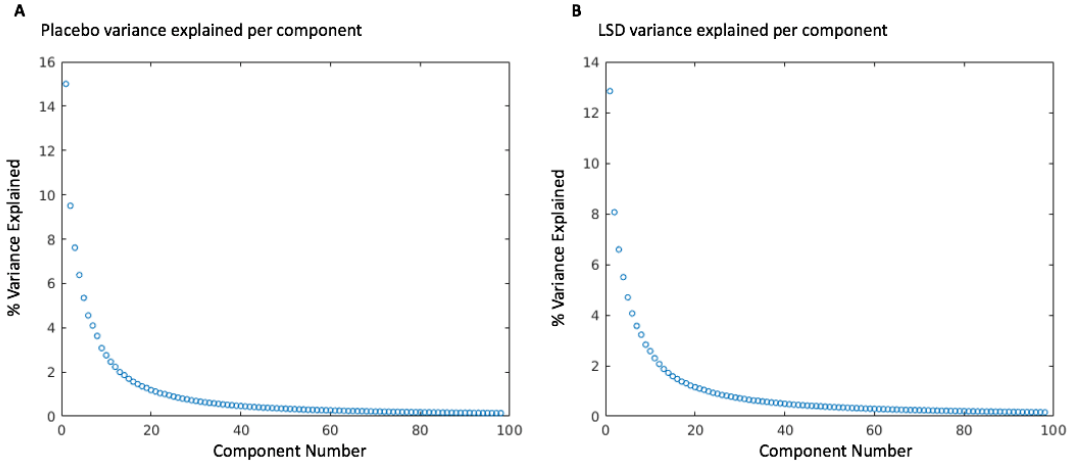
Supplementary Figures



Supplementary Figure 1. Mean variance explained for each embedding component (gradient), for Placebo (A), and LSD (B) conditions.