Electrophysiological effects of psilocybin co-administered with midazolam

Michael H. Sutherland[1,2], Christopher R. Nicholas[3,4], Richard L. Lennertz[1,4], Cody J. Wenthur[4,5], Bryan M. Krause[1], Christina J. Sauder[2], Brady A. Riedner[2,6], Richard F. Smith[2,6], Paul R. Hutson[4,5], Charles L. Raison[2,4], Matthew I. Banks[1,4,7]

[1] Department of Anesthesiology, School of Medicine and Public Health, University of Wisconsin, University of Wisconsin, Madison, WI 53792

[2] Department of Psychiatry, School of Medicine and Public Health, University of Wisconsin, University of Wisconsin, Madison, WI 53719

[3] Department of Family Medicine and Community Health, School of Medicine and Public Health, University of Wisconsin, Madison, WI 53705

[4] Transdisciplinary Center for Research in Psychoactive Substances, University of Wisconsin, Madison, WI 53705

[5] School of Pharmacy, University of Wisconsin, Madison, WI 53705

[6] Wisconsin Institute for Sleep and Consciousness, University of Wisconsin, Madison, WI 53706

[7] Department of Neuroscience, School of Medicine and Public Health, University of Wisconsin, University of Wisconsin, Madison, WI 53705

Running Title: EEG effects of psilocybin co-administered with midazolam

Corresponding author:

Mathew I. Banks, PhD
Department of Anesthesiology
School of Medicine and Public Health
University of Wisconsin-Madison
600 Highland Ave B6/319
Madison, WI 53792
mibanks@wisc.edu

Abstract

The serotonergic psychedelic psilocybin induces glutamatergic neural plasticity and profoundly alters consciousness. Midazolam, a benzodiazepine and positive allosteric modulator of $GABA_A$ receptors, blunts neural plasticity and induces conscious amnesia. In our recent open label pilot study, we co-administered psilocybin (25 mg) with intravenous midazolam, targeting midazolam doses that allowed maintained subjective experience (mild to moderate sedation) while blocking memory encoding. We reported preliminary results from high density scalp electroencephalography (EEG) recorded during dosing session. Here, we used linear mixed effects models to examine changes in EEG band power (delta, theta, alpha, beta, gamma), normalized Lempel Ziv complexity (LZCn), and spectral exponent (SE) as a function of time during the dosing session, and relate these changes to the subjective effects of the drugs measured with the Observer's Assessment of Arousal and Sedation (OAA/S) and the Altered States of Consciousness (ASC) questionnaire. We found that SE, LZCn, and power in all frequency bands changed during drug administration, with an early (15-30 mins) increase in beta power and decrease in SE followed by increases in LZCn and SE and broadband decreases in power over the next 6 hours. OAA/S improved model fits for alpha power while ASC improved the model fits for SE and LZCn. These data are further evidence that the effects of psilocybin are maintained in the presence of midazolam, and that aspects of the subjective experience are captured by EEG measures.

Introduction

Psilocybin, a serotonergic psychedelic, and midazolam, a benzodiazepine sedative and positive allosteric modulator of $GABA_A$ receptors, have divergent functional effects on the brain and subjective experience. Psilocybin induces neural plasticity and metaplasticity (de la Fuente Revenga et al., 2021; Hesselgrave et al., 2021; Nardou et al., 2023; Shao et al., 2021; Vargas et al., 2023) and profoundly alters consciousness (Griffiths et al., 2011; Roseman et al., 2017). Midazolam blunts neural plasticity and induces conscious amnesia (del Cerro et al., 1992; Evans & Viola-McCabe, 1996; Puig-Bosch et al., 2022; Veselis et al., 2009). Recently, we reported the results of an open label pilot study (Nicholas et al., 2024) in which we co-administered psilocybin (25 mg) with midazolam at doses that induced mild to moderate sedation while blocking memory encoding. The subjective effects of the two drugs appeared to combine: the subjective quality of the psychedelic experience was maintained, while memory for the experience could in some cases be blunted. During the dosing session, we monitored brain activity with high density electroencephalography (EEG), sedation level and the intensity of the subjective experience. Preliminary results showed that at least some of the electrophysiological effects (via EEG) of psilocybin were maintained in the presence of midazolam. Here, we report a more in-depth analysis of those EEG effects.

We focus on three EEG metrics that have previously been linked to changes in arousal state and modulation by psychedelics and anesthetic agents: frequency band power, spectral exponent (SE), and Lempel Ziv complexity (LZC). Frequency band-specific changes in EEG signal power associate with changes in arousal and behavioral state (Buzsaki, 2006). For example, psilocybin has been shown to acutely induce a broadband decrease in signal power, with effects especially apparent in reducing the alpha band (8-13 Hz) spectral peak that is often observed in the resting state (Muthukumaraswamy et al., 2013; Ort et al., 2023; Timmermann et al., 2019). Midazolam is also reported to reduce alpha band power, but, in contrast to serotonergic psychedelics, increases delta band (1-4 Hz) and beta band (20-30 Hz) power (Breimer et al., 1990; Hotz et al., 2000). SE describes the decay of the aperiodic component of EEG signal power with frequency. Computational models suggest that SE varies with excitation/inhibition (E/I) balance (Gao et al., 2017; Lombardi et al., 2017). Consistent with these models, SE decreases during sleep (Aamodt et al., 2022; Höhn et al., 2024; Kozhemiako et al., 2022; Lendner et al., 2020) and anesthesia (Colombo et al., 2019; Gao et al., 2017), both of which have been linked to increased GABAergic inhibition (Franks, 2008; Park et al., 2020; Tossell et al., 2023). Conversely, SE increases after administration of serotonergic psychedelics (Muthukumaraswamy & Liley, 2018),

which promote neural excitability via 5-HT_{2A} receptor activation (Banks et al., 2021; Celada et al., 2013; Wallach et al., 2023).

LZC measures the compressibility of time-varying signals by counting the number of non-redundant binary patterns contained in the signal (Lempel & Ziv, 1976), and is associated with the repertoire of brain network states (Sarasso et al., 2021). Similar to SE, LZC reliably decreases with depth of sleep (Aamodt et al., 2021; Aamodt et al., 2022; Abásolo et al., 2015; Andrillon et al., 2016; Schartner, Pigorini, et al., 2017) and anesthesia (Hudetz et al., 2016; Maschke et al., 2024; Schartner et al., 2015; Wenzel et al., 2019; Zhang et al., 2001), and increases upon administration of serotonergic psychedelics (Mediano et al., 2024; Ort et al., 2023; Schartner, Carhart-Harris, et al., 2017; Timmermann et al., 2019).

Here, we measured band power, normalized LZC (LZCn), and SE at several timepoints throughout the dosing session. We sought to test: 1) whether psilocybin-induced changes in band power, LZCn, and SE observed in previous reports with psilocybin monotherapy remain with co-administration of midazolam, and 2) how band power, LZCn, and SE relate to arousal state and the subjective quality of the psychedelic experience during the dosing session, measured using the Observer's Assessment of Arousal and Sedation (OAA/S) (Chernik et al., 1990) and a selection of questions from the Altered States of Consciousness (ASC) questionnaire (Studerus et al., 2010) respectively. We ran linear mixed effect models predicting band power, LZCn, and SE over time, and used nested model comparisons to assess the contribution of OAA/S and ASC measurements to predicting band power, LZCn, and SE. We found significant changes in beta power and SE shortly after midazolam administration before the onset of psilocybin's subjective effects, as well as changes in delta, theta, and alpha power, and LZCn and SE once psilocybin's subjective effects began. This suggests that band power, LZCn, and SE track acute neural effects of psilocybin when co-administered with midazolam. Moreover, adding ASC scores to the models improved model fit, explaining variance in LZCn and SE, but not band power, beyond time and OAA/S. This suggests that aspects of the subjective experience of psilocybin may be explained by changes in LZCn and SE.

Methods

Dosing session and behavioral measures

Psilocybin and midazolam were co-administered to 8 healthy participants. Subjects were administered an oral 25 mg dose of psilocybin and IV midazolam doses targeting plasma concentrations of 25-95 mg/ml. The oral psilocybin and initial IV midazolam bolus were simultaneous. The initial midazolam bolus was chosen based on subject age and weight, with additional bolus adjustments over the next 3.5 hours to maintain mild to moderate sedation with memory impairment. Memory impairment was assessed with California Verbal Learning Test (CVLT) [(Woods et al., 2006)], with the goal of achieving memory impairment such that CVLT scores were < 25%. Sedation was measured by scores on the Observer's Assessment of Alertness/Sedation Scale (OAA/S) [(Chernik et al., 1990)], with the goal of maintaining arousal level ≥ 3. OAA/S measurements were collected repeatedly between 5- and 210-minutes post-dosing. Subjective experience was assessed with 6 items from the Altered States of Consciousness (ASC) questionnaire (Studerus et al., 2010) previously shown to predict antidepressant effect induced by psilocybin (Roseman et al., 2017). ASC items were collected at 60-, 90-, 120-, 160-, 210-, and 360-minutes post-dosing. For more details and a diagram outlining the dosing session, see (Nicholas et al., 2024).

EEG and behavioral assessments

High-density EEG (hdEEG) was collected continuously from 20-minutes pre-dosing to 360-minutes post-dosing using a NetAmps 300 amplifier, NetStation software, and 256 channels sampled at 500Hz (Electrical Geodesics, Inc., Eugene, OR). From these data, 11 time points were chosen throughout the dosing session for hdEEG analysis: two baseline time points – music-on and music-off – and 9 post-dosing time points: 15-, 30-, 60-, 90-, 120-, 130-, 160-, 210-, and 360-minutes. Time points were chosen based on continuous (relatively) artifact free hdEEG and to match time points in which behavioral measures (i.e. OAA/S and ASC) were assessed. All time points were periods with eyes-closed, and post-dosing time points were periods with music-on except for 30 and 120 minutes, which were music-off. In each hdEEG time point, 0.11-8.92 minutes of data immediately preceding the stated time was used for analysis (mean 4.04 minutes).

Preprocessing of high-density EEG data

Preprocesssing of EEG data was done using MATLAB and EEGLAB (Delorme & Makeig, 2004) software. Electrodes located on the face or neck were excluded leaving 185 channels per subject. A first-order high-pass (single pole IIR, 0.1 Hz) and band-pass filter (2-way least squares FIR, 1–50 Hz) were applied. Bad channels and artifactual segments were rejected by visual inspection using EEGLAB. EEG data were denoised using Adaptive Mixture ICA (AMICA) in EEGLAB. Data were then visually inspected in component space and if artifactual segments remained, artifact rejection was performed again on the original data. If necessary, AMICA was run again on the original data with the additional noisy segments removed. Components labelled as eye, muscle, or channel artifact, and those labelled as neural but below a confidence threshold of 85% were removed. Rejected channels were then interpolated using spherical splines method and average referenced.

Band power

Power spectral density (PSD) of the EEG signal was performed using Welch's method with 4-second nonoverlapping windows. The PSD was separated into canonical bands: delta 1-4 Hz, theta 4-8 Hz, alpha 8-14 Hz, beta 14-30 Hz, and gamma 30-50 Hz. We computed the log10(power) and averaged across frequencies within each band.

Lempel Ziv complexity

Brain signal diversity was measured using time-domain LZC (Lempel & Ziv, 1976). For each channel, the hdEEG data were first split up into 4 second nonoverlapping segments (2000 samples each). The signal (each segment of each channel separately) was binarized using the median amplitude of the Hilbert transform and converted to a string. LZC of the binarized string was computed with the LZ76 algorithm (Lempel & Ziv, 1976). Starting from the beginning of the string, the algorithm iteratively compares a "search" substring to all prior substrings seen in the signal. If the search substring is already contained in earlier substring patterns, the search window extends. If the search substring is a unique pattern, the complexity count is increased by one. The search continues until the entire string has been assessed. The total complexity count was then normalized by dividing by the string's theoretical upper bound, $N/\log_2(N)$, where N is the length of the segmented signal. This returns a value, LZC, which typically falls between 0 and 1. To assess LZC above and beyond the influence of spectral power, a phase-shuffled surrogate signal was created by computing the Fourier transform of the original signal, randomly shuffling its phase, and computing the inverse Fourier transform to return to the time domain (Schartner et al., 2015). The same binarization and LZC computation procedure followed as above. This

process was repeated 100 times, and LZC was normalized by the average phase-shuffled LZC, returning the measure LZCn.

Spectral exponent

Spectral exponent (SE) was computed with the FOOOF algorithm (Donoghue et al., 2020). First, each 4 second PSD segment (frequency range 1 – 50 Hz) was fit with an estimation of its power law (aperiodic) component by taking the slope between power values at the first and last frequencies of the spectrum in log-log space. This fit is then subtracted from the PSD, leaving a flattened spectrum without the aperiodic component. A threshold of the 2.5 percentile of the flattened spectrum is defined, and any power values falling below the threshold are considered part of the aperiodic component. A second fit is then performed on the PSD by taking the slope between all power values within the aperiodic component. This component is removed from the PSD, leaving a flattened spectrum thought to contain periodic components and noise. The maximum peak in the flattened spectrum is then found and fit with a Gaussian if it exceeds a defined threshold of 2.2 standard deviations of the flattened spectrum. The fit Gaussian is removed from the spectrum, and the algorithm begins its peak search again. The search continues until no more peaks in the flattened spectrum exceed the threshold. The Gaussian fits are combined to create a periodic fit which is subtracted from the original PSD. A final aperiodic fit is then performed on the remaining PSD which now contains a full spectrum aperiodic component estimation. SE was taken as the – (final fit).

Statistical modeling

Statistical analysis was performed using linear mixed effects models (nlme package) in R. To test the influence of time on band power, LZCn, and SE, we ran models predicting each measure using time and music as fixed factors and subjects as a random effect. To account for serial autocorrelation within subjects across time, we included a continuous-time first order autoregressive covariance structure (corCAR1). Music was chosen as a fixed factor due to preliminary analyses showing music not differentially affecting band power, LZC nor SE in separate time points. Before model fitting, band power, LZCn and SE were averaged across EEG channels per time point. Post model fitting, marginal mean band power, LZCn, and SE values for each time point and contrasts between time points were estimated using the emmeans package in R. Multivariate t distributions were used to correct for multiple comparisons between time points. To test the influence of OAA/S and ASC on band power, LZCn, and SE, OAA/S measurements were first added to the model as

a fixed effect. Then, ASC was added to the model as a fixed effect to assess the influence of ASC above and beyond time and OAA/S. To assess the contribution of OAA/S and ASC to the model fit, models were compared with likelihood ratio tests.

Results

Administration of psilocybin with midazolam revealed consistent changes in EEG power spectral density (**Figure 1A**). Band- and time point-specific changes were identified using linear mixed effect models. To assess the influence of time on band power, estimated marginal mean power values from each band were averaged across channels and plotted across time (**Figure 1B-F**). Time had a significant effect on all bands (all $p < 0.0001$). For each band, post hoc comparisons were computed between post-dosing time points and baseline (**Supplementary Table 1**). Delta power significantly decreased at all time points between 60 – 360 minutes. Theta and alpha power decreased at all post-dosing time points. Beta power increased at 15 and 30 minutes and decreased at 160- through 360-minutes. Gamma power decreased at 30-, 60-, and 120- minutes. We then compared the influence of sedation (OAA/S) and subjective intensity (ASC) on band power. First, we added OAA/S to the previous models to compare model fits with and without OAA/S using a likelihood ratio test. OAA/S improved model fits for alpha power only ($p = 0.0367$). There was no effect of including ASC to the model for any bands. Next, we ran linear mixed effect models predicting band power with time as a fixed effect and subject as a random effect (as stated above), but for each EEG electrode separately.

Marginal mean power values for each channel are depicted on separate topo plots for each band and time point separately (**Figure 2**). Changes in power throughout dosing day were significant globally. This was especially the case with delta, theta, and alpha power, which maximally decreased between 120- through 210- minutes, and beta power, which significantly increased at 15 minutes post-dosing.

LZCn and SE consistently changed after administration of psilocybin with midazolam (**Figure 3**). A linear mixed effect model was run to assess the influence of time and music

on LZCn, and the estimated marginal means were plotted across time (**Figure 3A**). Time had a significant effect on LZCn ($p < 0.0001$), whereas music did not ($p = 0.05958$). After running post-hoc comparisons between post-dosing time points and pre-dosing baseline, we found LZCn to be significantly increased from baseline at 30- through 210- minutes post-dosing, returning to baseline at 360 minutes (**Supplementary Table 2**). We then assessed the influence of OAA/S and ASC on LZCn by including each measurement to the model as a fixed effect. When comparing models with each additional measurement to null models using a likelihood ratio test, OAA/S failed to improve the model fit for LZCn ($p = 0.3160$). By contrast, ASC significantly improved the model fit for LZCn ($p = 0.0332$). Model prediction of LZCn was plotted against the significant predictor ASC (**Figure 3B**), showing that increased ASC scores predicted increased LZCn. A linear mixed effect model was then run to predict SE with time and music as fixed effects and subject as random effect. Marginal means are plotted across time (**Figure 3C**), showing an increase in SE (i.e., a flatter spectrum) during the dosing session. Time ($p < 0.0001$) but not music (0.8948) had a significant effect on predicting SE. After running posthoc tests comparing post-dosing time points to baseline, we found a significant decrease from baseline at 15 minutes and significant increases at 90- through 360- minutes (**Supplementary Table 3**). After assessing the additional contribution of OAA/S and ASC to SE beyond time, OAA/S failed to improve model fit ($p = 0.4140$) whereas ASC improved model fit for SE ($p = 0.0015$). The relationship of the model-predicted SE versus the predictor ASC is shown in **Figure 3D**, showing that increased ASC scores predicted increased SE.

Discussion

Summary

Changes in EEG spectral band power, complexity, and spectral exponent have previously been used to track changes in arousal, cognition, and consciousness, providing a link between neurophysiology and behavior (Aamodt et al., 2022; Buzsáki, 2006; Colombo et al., 2019; Lendner et al., 2020; Muthukumaraswamy et al., 2013; Muthukumaraswamy & Liley, 2018; Ort et al., 2023; Schartner et al., 2015; Schartner, Carhart-Harris, et al., 2017; Timmermann et al., 2019). Here, we show that the concurrent administration of psilocybin and midazolam in healthy individuals precedes changes in neural activity and phenomenology in a manner that is broadly consistent with these previous results. Broadband spectral power decreased during the dosing session, largely driven by decreases in delta, theta, and alpha bands. We observed a transient elevation of beta

power that peaked at 15 minutes, when midazolam was already estimated to reach its steady plasma target concentration but before the subjective effects of psilocybin were apparent. LZCn increased during the dosing session before returning to baseline at 360 minutes, consistent with the time course of the acute effects of psilocybin. SE decreased immediately post-dosing and then increased throughout the dosing session, remaining elevated at 360 minutes, broadly consistent with expected effects of both psilocybin and midazolam.

Band power

At 15 minutes post-dosing, the most prominent effect of band power was an increase in beta power, although theta and alpha power were decreased as well, in agreement with prior studies on the effects of midazolam alone on EEG signal power (Breimer et al., 1990; Hotz et al., 2000). Once the effects of psilocybin became apparent, all bands decreased in power during the dosing session and remained decreased at 360 minutes except for gamma, which only decreased at 30-, 60-, and 120- minutes. Alpha power has been associated with the subjective effects of psilocybin, consistently decreasing following the time course of acute drug effect (Muthukumaraswamy et al., 2013; Ort et al., 2023; Schartner, Carhart-Harris, et al., 2017; Timmermann et al., 2019). Here, alpha power reached its nadir at 120 minutes, consistent with a typical time-window of peak subjective effects. However, including ASC scores in our model predicting alpha power did not significantly improve model fit. By contrast, we found that including ASC scores to our models predicting LZCn and SE did significantly improve model fit. For LZCn, this tracks with prior research finding (weak) correlations between LZC and various subjective questionnaire responses (Schartner, Carhart-Harris, et al., 2017; Timmermann et al., 2019). To our knowledge, this is the first report showing a significant relationship between aspects of subjective experience and SE (e.g. (Aamodt et al., 2022)) as opposed to the capacity for consciousness more broadly (Colombo et al., 2019; Zhao et al., 2025). Taken together, these results confirm the initial analysis done in our pilot paper and provides further support for the idea that although midazolam blocked some aspects of memory during and after the acute experience, the subjective experience itself was not attenuated.

LZCn

LZCn has previously been shown to decrease with anesthetics and sleep depth and increase with serotonergic psychedelics (Aamodt et al., 2021; Aamodt et al., 2022; Abásolo et al., 2015; Andrillon et al., 2016; Hudetz et al., 2016; Maschke et al., 2024; Mediano et al.,

2024; Ort et al., 2023; Sarasso et al., 2021; Schartner et al., 2015; Schartner, Carhart-Harris, et al., 2017; Schartner, Pigorini, et al., 2017; Timmermann et al., 2019; Wenzel et al., 2019; Zhang et al., 2001), likely reflecting changes in the temporal differentiation of underlying neural activity and the repertoire of network states. Given that deep sleep and some anesthetics are linked to enhanced GABAergic inhibition (Franks, 2008; Park et al., 2020; Tossell et al., 2023) and show decreased LZC, midazolam, through its $GABA_A$ receptor modulation, is also expected to decrease LZC. This decrease in complexity with midazolam administration has been shown previously in spontaneous EEG (Murphy et al., 2023) and TMS evoked EEG (Ferrarelli et al., 2010), although this is not always the case (Puglia et al., 2021). Serotonergic psychedelics, on the other hand, likely increase LZC through their purported 5-HT2A receptor-dependent increase in neural excitability and network connectivity (Banks et al., 2021; Celada et al., 2013; Siegel et al., 2024; Wallach et al., 2023). Given the contrasting mechanisms of each drug, it was unclear what would transpire when co-administered. Our observation of an increase in LZCn point to the pro-excitatory effects of the drug combination winning out, although implications for psilocybin induced plasticity remain unclear. We also found that including ASC scores in our model improved model fit for predicting LZCn. The relationship between LZC and subjective experience has been posited before, with the argument that LZC may broadly measure the diversity of network states, so if LZC increases under administration of psychedelics, perhaps this is what underlies the increased diversity of conscious states when compared to wake (Carhart-Harris, 2018). Some evidence points to this by correlating LZC with aspects of the subjective experience of psychedelics (e.g. richness or intensity of experience) (Schartner, Carhart-Harris, et al., 2017; Timmermann et al., 2019), however, correlations are weak or minimal. Additionally, one study assessing the complexity of TMS evoked EEG responses found no change in the Perturbational Complexity Index (PCI) but found an increase in spontaneous LZC when compared to wake (Ort et al., 2023). PCI is arguably the most accurate measure of level of consciousness to date (Casali et al., 2013), suggesting that the psychedelic-induced increase in spontaneous LZC does not have to do with level of consciousness, broadly. Moreover, another prior study tested whether LZC could distinguish between within-state NREM2 sleep dream recall vs no dream recall upon awakening (Aamodt et al., 2022). There was no difference in LZC between conscious and unconscious sleepers, and LZC did not track aspects of the dream experience within the dream recall group. Taken together, these results suggest that LZC is not always reliable tracker of subjective experience. This could be due to LZC being a rather coarse measure, missing out on changes to neural dynamics that don't survive the binarization, or it could be due to flawed assumptions between diversity of spontaneous neural activity tracking the diversity of consciousness.

SE

SE has previously been shown to decrease in sleep and anesthesia (Aamodt et al., 2022; Colombo et al., 2019; Gao et al., 2017; Höhn et al., 2024; Kozhemiako et al., 2022; Lendner et al., 2020). To our knowledge, only one study to date applied SE to data recorded during administration of serotonergic psychedelics, and it showed an increase from baseline resting state (Muthukumaraswamy & Liley, 2018). SE has been linked to E/I balance in computational modeling (Gao et al., 2017; Lombardi et al., 2017), and SE changes in contexts of purported shifts in E/I balance, e.g., decreasing with shifts toward more inhibition. Our results track this, as midazolam's GABAergic action is expected to shift this balance toward inhibition, with concomitant decreases in neural plasticity and memory. SE, unlike LZCn, significantly decreased at 15 minutes post-dosing, where brain activity is more likely to reflect the effects of midazolam without contributions from psilocybin. However, our behavioral measure of midazolam sedation, OAA/S, remained an insignificant contributor in predicting SE, which may reflect the lower dose requirement for amnesia compared to sedation (Veselis et al., 2009). Additionally, unlike LZCn, SE remained significantly elevated at 360 minutes, consistent with elevated subjective effects of psilocybin observed in 4/8 participants at 360 minutes (Nicholas et al., 2024) and the significant contribution of ASC to predicting SE. Broadband SE has been shown to correlate with level of consciousness, specifically being higher in states in which conscious report is available (e.g., typical waking consciousness, dreaming, ketamine anesthesia) vs unavailable (e.g., under propofol anesthesia) (Colombo et al., 2019). We note, however, that more rigorous analysis is required to assess the direct electrophysiological contributors to SE.

Limitations

There are several important limitations in this study that should be considered when interpreting results. The open-label design, small sample size, and substantial individual variability observed among participants limit generalizability. As the goal of the pilot study was finding an optimal midazolam dose to induce amnesia while allowing the psychedelic experience to unfold, different dosing regimens were used across participants, with the second 4 receiving higher doses than the first 4. This variability presented an opportunity, however, which we leveraged here to investigate the potential interactions between midazolam and psilocybin.

The LZC and SE measures employed also have inherent limitations. LZC requires binarization of the data that may result in information loss and reduced sensitivity.

Moreover, LZC is influenced by changes in spectral power, especially in the low frequencies, though here we accounted for this by normalizing with a phase-shuffled surrogate signal. Low frequency power is also thought to contribute substantially to SE, especially when fitting broadband exponents. Moreover, SE is subject to contamination by scalp muscle activity which may not be entirely removed during preprocessing, as suggested by a recent study investigating the effect on SE of changes in muscle tone during sleep (Kozhemiako et al., 2022). In the current study, we do not expect dramatic changes in scalp muscle tone, but further investigation is required.

Conclusions

Previous evidence suggests that psilocybin and midazolam exert opposing electrophysiological effects. We tested the effects of the co-administration of psilocybin and midazolam on the common EEG measures of band power, LZCn, and SE. We found decreased broadband power, increased LZCn, and increased SE throughout the dosing session, with LZCn and SE being at least partially dependent on the subjective quality of the psychedelic experience. In our upcoming randomized, placebo-controlled follow-up study (RECAP2, https://clinicaltrials.gov/study/NCT06692192), we will systematically investigate the acute and post-acute electrophysiological effects of psilocybin and midazolam utilizing resting state and TMS-evoked EEG activity, and link these neurophysiological effects to subjective experience and long-term behavioral outcomes of psilocybin.

References

Aamodt, A., Nilsen, A. S., Thürer, B., Moghadam, F. H., Kauppi, N., Juel, B. E., & Storm, J. F. (2021). EEG Signal Diversity Varies With Sleep Stage and Aspects of Dream Experience. *Front Psychol*, *12*, 655884. https://doi.org/10.3389/fpsyg.2021.655884

Aamodt, A., Sevenius Nilsen, A., Markhus, R., Kusztor, A., HasanzadehMoghadam, F., Kauppi, N., Thürer, B., Storm, J. F., & Juel, B. E. (2022). EEG Lempel-Ziv complexity varies with sleep stage, but does not seem to track dream experience. *Front Hum Neurosci*, *16*, 987714. https://doi.org/10.3389/fnhum.2022.987714

Abásolo, D., Simons, S., Morgado da Silva, R., Tononi, G., & Vyazovskiy, V. V. (2015). Lempel-Ziv complexity of cortical activity during sleep and waking in rats. *J Neurophysiol*, *113*(7), 2742-2752. https://doi.org/10.1152/jn.00575.2014

Andrillon, T., Poulsen, A. T., Hansen, L. K., Léger, D., & Kouider, S. (2016). Neural Markers of Responsiveness to the Environment in Human Sleep. *J Neurosci*, *36*(24), 6583-6596. https://doi.org/10.1523/jneurosci.0902-16.2016

Banks, M. I., Zahid, Z., Jones, N. T., Sultan, Z. W., & Wenthur, C. J. (2021). Catalysts for change: the cellular neurobiology of psychedelics. *Mol Biol Cell*, *32*(12), 1135-1144. https://doi.org/10.1091/mbc.E20-05-0340

Breimer, L. T., Hennis, P. J., Burm, A. G., Danhof, M., Bovill, J. G., Spierdijk, J., & Vletter, A. A. (1990). Quantification of the EEG effect of midazolam by aperiodic analysis in volunteers. Pharmacokinetic/pharmacodynamic modelling. *Clin Pharmacokinet*, *18*(3), 245-253. https://doi.org/10.2165/00003088-199018030-00006

Buzsaki, G. (2006). *Rhythms of the Brain*. Oxford University Press.

Buzsáki, G. (2006). *Rhythms of the Brain*. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780195301069.001.0001

Carhart-Harris, R. L. (2018). The entropic brain - revisited. *Neuropharmacology*, *142*, 167-178. https://doi.org/10.1016/j.neuropharm.2018.03.010

Casali, A. G., Gosseries, O., Rosanova, M., Boly, M., Sarasso, S., Casali, K. R., Casarotto, S., Bruno, M. A., Laureys, S., Tononi, G., & Massimini, M. (2013). A theoretically based index of consciousness independent of sensory processing and behavior. *Sci Transl Med*, *5*(198), 198ra105. https://doi.org/10.1126/scitranslmed.3006294

Celada, P., Puig, M. V., & Artigas, F. (2013). Serotonin modulation of cortical neurons and networks. *Front Integr Neurosci*, *7*, 25. https://doi.org/10.3389/fnint.2013.00025

Chernik, D. A., Gillings, D., Laine, H., Hendler, J., Silver, J. M., Davidson, A. B., Schwam, E. M., & Siegel, J. L. (1990). Validity and reliability of the Observer's Assessment of Alertness/Sedation Scale: study with intravenous midazolam. *J Clin Psychopharmacol*, *10*(4), 244-251. https://www.ncbi.nlm.nih.gov/pubmed/2286697

Colombo, M. A., Napolitani, M., Boly, M., Gosseries, O., Casarotto, S., Rosanova, M., Brichant, J. F., Boveroux, P., Rex, S., Laureys, S., Massimini, M., Chieregato, A., & Sarasso, S. (2019). The spectral exponent of the resting EEG indexes the presence of consciousness during unresponsiveness induced by propofol, xenon, and ketamine. *Neuroimage*, *189*, 631-644. https://doi.org/10.1016/j.neuroimage.2019.01.024

de la Fuente Revenga, M., Zhu, B., Guevara, C. A., Naler, L. B., Saunders, J. M., Zhou, Z., Toneatti, R., Sierra, S., Wolstenholme, J. T., Beardsley, P. M., Huntley, G. W., Lu, C., & González-Maeso, J. (2021). Prolonged epigenomic and synaptic plasticity alterations following single exposure to a psychedelic in mice. *Cell Rep*, *37*(3), 109836. https://doi.org/10.1016/j.celrep.2021.109836

del Cerro, S., Jung, M., & Lynch, G. (1992). Benzodiazepines block long-term potentiation in slices of hippocampus and piriform cortex. *Neuroscience*, *49*(1), 1-6. (Not in File)

Delorme, A., & Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J Neurosci Methods*, *134*(1), 9-21. https://doi.org/10.1016/j.jneumeth.2003.10.009

Donoghue, T., Haller, M., Peterson, E. J., Varma, P., Sebastian, P., Gao, R., Noto, T., Lara, A. H., Wallis, J. D., Knight, R. T., Shestyuk, A., & Voytek, B. (2020). Parameterizing neural power spectra into periodic and aperiodic components. *Nat Neurosci*, *23*(12), 1655-1665. https://doi.org/10.1038/s41593-020-00744-x

Evans, M. S., & Viola-McCabe, K. E. (1996). Midazolam inhibits long-term potentiation through modulation of GABAA receptors. *Neuropharmacology*, *35*(3), 347-357. https://doi.org/10.1016/0028-3908(95)00182-4

Ferrarelli, F., Massimini, M., Sarasso, S., Casali, A., Riedner, B. A., Angelini, G., Tononi, G., & Pearce, R. A. (2010). Breakdown in cortical effective connectivity during midazolam-induced loss of consciousness. *Proc Natl Acad Sci USA*, *107*(6), 2681-2686. https://doi.org/0913008107 [pii];10.1073/pnas.0913008107 [doi] (Not in File)

Franks, N. P. (2008). General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. *Nat Rev Neurosci*, *9*(5), 370-386. https://doi.org/10.1038/nrn2372

Gao, R., Peterson, E. J., & Voytek, B. (2017). Inferring synaptic excitation/inhibition balance from field potentials. *Neuroimage*, *158*, 70-78. https://doi.org/10.1016/j.neuroimage.2017.06.078

Griffiths, R. R., Johnson, M. W., Richards, W. A., Richards, B. D., McCann, U., & Jesse, R. (2011). Psilocybin occasioned mystical-type experiences: immediate and persisting dose-related effects. *Psychopharmacology (Berl)*, *218*(4), 649-665. https://doi.org/10.1007/s00213-011-2358-5

Hesselgrave, N., Troppoli, T. A., Wulff, A. B., Cole, A. B., & Thompson, S. M. (2021). Harnessing psilocybin: antidepressant-like behavioral and synaptic actions of psilocybin are independent of 5-HT2R activation in mice. *Proc Natl Acad Sci U S A*, *118*(17). https://doi.org/10.1073/pnas.2022489118

Höhn, C., Hahn, M. A., Lendner, J. D., & Hoedlmoser, K. (2024). Spectral Slope and Lempel-Ziv Complexity as Robust Markers of Brain States during Sleep and Wakefulness. *eNeuro*, *11*(3). https://doi.org/10.1523/eneuro.0259-23.2024

Hotz, M. A., Ritz, R., Linder, L., Scollo-Lavizzari, G., & Haefeli, W. E. (2000). Auditory and electroencephalographic effects of midazolam and alpha-hydroxy-midazolam in healthy subjects. *British Journal of Clinical Pharmacology*, *49*(1), 72-79. (Not in File)

Hudetz, A. G., Liu, X., Pillay, S., Boly, M., & Tononi, G. (2016). Propofol anesthesia reduces Lempel-Ziv complexity of spontaneous brain activity in rats. *Neurosci Lett*, *628*, 132-135. https://doi.org/10.1016/j.neulet.2016.06.017

Kozhemiako, N., Mylonas, D., Pan, J. Q., Prerau, M. J., Redline, S., & Purcell, S. M. (2022). Sources of Variation in the Spectral Slope of the Sleep EEG. *eNeuro*, *9*(5). https://doi.org/10.1523/ENEURO.0094-22.2022

Lempel, A., & Ziv, J. (1976). On the Complexity of Finite Sequences [Article]. *Ieee Transactions on Information Theory*, *22*(1), 75-81. https://doi.org/10.1109/tit.1976.1055501

Lendner, J. D., Helfrich, R. F., Mander, B. A., Romundstad, L., Lin, J. J., Walker, M. P., Larsson, P. G., & Knight, R. T. (2020). An electrophysiological marker of arousal level in humans. *Elife*, *9*. https://doi.org/10.7554/eLife.55092

Lombardi, F., Herrmann, H. J., & de Arcangelis, L. (2017). Balance of excitation and inhibition determines 1/f power spectrum in neuronal networks. *Chaos*, *27*(4), 047402. https://doi.org/10.1063/1.4979043

Maschke, C., O'Byrne, J., Colombo, M. A., Boly, M., Gosseries, O., Laureys, S., Rosanova, M., Jerbi, K., & Blain-Moraes, S. (2024). Critical dynamics in spontaneous EEG predict anesthetic-induced loss of consciousness and perturbational complexity. *Commun Biol*, *7*(1), 946. https://doi.org/10.1038/s42003-024-06613-8

Mediano, P. A. M., Rosas, F. E., Timmermann, C., Roseman, L., Nutt, D. J., Feilding, A., Kaelen, M., Kringelbach, M. L., Barrett, A. B., Seth, A. K., Muthukumaraswamy, S., Bor, D., & Carhart-Harris, R. L. (2024). Effects of External Stimulation on Psychedelic State Neurodynamics. *ACS Chem Neurosci*, *15*(3), 462-471. https://doi.org/10.1021/acschemneuro.3c00289

Murphy, N., Tamman, A. J. F., Lijffijt, M., Amarneh, D., Iqbal, S., Swann, A., Averill, L. A., O'Brien, B., & Mathew, S. J. (2023). Neural complexity EEG biomarkers of rapid and post-rapid ketamine effects in late-life treatment-resistant depression: a randomized control trial. *Neuropsychopharmacology*, *48*(11), 1586-1593. https://doi.org/10.1038/s41386-023-01586-4

Muthukumaraswamy, S. D., Carhart-Harris, R. L., Moran, R. J., Brookes, M. J., Williams, T. M., Errtizoe, D., Sessa, B., Papadopoulos, A., Bolstridge, M., Singh, K. D., Feilding, A., Friston, K. J., & Nutt, D. J. (2013). Broadband cortical desynchronization underlies the human psychedelic state. *J Neurosci*, *33*(38), 15171-15183. https://doi.org/10.1523/jneurosci.2063-13.2013

Muthukumaraswamy, S. D., & Liley, D. T. (2018). 1/f electrophysiological spectra in resting and drug-induced states can be explained by the dynamics of multiple oscillatory relaxation processes. *Neuroimage*, *179*, 582-595. https://doi.org/10.1016/j.neuroimage.2018.06.068

Nardou, R., Sawyer, E., Song, Y. J., Wilkinson, M., Padovan-Hernandez, Y., de Deus, J. L., Wright, N., Lama, C., Faltin, S., Goff, L. A., Stein-O'Brien, G. L., & Dölen, G. (2023). Psychedelics reopen the social reward learning critical period. *Nature*, *618*(7966), 790-798. https://doi.org/10.1038/s41586-023-06204-3

Nicholas, C. R., Banks, M. I., Lennertz, R. L., Wenthur, C. J., Krause, B. M., Riedner, B. R., Smith, R. F., Hutson, P. R., Sauder, C. J., Dunne, J. D., & Raison, C. L. (2024). Co-administration of midazolam and psilocybin: Differential effects on subjective quality versus memory of the psychedelic experience. *bioRxiv*, 2024.2006.2013.598878. https://doi.org/10.1101/2024.06.13.598878

Ort, A., Smallridge, J. W., Sarasso, S., Casarotto, S., von Rotz, R., Casanova, A., Seifritz, E., Preller, K. H., Tononi, G., & Vollenweider, F. X. (2023). TMS-EEG and resting-state EEG applied to altered states of consciousness: oscillations, complexity, and phenomenology. *iScience*, *26*(5), 106589. https://doi.org/10.1016/j.isci.2023.106589

Park, S., Kang, I., Edden, R. A. E., Namgung, E., Kim, J., & Kim, J. (2020). Shorter sleep duration is associated with lower GABA levels in the anterior cingulate cortex. *Sleep Med*, *71*, 1-7. https://doi.org/10.1016/j.sleep.2020.02.018

Puglia, M. P., Li, D., Leis, A. M., Jewell, E. S., Kaplan, C. M., Therrian, M., Kim, M., Lee, U., Mashour, G. A., & Vlisides, P. E. (2021). Neurophysiologic Complexity in Children Increases with Developmental Age and Is Reduced by General Anesthesia. *Anesthesiology*, *135*(5), 813-828. https://doi.org/10.1097/aln.0000000000003929

Puig-Bosch, X., Bieletzki, S., Zeilhofer, H. U., Rudolph, U., Antkowiak, B., & Rammes, G. (2022). Midazolam at Low Nanomolar Concentrations Affects Long-term Potentiation and Synaptic Transmission Predominantly via the alpha1-gamma-

Aminobutyric Acid Type A Receptor Subunit in Mice. *Anesthesiology*, *136*(6), 954-969. https://doi.org/10.1097/ALN.0000000000004202

Roseman, L., Nutt, D. J., & Carhart-Harris, R. L. (2017). Quality of Acute Psychedelic Experience Predicts Therapeutic Efficacy of Psilocybin for Treatment-Resistant Depression. *Front Pharmacol*, *8*, 974. https://doi.org/10.3389/fphar.2017.00974

Sarasso, S., Casali, A. G., Casarotto, S., Rosanova, M., Sinigaglia, C., & Massimini, M. (2021). Consciousness and complexity: a consilience of evidence. *Neurosci Conscious*, *7*(2), 1-24. https://doi.org/10.1093/nc/niab023

Schartner, M., Seth, A., Noirhomme, Q., Boly, M., Bruno, M. A., Laureys, S., & Barrett, A. (2015). Complexity of Multi-Dimensional Spontaneous EEG Decreases during Propofol Induced General Anaesthesia. *PLoS One*, *10*(8), e0133532. https://doi.org/10.1371/journal.pone.0133532

Schartner, M. M., Carhart-Harris, R. L., Barrett, A. B., Seth, A. K., & Muthukumaraswamy, S. D. (2017). Increased spontaneous MEG signal diversity for psychoactive doses of ketamine, LSD and psilocybin. *Sci Rep*, *7*, 46421. https://doi.org/10.1038/srep46421

Schartner, M. M., Pigorini, A., Gibbs, S. A., Arnulfo, G., Sarasso, S., Barnett, L., Nobili, L., Massimini, M., Seth, A. K., & Barrett, A. B. (2017). Global and local complexity of intracranial EEG decreases during NREM sleep. *Neurosci Conscious*, *2017*(1), niw022. https://doi.org/10.1093/nc/niw022

Shao, L. X., Liao, C., Gregg, I., Davoudian, P. A., Savalia, N. K., Delagarza, K., & Kwan, A. C. (2021). Psilocybin induces rapid and persistent growth of dendritic spines in frontal cortex in vivo. *Neuron*, *109*(16), 2535-2544 e2534. https://doi.org/10.1016/j.neuron.2021.06.008

Siegel, J. S., Subramanian, S., Perry, D., Kay, B. P., Gordon, E. M., Laumann, T. O., Reneau, T. R., Metcalf, N. V., Chacko, R. V., Gratton, C., Horan, C., Krimmel, S. R., Shimony, J. S., Schweiger, J. A., Wong, D. F., Bender, D. A., Scheidter, K. M., Whiting, F. I., Padawer-Curry, J. A.,...Dosenbach, N. U. F. (2024). Psilocybin desynchronizes the human brain. *Nature*, *632*(8023), 131-138. https://doi.org/10.1038/s41586-024-07624-5

Studerus, E., Gamma, A., & Vollenweider, F. X. (2010). Psychometric evaluation of the altered states of consciousness rating scale (OAV). *PLoS One*, *5*(8), e12412. https://doi.org/10.1371/journal.pone.0012412

Timmermann, C., Roseman, L., Schartner, M., Milliere, R., Williams, L. T. J., Erritzoe, D., Muthukumaraswamy, S., Ashton, M., Bendrioua, A., Kaur, O., Turton, S., Nour, M. M., Day, C. M., Leech, R., Nutt, D. J., & Carhart-Harris, R. L. (2019). Neural correlates of the DMT experience assessed with multivariate EEG. *Sci Rep*, *9*(1), 16324. https://doi.org/10.1038/s41598-019-51974-4

Tossell, K., Yu, X., Giannos, P., Anuncibay Soto, B., Nollet, M., Yustos, R., Miracca, G., Vicente, M., Miao, A., Hsieh, B., Ma, Y., Vyssotski, A. L., Constandinou, T., Franks, N. P., & Wisden, W. (2023). Somatostatin neurons in prefrontal cortex initiate sleep-preparatory behavior and sleep via the preoptic and lateral hypothalamus. *Nat Neurosci*, *26*(10), 1805-1819. https://doi.org/10.1038/s41593-023-01430-4

Vargas, M. V., Dunlap, L. E., Dong, C., Carter, S. J., Tombari, R. J., Jami, S. A., Cameron, L. P., Patel, S. D., Hennessey, J. J., Saeger, H. N., McCorvy, J. D., Gray, J. A., Tian, L., &

Olson, D. E. (2023). Psychedelics promote neuroplasticity through the activation of intracellular 5-HT2A receptors. *Science*, *379*(6633), 700-706. https://doi.org/10.1126/science.adf0435

Veselis, R. A., Pryor, K. O., Reinsel, R. A., Li, Y., Mehta, M., & Johnson, R., Jr. (2009). Propofol and midazolam inhibit conscious memory processes very soon after encoding: an event-related potential study of familiarity and recollection in volunteers. *Anesthesiology*, *110*(2), 295-312. https://doi.org/10.1097/ALN.0b013e3181942ef0

Wallach, J., Cao, A. B., Calkins, M. M., Heim, A. J., Lanham, J. K., Bonniwell, E. M., Hennessey, J. J., Bock, H. A., Anderson, E. I., Sherwood, A. M., Morris, H., de Klein, R., Klein, A. K., Cuccurazzu, B., Gamrat, J., Fannana, T., Zauhar, R., Halberstadt, A. L., & McCorvy, J. D. (2023). Identification of 5-HT(2A) receptor signaling pathways associated with psychedelic potential. *Nat Commun*, *14*(1), 8221. https://doi.org/10.1038/s41467-023-44016-1

Wenzel, M., Han, S., Smith, E. H., Hoel, E., Greger, B., House, P. A., & Yuste, R. (2019). Reduced Repertoire of Cortical Microstates and Neuronal Ensembles in Medically Induced Loss of Consciousness. *Cell Syst*, *8*(5), 467-474.e464. https://doi.org/10.1016/j.cels.2019.03.007

Woods, S. P., Delis, D. C., Scott, J. C., Kramer, J. H., & Holdnack, J. A. (2006). The California Verbal Learning Test--second edition: test-retest reliability, practice effects, and reliable change indices for the standard and alternate forms. *Arch Clin Neuropsychol*, *21*(5), 413-420. https://doi.org/10.1016/j.acn.2006.06.002

Zhang, X. S., Roy, R. J., & Jensen, E. W. (2001). EEG complexity as a measure of depth of anesthesia for patients. *IEEE Trans Biomed Eng*, *48*(12), 1424-1433. https://doi.org/10.1109/10.966601

Zhao, Y., Wang, A., Zhao, W., Hu, N., Laureys, S., & Di, H. (2025). The Neural Correlates of Consciousness: A Spectral Exponent Approach to Diagnosing Disorders of Consciousness. *Brain Sci*, *15*(4). https://doi.org/10.3390/brainsci15040377

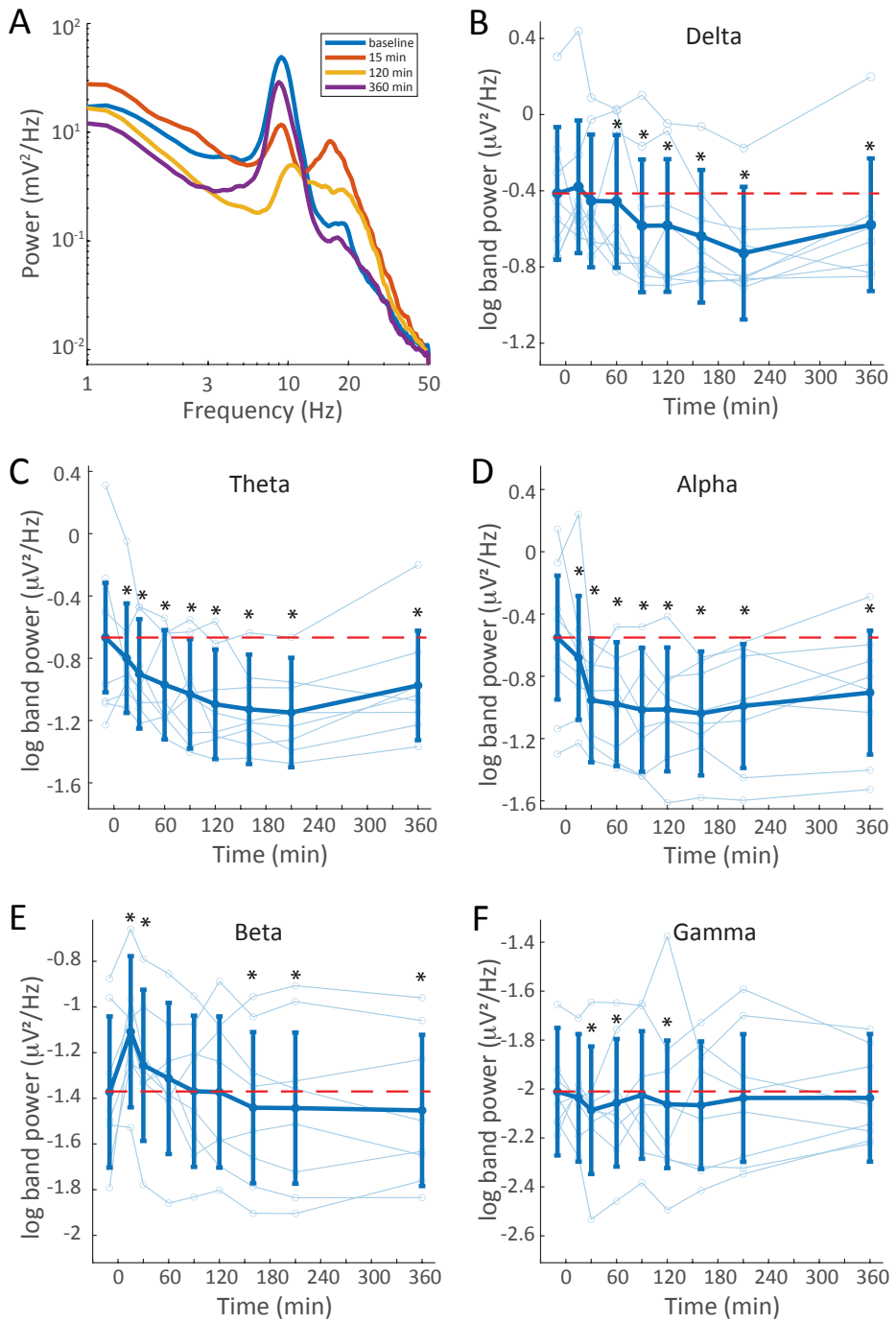

Figure 1. EEG power spectral density changes throughout dosing day. A. Lines represent eyes closed PSD from a single subject (PR004) at baseline, 15-, 120-, and 360- minutes post dosing. **B-F.** Time series plots of band power. Light blue lines and symbols show individual participant data; black lines and symbols show model-derived marginal means and confidence intervals. Panels (B) – (F) show delta (1-4 Hz), theta (4-8 Hz), alpha (8-14 Hz), beta (14-30 Hz), and gamma (30-50 Hz), respectively.

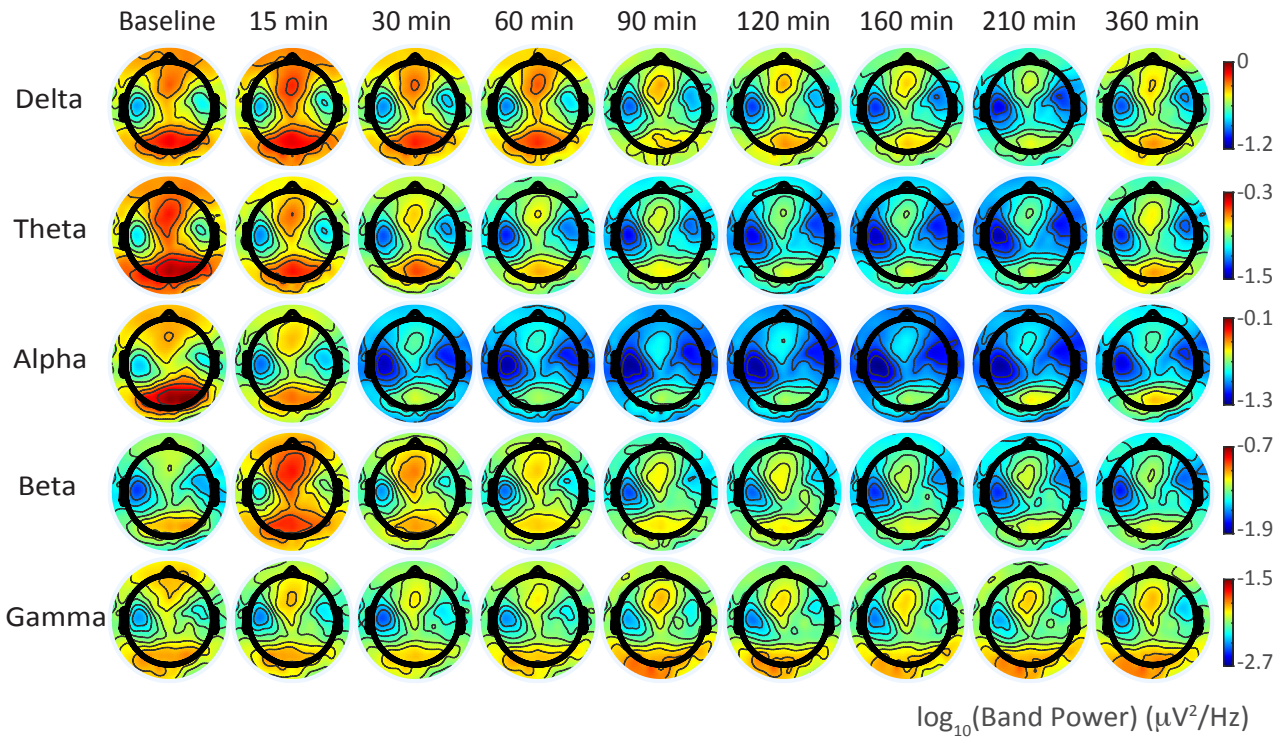

Figure 2. Topography of EEG power changes during administration of psilocybin with midazolam. Sensor-level topo plots for the data in Figure 1B-F**.** Each row is a frequency band; each column is a time point.

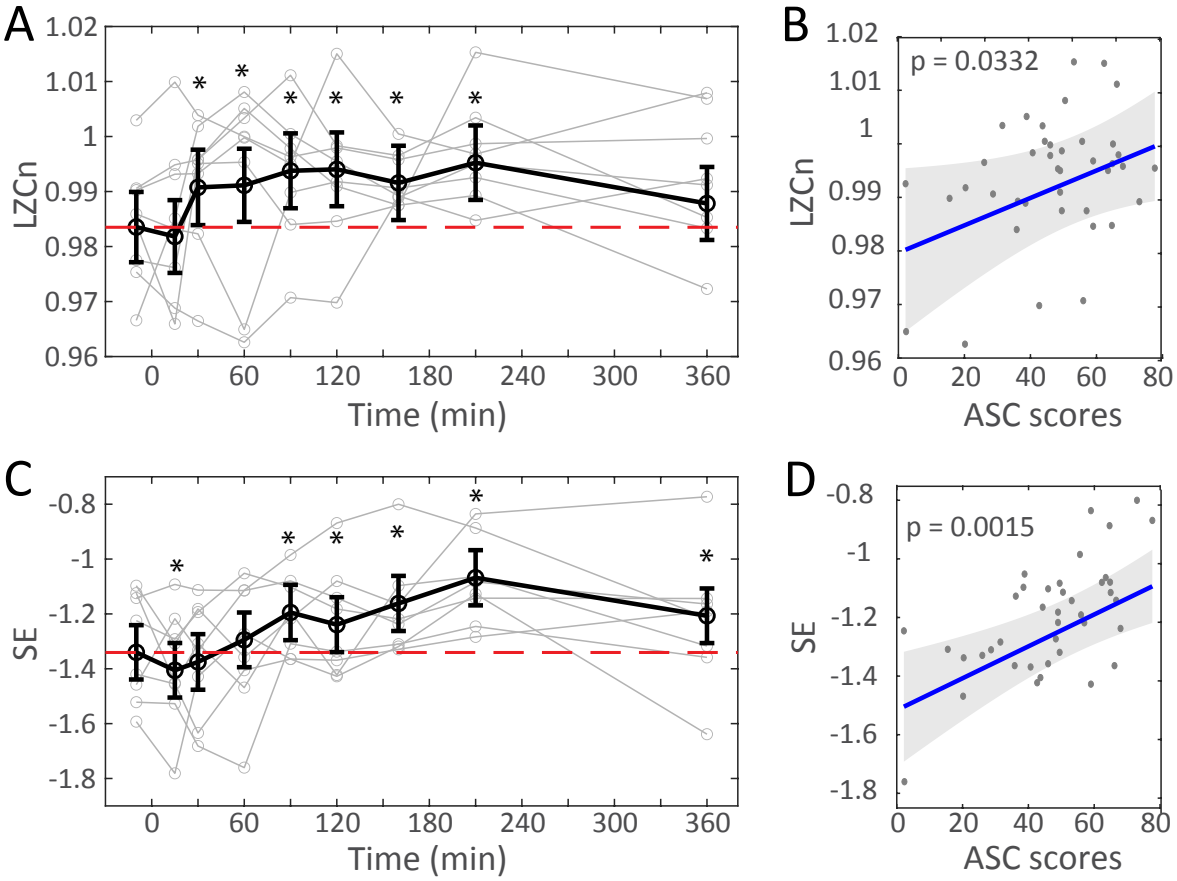

Figure 3. Complexity and spectral slope change systematically during administration of psilocybin with midazolam and depend on the subjective quality of the psychedelic experience. Shown are time series plots (A, C) of model-derived marginal means (black lines and symbols) and individual participant measurements (grey lines and symbols) for normalized Lempel-Ziv complexity (LZCn; **A**) and spectral exponent (SE; **B**). (**B, D**) Model prediction of relationship between LZCn (B) and SE (D) with ASC scores.