EEG gamma band alterations and REM-like traits underpin the acute effect of the atypical psychedelic ibogaine in the rat

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SUPPLEMENTARY MATERIAL

Table S1. Electrode Location.

Electrode Label	Coordinates*	
	Lateral	Antero-posterior
OBr	+1.25 mm	+7.5 mm
M1r	+2.5 mm	+2.5 mm
M1l	-2.5 mm	+2.5 mm
S1r	+2.5 mm	-2.5 mm
S1l	-2.5 mm	-2.5 mm
V2r	+2.5 mm	-7.5 mm
V2l	-2.5 mm	-7.5 mm

*All coordinates referenced to Bregma (Lateral: 0 mm, Antero-posterior: 0 mm) ("Paxinos, G. and Watson, C. (2007) The Rat Brain in Stereotaxic Coordinates. 6th Edition, Academic Press, San Diego.," n.d.).

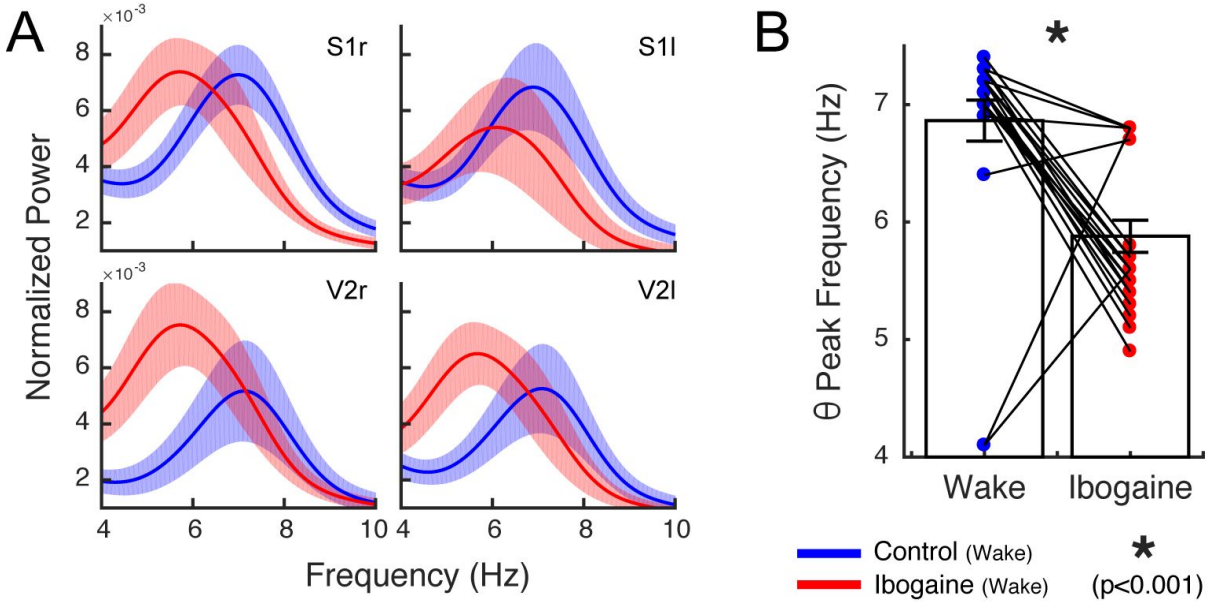


Figure S1. Ibogaine slows down theta peak frequency. A Normalized power spectrum in the theta range (4-10 Hz) for the four posterior electrodes. The solid line represents the mean (n=6 animals) for the first 2 hours post-injection; the shaded area depicts S.E.M.. Blue: control wakefulness. Red: ibogaine wakefulness. **B** Theta peak frequency during control and ibogaine wakefulness. Each dot is a cortical location of an individual animal (4 locations per animal). Bars represent mean ± S.E.M. *p<0.001, paired t-test.

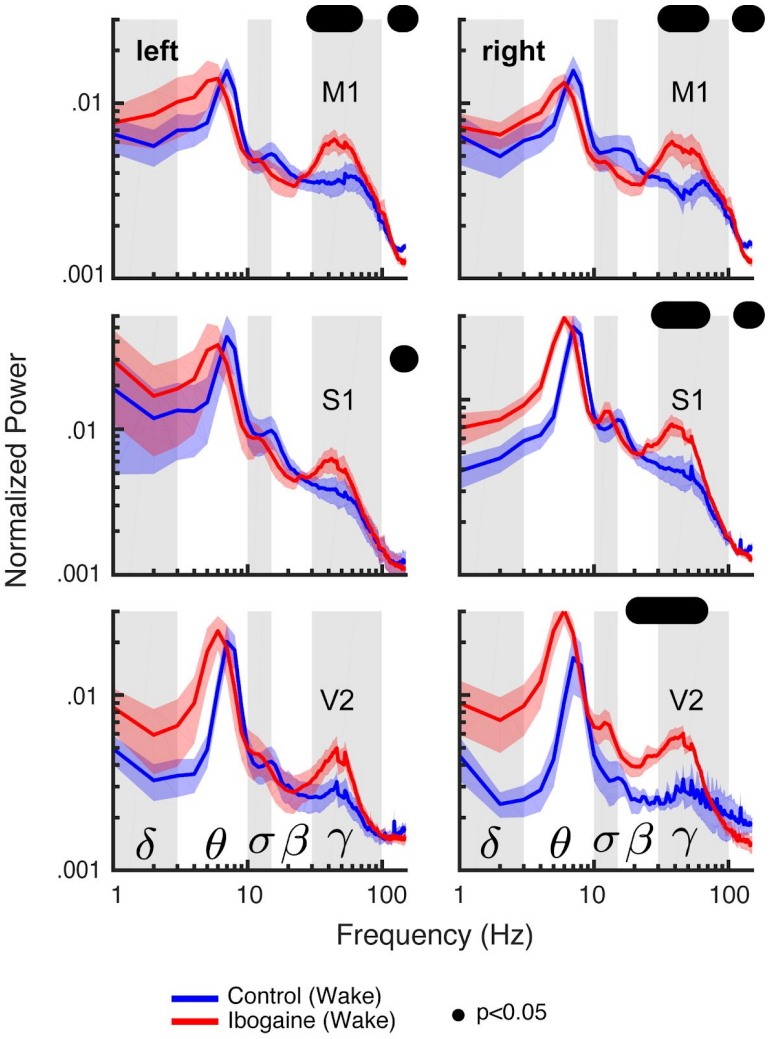


Figure S2. Ibogaine induces similar power changes in the left and right hemispheres. Blue: control wakefulness. Red: ibogaine wakefulness. The solid line represents the mean (n=6 animals) for the first 2 hours post-injection; the shaded area depicts S.E.M.. The black dots depict the statistically significant frequencies ($p<0.05$) corrected by a cluster-based permutation test. The spectra were whitened by multiplying power at each frequency by the frequency itself, and then normalized by dividing each frequency by the sum across frequencies.

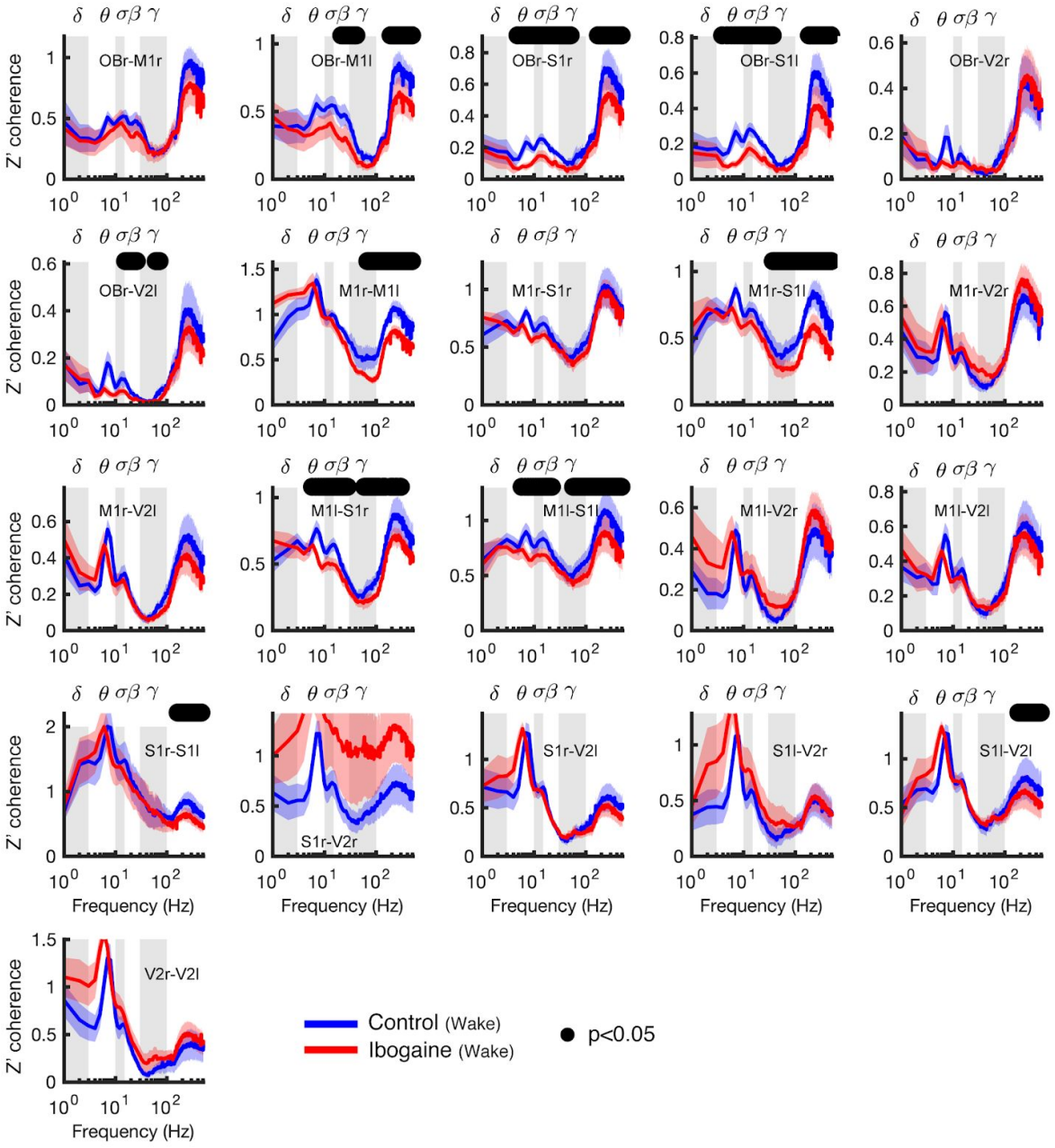


Figure S3. Coherence between all the electrode pairs during wakefulness. The solid line represents the mean (n=6 animals) for the first 2 hours post-injection; the shaded area depicts S.E.M. (r: right; l: left). The black dots depict the statistically significant frequencies (p<0.05) corrected by a cluster-based permutation test.

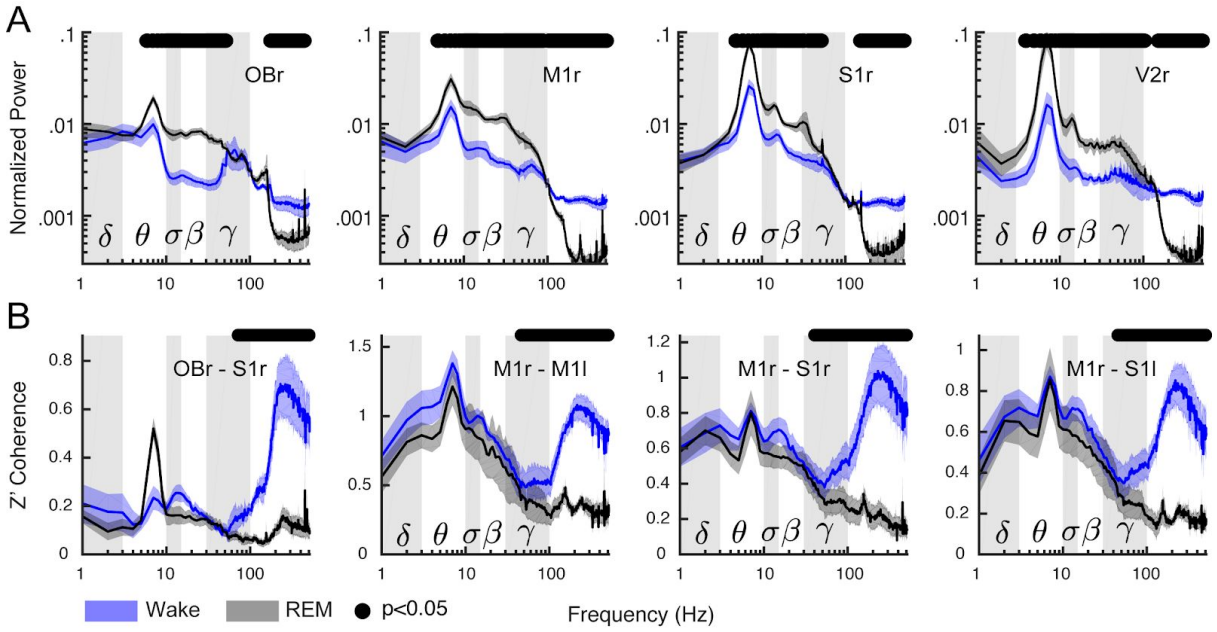


Figure S4. Gamma power and coherence during physiological wakefulness and REM sleep. (A) Power spectrum comparisons between physiological wakefulness (blue) and REM sleep (black); only the right hemisphere is shown. **(B)** Coherence spectra are shown for both normal wakefulness (blue) and REM sleep (black). The solid line represents the mean (n=6 animals); the shaded area depicts the S.E.M.. The black dots mark the statistically significant frequencies ($p<0.05$) corrected by a cluster-based permutation test (n=6 animals).

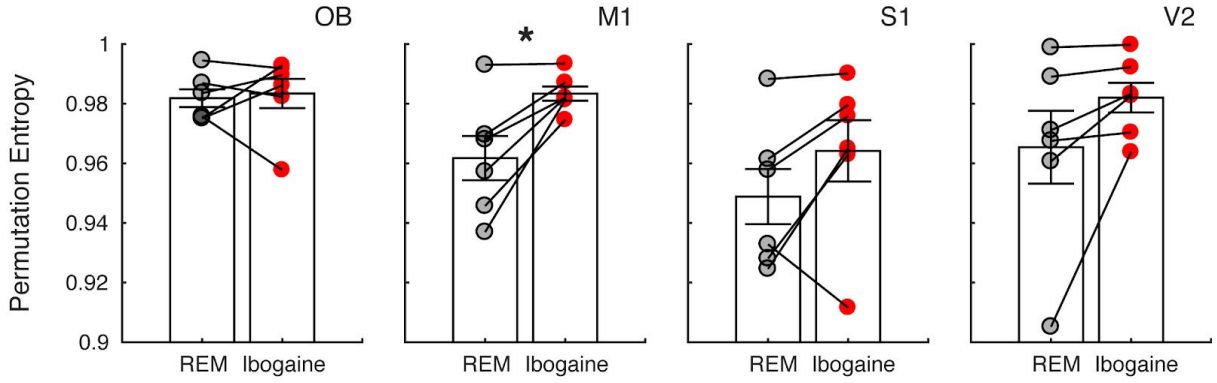


Figure S5. Permutation entropy during REM sleep and ibogaine wakefulness. Permutation entropy was employed to quantify the iEEG temporal complexity in physiological REM sleep (gray) and ibogaine wakefulness (red). Each dot shows the average permutation entropy of an animal (same electrodes as in Figure 1). Bars represent mean ± S.E.M.. *$p<0.05$, paired t-test (n=6 animals).

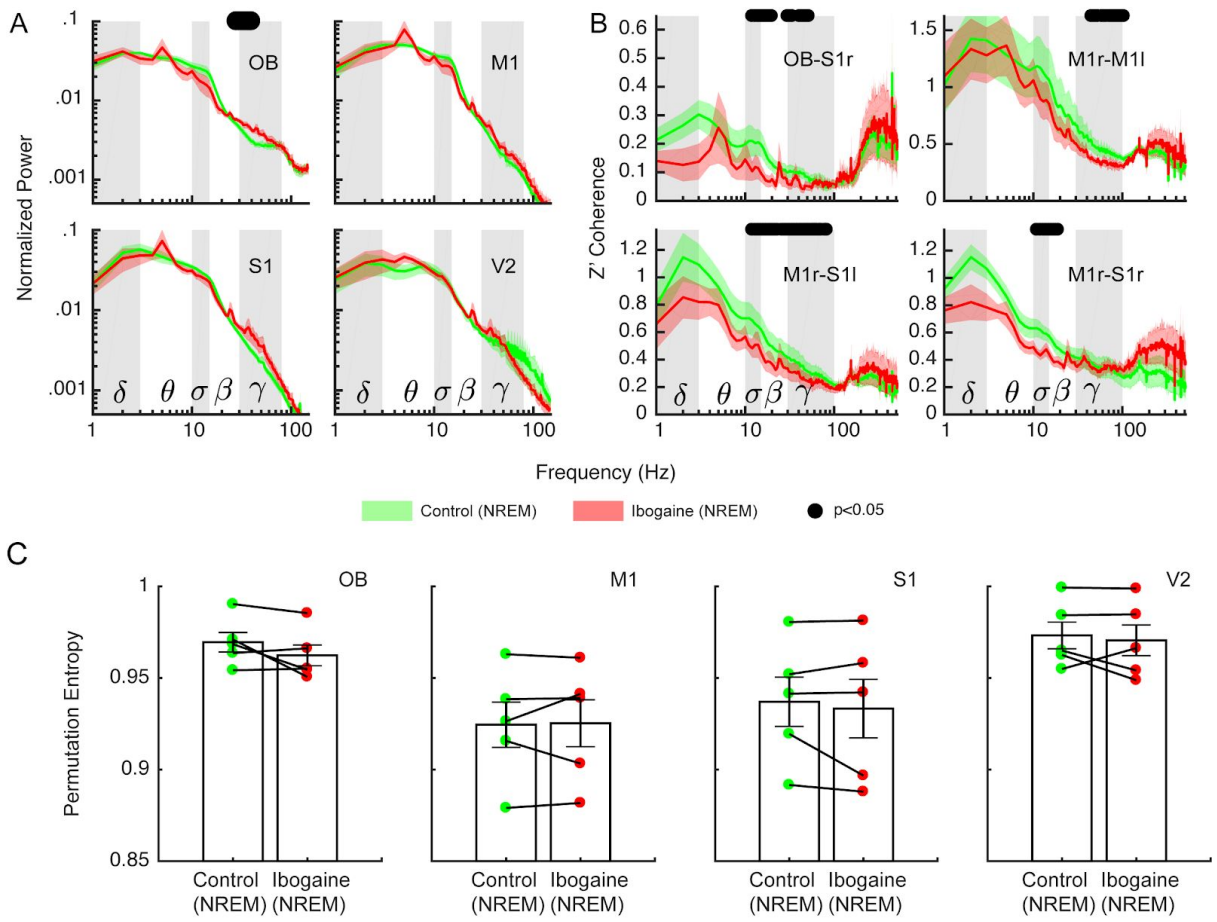


Figure S6. Ibogaine decreases inter-regional synchronization during NREM sleep. A,B Normalized power (**A**) and coherence (**B**) spectra for the physiological (green) and ibogaine NREM sleep (red). The solid line represents the mean (n=6 animals) for all NREM epochs within the first 6 hours post-injection; the shaded area depicts S.E.M. (r: right; l: left). The black dots depict the statistically significant frequencies ($p<0.05$) corrected by a cluster-based permutation test. **C** Permutation entropy comparisons between physiological NREM sleep (Control) and ibogaine NREM sleep (Ibogaine). Each dot shows the average permutation entropy of an animal (same electrodes as in Figure 1). Bars represent mean ± S.E.M.. No significant differences were found, paired t-test (n = 5 animals, one animal did not reach the minimum time required for the analysis).

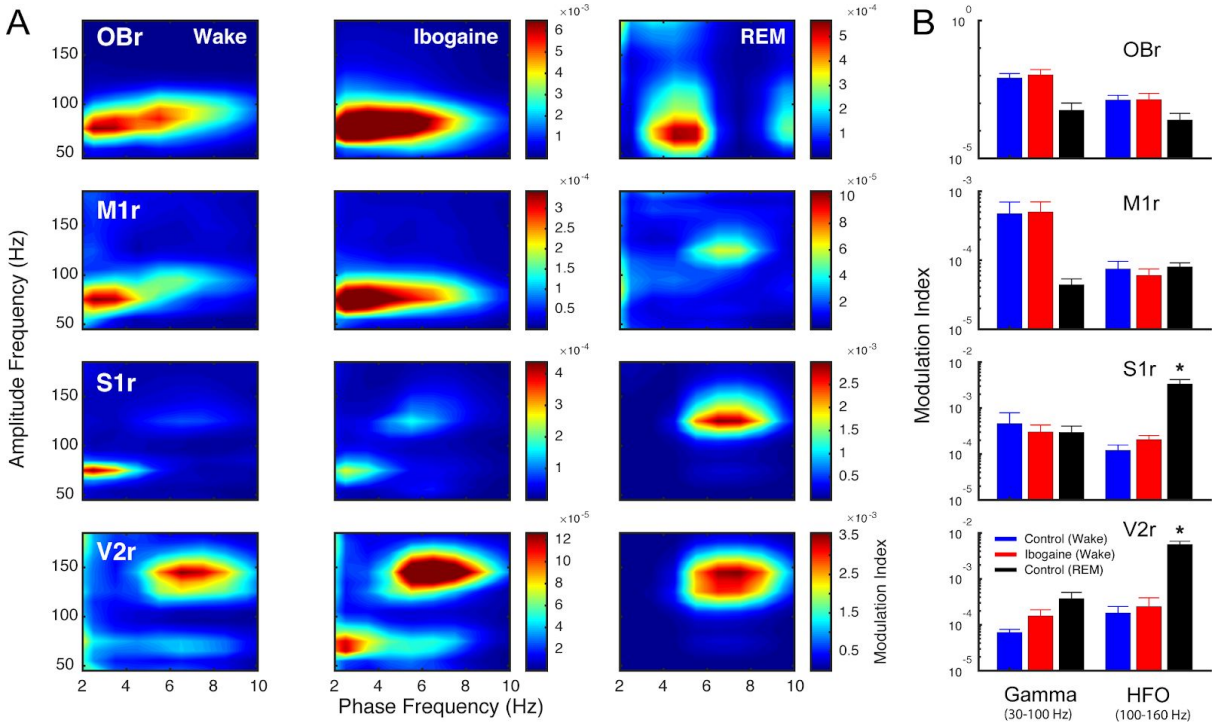
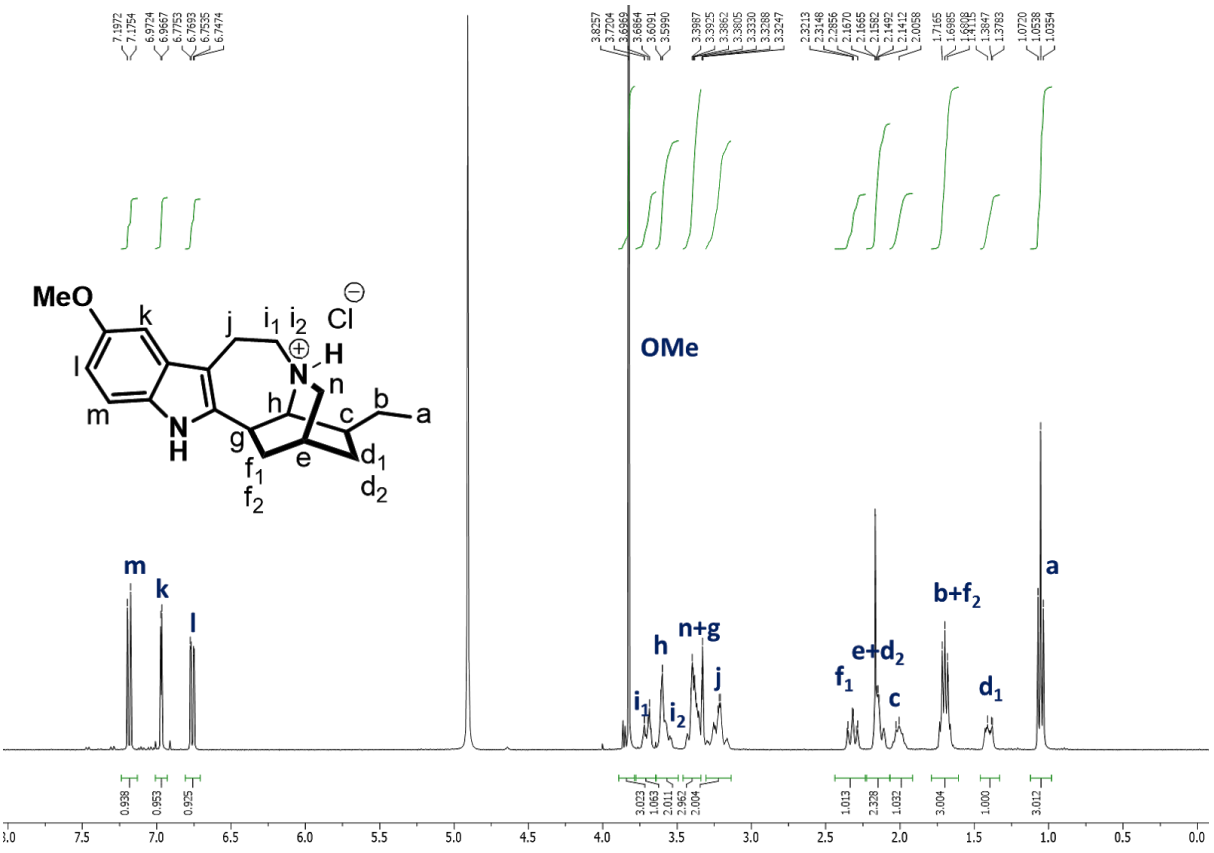


Figure S7. Gamma band cross-frequency coupling remains unaltered after ibogaine administration. **(A)** Population averaged co-modulation maps for control wakefulness, ibogaine wakefulness and REM sleep. Red colors indicate high phase-amplitude coupling (see Material and Methods). **(B)** Modulation index for theta-gamma (30-100 Hz) and theta-high-frequency oscillation (100-160 Hz, also known as the fast-gamma band) coupling. For each animal and state, the maximal modulation index value within the analyzed frequency ranges was taken for each cortex. Bars represent mean ± S.E.M. over animals. *p<0.05 paired t-test against ibogaine wakefulness state (n = 5 animals, one animal had large co-modulation artifacts).

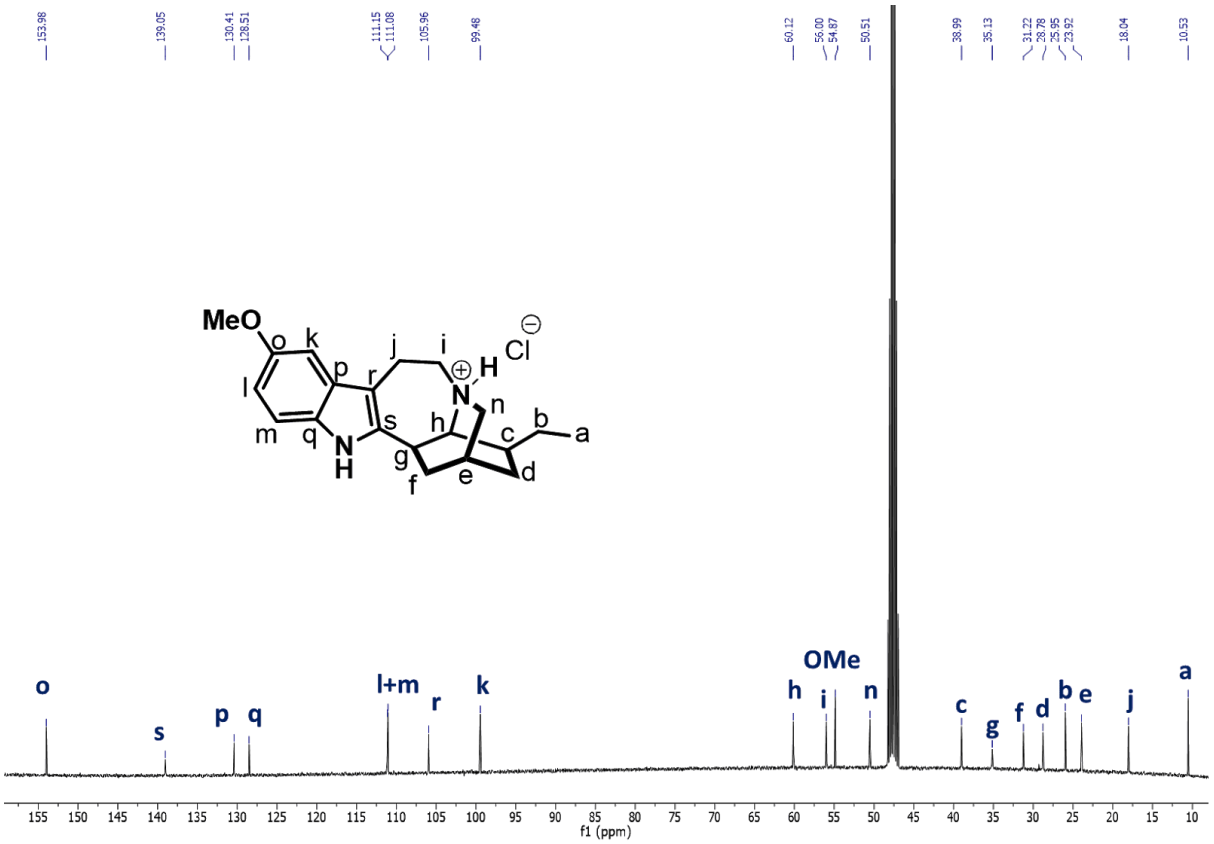
IBOGAINE SUPPLEMENTARY INFORMATION

Total iboga alkaloid extract from the root bark of *T. iboga* was purified as follows. The material was suspended in aqueous 10% NaOH solution, which was extracted with ethyl acetate (4 × 200 ml). The combined organic layers were dried with Na_2SO_4 and evaporated *in vacuo*. The residue was purified by column chromatography (SiO_2, CH_2Cl_2:MeOH 9:1 + 0.1% NH_4OH). The obtained free base was further crystallized from ethanol. Ibogaine HCl was prepared dissolving the free base in dried acetone under Argon atmosphere and the equivalent amount of HCl (aq, 36%) was added. Ibogaine hydrochloride was filtered, washed with cold acetone, dried under vacuum, and characterized by ^{1}H- and ^{13}C-NMR. Purity was determined as 96.4% by GC–MS.

Ibogaine NMR structure elucidation. Nuclear Magnetic Resonance spectra were obtained in CD_3OD on a Bruker Avance DPX-400 instrument. **^{1}H NMR** (400 MHz, CD_3OD) δ (ppm) =7.19 (d, *J* = 8.9 Hz, 1H), 6.97 (d, *J* = 2.4 Hz, 1H), 6.76 (dd, *J* = 8.8, 2.5 Hz, 1H), 3.87 (s, 3H), 3.70 (dt, *J*= 13.4, 4.2 Hz, 1H), 3.63 – 3.53 (m, 2H), 3.45 – 3.34 (m, 3H), 3.31 – 3.14 (m, 2H), 2.32 (ddt, 13.5, 12.1, 2.7 Hz, 1H), 2.19 – 2.09 (m, 2H), 2.06 (hept, *J* = 7.5 Hz, 1H), 1.74 – 1.65 (m, 3H), 1.46 – 1.34 (m, 1H), 1.03 (t, *J* = 7.3 Hz, 3H) **^{13}C NMR** (100 MHz, CD_3OD) δ(ppm) = 153.0, 139.1, 130.4, 128.5, 111.2, 111.1, 106.0, 99.5, 60.1, 56.0, 54.9, 50.5, 39.0, 35.1, 31.2, 28.8, 26.0, 23.9, 18,0, 10.5



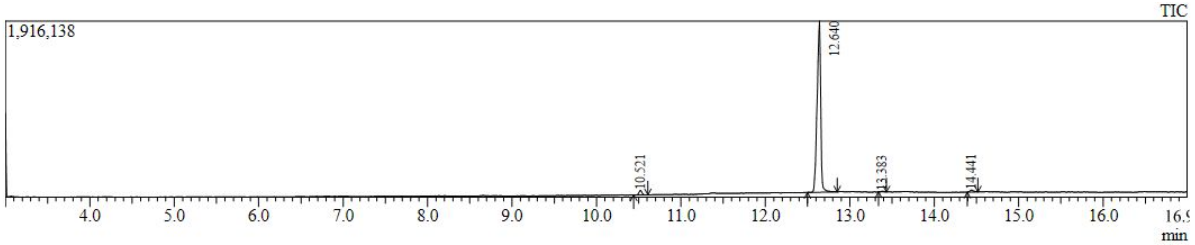
Ibogaine-HCl ^{1}H NMR. Diastereotopic protons are labeled as $x_{1/}x_{2.}$ Solvent residual peak for MeOH is found at 3.33 ppm, and H_2O at 4.90.



Ibogaine-HCl ^{13}C NMR.

Ibogaine relative purity analysis by GC-MS. Gas Chromatography analysis was carried out in a GC-MS Shimadzu QP 1100 EX instrument using the electron impact mode, 70 eV. Column HP-5MS (30m x 0.25mm x 0.25um) Temperature Program 200 ºC (Hold time, 2 minutes) to 300 ºC (Hold time, 5 minutes) with a rate of 10 ºC/min. Ibogaine purity was determined as 96.4%, and ibogamine and ibogaline were detected as trace impurities.

Retention Time (minutes)	% Area	Structure
10.521	2.05	Ibogamine
12.640	96.38	Ibogaine
13.383	0.34	Unkown
14.441	1.23	Ibogaline



Ibogaine-HCl GC-MS chromatogram