frontiers

Supplementary Material

Ibogaine administration modifies GDNF and BDNF expression in brain regions involved in mesocorticolimbic and nigral dopaminergic circuits.

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Supplementary Data

1.1 Ibogaine-HCl Preparation. Nuclear Magnetic Resonance spectra were obtained on a Bruker Avance DPX-400 instrument. In both cases samples were characterized as voacangine or ibogaine according to the following NMR data

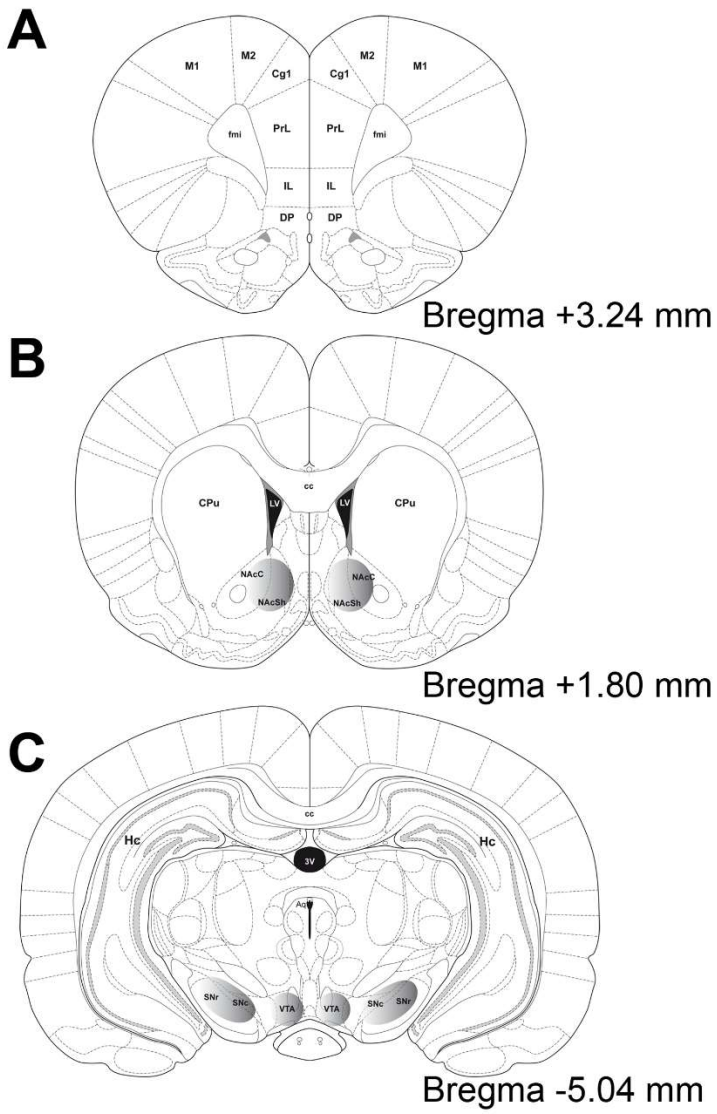
Voacangine (12-methoxy-16-carbomethoxyibogamine) isolated from *Voacanga Africana* root bark (see Material and Methods section in the manuscript)
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.13 (d, *J* = 8.7 Hz, 1H), 6.93 (d, *J* = 2.3 Hz, 1H), 6.80 (dd, J = 8.7, 2.4 Hz, 1H), 3.84 (s, 3H), 3.70 (s, 3H), 3.55 (s, 1H), 3.44 – 3.33 (m, 1H), 3.26 – 3.08 (m, 2H), 3.03 – 2.86 (m, 2H), 2.81 (d, *J* = 8.5 Hz, 1H), 2.58 (dd, *J* = 11.8, 2.1 Hz, 1H), 1.94 – 1.81 (m, 2H), 1.73 (t, *J* = 11.1 Hz, 1H), 1.56 (dt, *J* = 22.0, 7.4 Hz, 1H), 1.49 – 1.39 (m, 1H), 1.37 – 1.28 (m, 2H), 1.15 – 1.08 (m, 1H), 0.90 (t, *J* = 7.3 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 175.71, 153.98, 137.51, 130.53, 129.19, 116.62, 111.81, 111.07, 110.12, 100.77, 99.99, 77.34, 77.03, 76.71, 57.52, 56.04, 55.14, 53.13, 52.59, 51.52, 39.15, 36.56, 32.03, 27.35, 26.76, 22.22, 11.68.

Ibogaine·HCl prepared by decarboxylation of voacangine (see Material and Methods section in the manuscript)
^{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

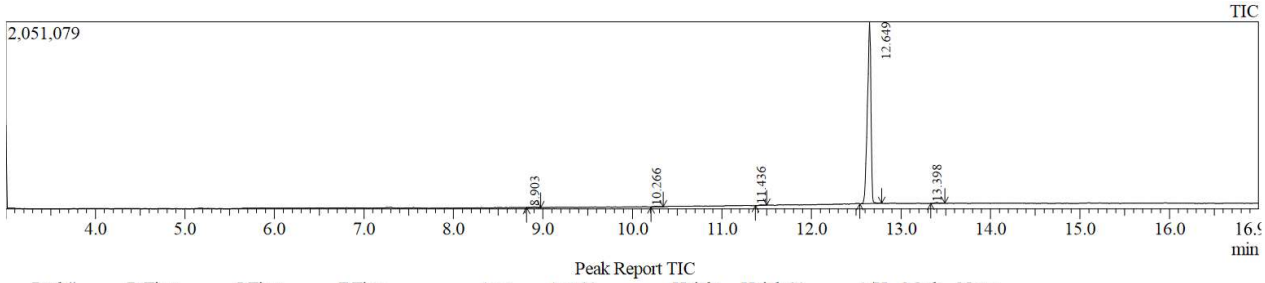
To determine ibogaine-HCl purity (after crystallization and purification procedures described in the Materials and Methods section of the manuscript) Gas Chromatography analysis was carried out in a GC-MS Shimadzu QP 1100 EX instrument using the electron impact mode, 70 eV. For analysis sample was previously dissolved in aqueous NaOH 10% and extracted with Ethyl Acetate. Conditions: 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 98.3% (See chromatogram below)

2 Supplementary Figures and Tables

2.1 Supplementary Figures



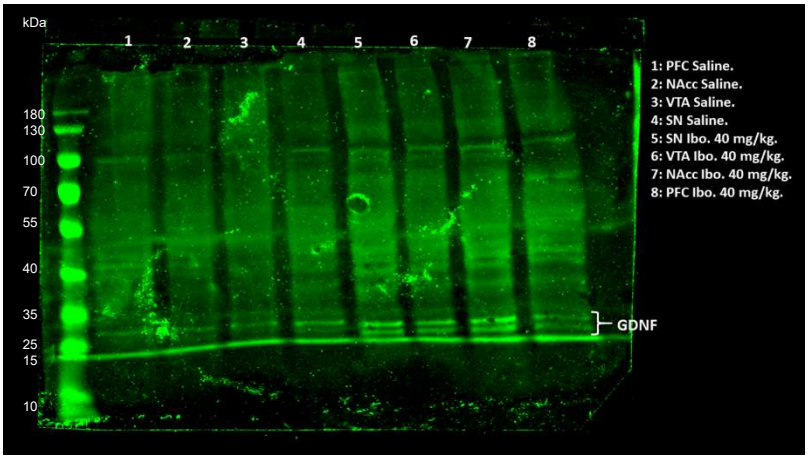
Supplementary Figure 1. Dissected brain areas. Figure shows a schematic diagram of coronal sections at three different levels. A: +3.24 for PFC, B: + 1.80 for NAcc, and C: -5.04 for VTA and SN, from Bregma) according to Paxinos and Watson (2005) atlas. In A, a complete slice (2 mm) was taken for PFC (including the mPFC). In B and C, gray areas indicate an approximately extension of the brain tissue dissected for NAcc ((this region was punched by using a sample corer with an inner diameter of 2 mm), VTA and SN, respectively. Cg, cingulate cortex; PrL, prelimbic; IL, infralimbic; M1 and M2, motor cortex; CPu, corpus striatum; NAcC and NAcSh, nucleus accumbens core and shell; Hc, hippocampus; CC, corpus callosum; 3V, third ventricle, SNc and SNr, compact and reticular subtantia nigra; VTA, ventral tegmental area.



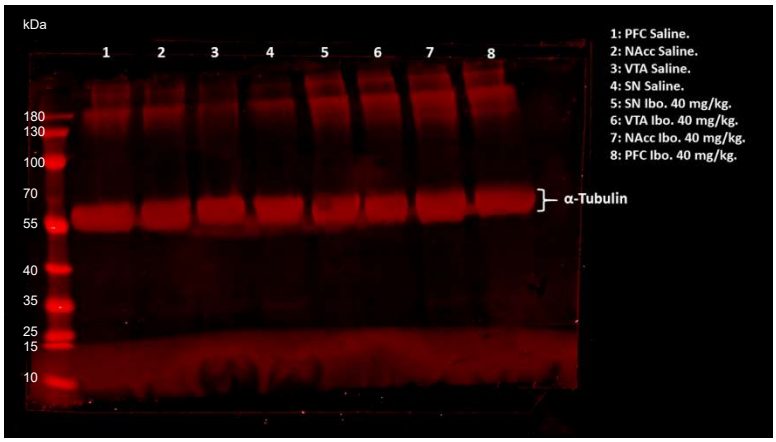
Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	8.903	8.817	8.975	32164	0.58	9633	0.48	3.34	MI	
2	10.266	10.208	10.342	16222	0.29	6610	0.33	2.45	MI	
3	11.436	11.375	11.500	16385	0.30	6125	0.30	2.68	MI	
4	12.649	12.542	12.783	5421252	98.31	1990692	98.49	2.72	MI	
5	13.398	13.333	13.492	28586	0.52	8238	0.41	3.47	MI	
				5514609	100.00	2021298	100.00			

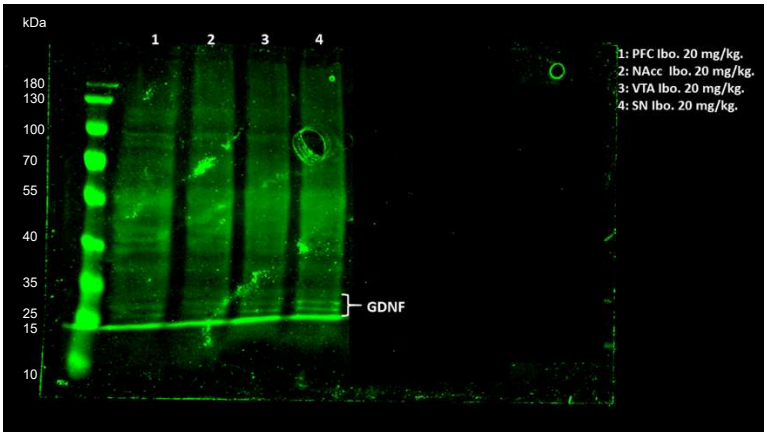
Supplementary Figure 2. Ibogaine-HCl GC-MS chromatogram.



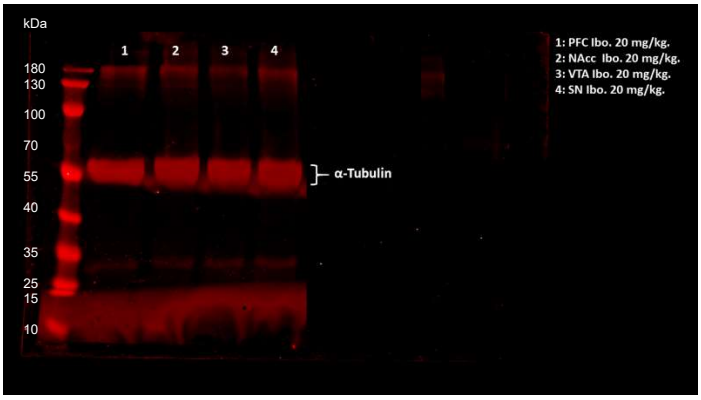
Supplementary Figure 3. Nitrocelulose membrane incubated with GDNF antibody, for Saline and Ibogaine 40 mg/kg samples.



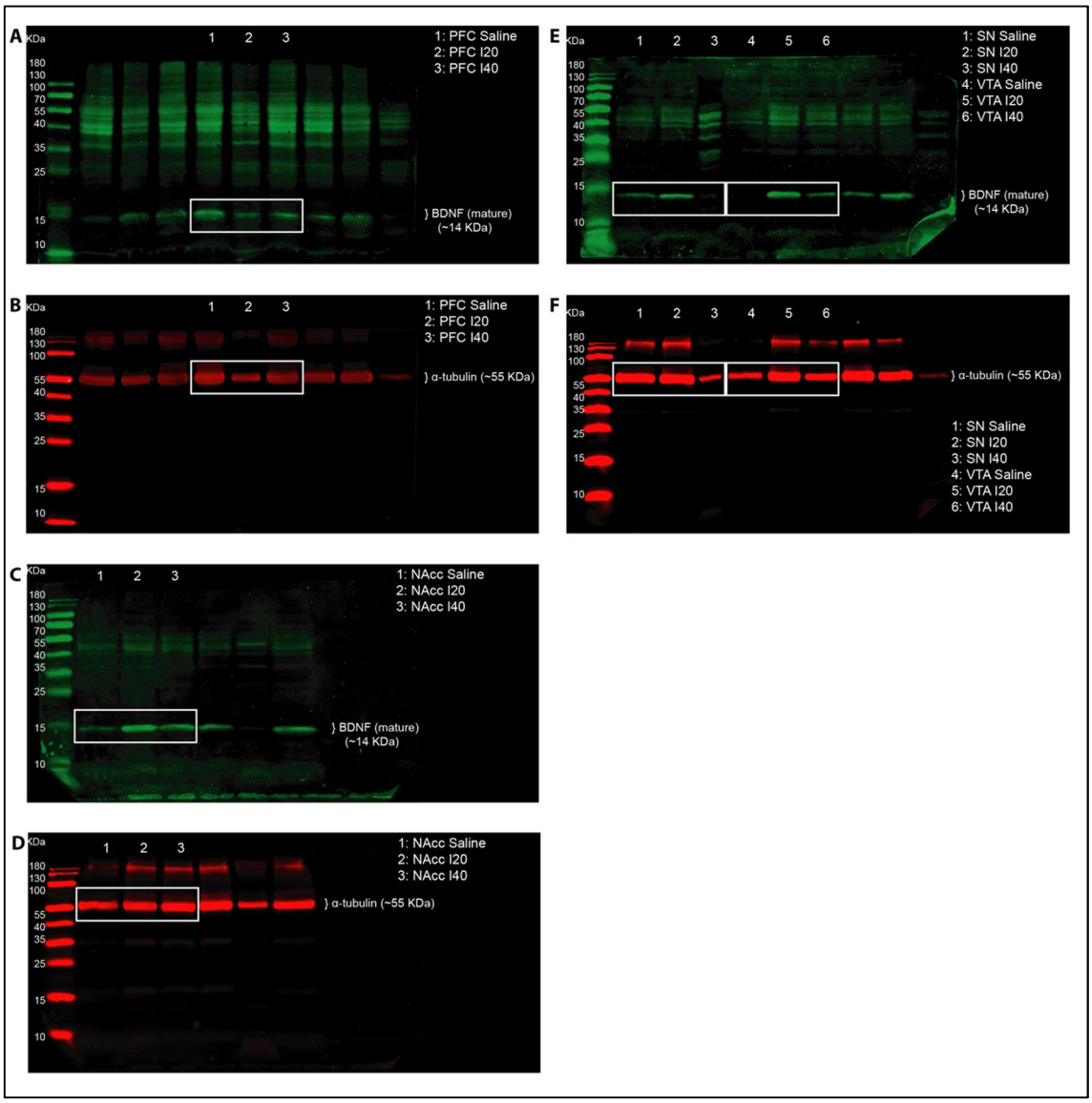
Supplementary Figure 4. Nitrocelulose membrane incubated with alpha-tubulin antibody, for Saline and Ibogaine 40 mg/kg samples.



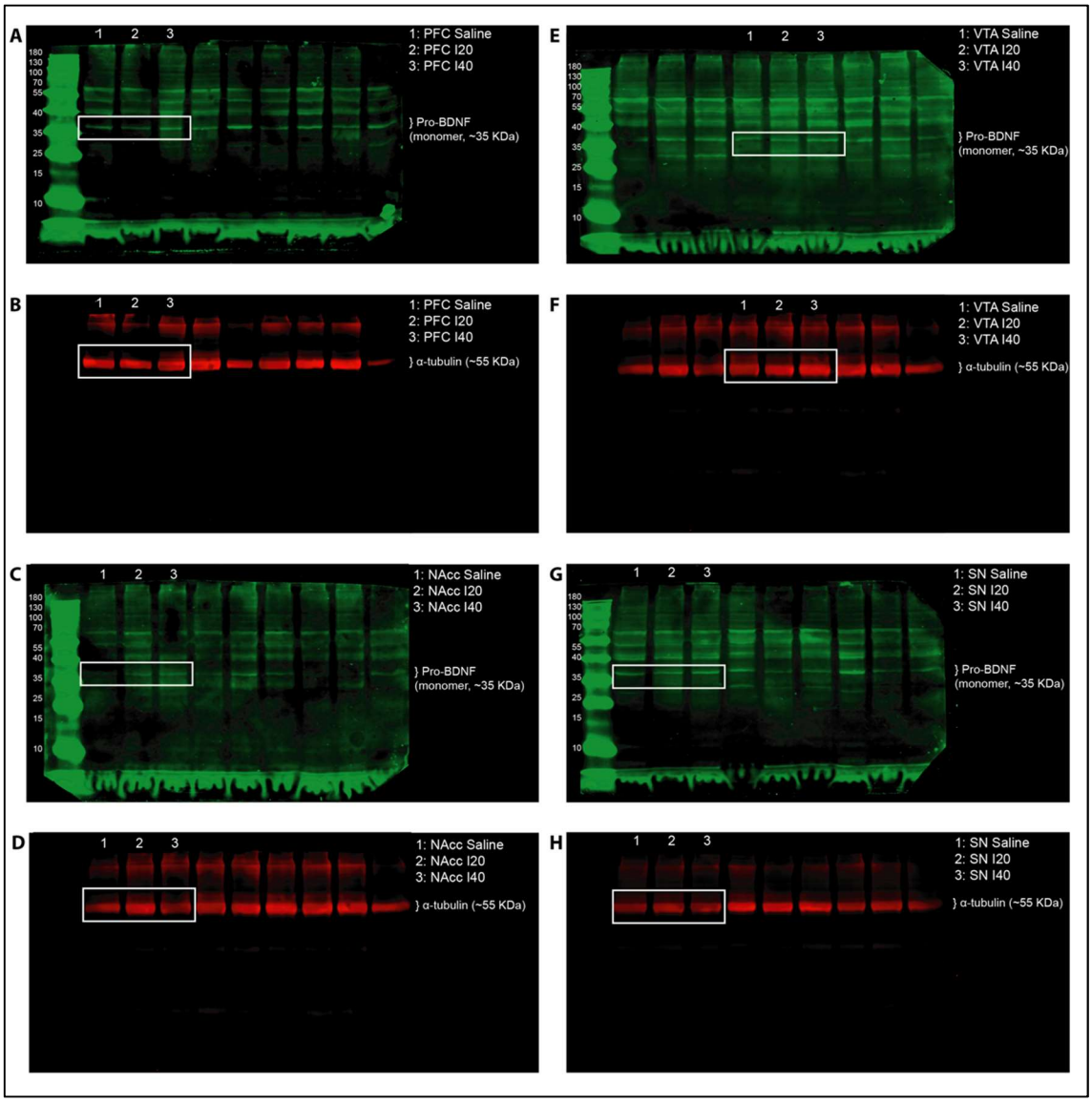
Supplementary Figure 5. Nitrocelulose membrane incubated with GDNF antibody, for Ibogaine 20 mg/kg samples.



Supplementary Figure 6. Nitrocelulose membrane incubated with alpha-tubulin antibody, for Ibogaine 20 mg/kg samples.



Supplementary Figure 7. Full-scanned images of western blots in main Figure 7-C. Nitrocellulose membranes corresponding to samples of PFC (**A-B),** NAcc **(C-D),** VTA and SN **(E-F)** from saline, I20 and I40-treated rats incubated with antibodies to BDNF **(A, C, E)** and α-tubulin **(B, D, F)**.



Supplementary Figure 8. Full-scanned images of western blots in main Figure 7-E. Nitrocellulose membranes corresponding to samples of PFC (**A-B),** NAcc **(C-D),** VTA **(E-F)** and SN **(G-H)** from saline, I20 and I40-treated rats incubated with antibodies to ProBDNF **(A, C, E, G)** and α-tubulin **(B, D, F, H)**.