A Single Administration of the Atypical Psychedelic Ibogaine or its Metabolite Noribogaine Induces an Antidepressant-like Effect in Rats

Paola Rodríguez[1,2], Jessika Urbanavicius[2], José Pedro Prieto[2], Sara Fabius[2], Ana Laura Reyes[3], Vaclav Havel[4], Dalibor Sames[4*], Cecilia Scorza[2*], Ignacio Carrera[1*]

[1]Laboratorio de Síntesis Orgánica, Departamento de Química Orgánica, Facultad de Química - Universidad de la República, Montevideo, Uruguay

[2]Departamento de Neurofarmacología Experimental, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay

[3]Centro Uruguayo de Imagenología Molecular, Montevideo, Uruguay

[4]Department of Chemistry, Columbia University, New York, USA

***Corresponding authors:** Dalibor Sames, Cecilia Scorza and Ignacio Carrera

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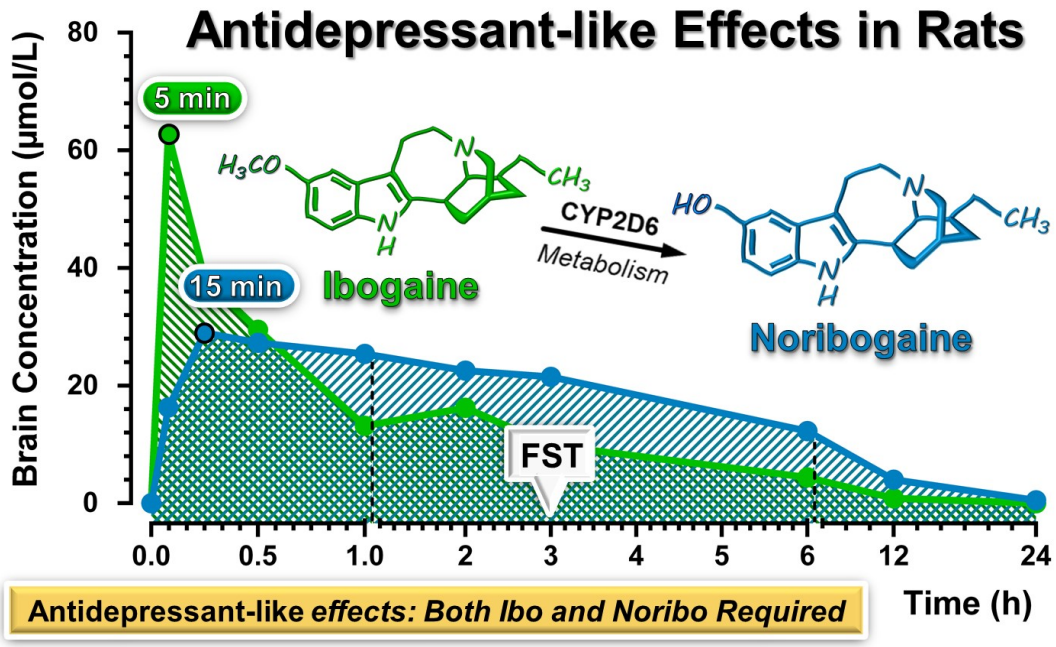

Abstract

Anecdotal reports and open label case studies in humans indicated that the psychedelic alkaloid ibogaine exert profound anti-addictive effects. Ample preclinical evidence demonstrated the efficacy of ibogaine, and its main metabolite noribogaine, in substance use disorder rodent models. In contrast to addiction research, depression-relevant effects of ibogaine or noribogaine in rodents have not been previously examined. We have recently reported that the acute ibogaine administration induced a long-term increase of brain-derived neurotrophic factor mRNA levels in the rat prefrontal cortex, which led us to hypothesize that ibogaine may elicit antidepressant-like effects in rats. Accordingly, we characterized behavioral effects (dose and time-dependence) induced by the acute ibogaine and noribogaine (20 and 40 mg/kg, i.p.) administration in rats using the forced swim test (FST). We also examined the correlation between plasma and brain concentrations of ibogaine and noribogaine and the elicited behavioral response. We found that ibogaine and noribogaine induced a dose- and time-dependent antidepressant-like effect without significant changes of animal locomotor activity. Noribogaine's FST effect was short lived (30 minutes) and correlated with high brain concentrations (estimated > 8 μM of free drug), while the ibogaine's antidepressant-like effect was significant at 3 hours. At this time point, both ibogaine and noribogaine were present in rat brain, at concentrations which cannot produce the same behavioral outcome on their own (ibogaine ~ 0.5 μM, noribogaine ~ 2.4 μM). Our data suggest a polypharmacological mechanism underpinning the antidepressant-like effects of ibogaine and noribogaine.

INTRODUCTION

Ibogaine is the main indole alkaloid isolated from the root bark of the African shrub *Tabernanthe iboga* (Figure 1).[1-3] It is considered an atypical psychedelic drug capable of inducing waking dream-like states (oneirogenic effects) and vivid memory recall and replay.[4,5] Anecdotal reports and open label case studies with volunteers seeking detoxification from heroin and cocaine indicated ibogaine's ability to interrupt the drug dependence phenotype via rapid and lasting relief of drug withdrawal symptoms and cravings.[6-8] Two recent open label observational clinical studies with subjects diagnosed with opioid dependence confirmed the earlier reports by showing a significant reduction of the withdrawal symptoms (3 days post-treatment) and an improvement of quality of life (up to 12 months) after a single ibogaine therapeutic session.[9,10] One of these studies also reported a sustained antidepressant effect (evaluated up to 12 months post-treatment)[10] consistent with the earlier observations of ibogaine's attenuation of depressive symptoms.[11] Extensive preclinical work recapitulated the clinical effects of ibogaine in rodent models of substance use disorders (SUDs), including attenuation of self-administration of opioids, cocaine, nicotine, and alcohol, as well as a reduction of opioid withdrawal symptoms in opioid-dependent animals.[12,13] In addition, it has been shown that noribogaine, ibogaine's major metabolite (Figure 1), exhibits a similar potency and efficacy profile in rodent models of SUDs, leading to a mechanistic model where noribogaine contributes to the observed anti-SUD effects.[14]

We recently reported in rats that ibogaine induces a dose-dependent upregulation of glial cell-derived neurotrophic factor (GDNF) in specific brain regions of the rat brain related to the mesocorticolimbic and nigral dopaminergic circuits,[15] an effect which was previously proposed as a molecular mechanism underlying the attenuation of drug self-administration and drug craving by ibogaine.[16,17] In addition, we also found a large upregulation of brain-derived neurotrophic factor (BDNF) expression in the prefrontal cortex after a single ibogaine administration, suggesting that ibogaine

may exert an antidepressant-like effect, since an increased BDNF signaling plays a critical role in the antidepressant-like efficacy of selective serotonin uptake inhibitors (SSRIs) in rodents.[18-20] Also, we previously showed that ibogaine administration in rats promoted an increase in wakefulness time and a long-lasting rapid eyes movement (REM) sleep suppression, a profile which is also shared with SSRIs.[21] Since it is well known that the ibogaine and noribogaine inhibit the plasma membrane serotonin transporter (SERT) (noribogaine displaying approximately ten-fold greater affinity for SERT, IC_{50} ~ 50-300 nM than ibogaine),[22-24] we therefore postulated that ibogaine administration in rats would exhibit antidepressant-like effects. In this manner, we hypothesized that the above noted physiological effects and the BDNF upregulation would be, at least in part, downstream consequences of the serotonin neurotransmission enhancement[23,25,26] generated by SERT inhibition. However, ibogaine can also block N-methyl-D-aspartate receptors (NMDA-R),[27] and NMDA-R non-competitive antagonists such as ketamine and dizocilpine were also shown to exhibit antidepressant-like effect in rodents.[28-31] Moreover, noribogaine was recently found to promote neuritogenesis in rat cortical primary cultures, which could also contribute to a potential antidepressant-like effect in rats.[32]

In contrast to the large body of literature on SUD models, to our knowledge the preclinical examination of antidepressant-like effects of ibogaine has not been reported. Considering the clinical observations and the mechanistic considerations mentioned above, we set out to examine the effects of ibogaine and noribogaine in the forced swim test (FST) in rats, a widely used preclinical assay, that measures coping strategy to an acute inescapable stress, with well-established responsiveness to a broad range of clinically efficacious antidepressants.[33-35] In this manner, the present study was designed to characterize the behavioral effect (dose and time-dependence) induced by acute administration of ibogaine and noribogaine in rats using the FST. Additionally, the plasma and brain concentration of ibogaine and noribogaine were

measured to associate the behavioral outcomes to the pharmacokinetic profiles of both drugs.

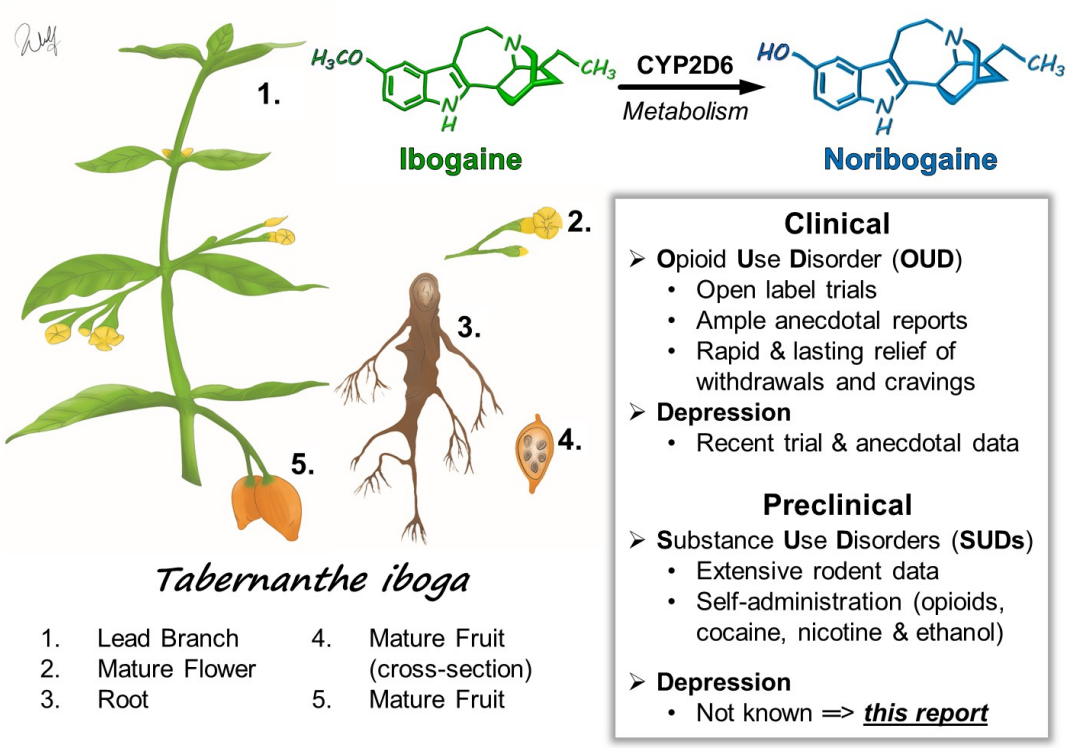

Figure 1. The psychoactive alkaloid ibogaine, its source, metabolism, and summary of clinical and preclinical effects. Illustration of different parts of *Tabernanthe iboga*. Ibogaine is isolated from the root bark. It is converted *in vivo* by the CYP2D6 isoform to its main active metabolite, noribogaine. In contrast to extensive examination of ibogaine in preclinical SUD models, antidepressant-like effects in rodents have not been investigated despite the clinical signals observed in anecdotal and open label studies.

RESULTS AND DISCUSSION

Ibogaine elicits dose-dependent and time-dependent reduction in the immobility time in the forced swim test. The FST has been extensively used as a behavioral screening test for antidepressant drugs with different mechanisms of action.[36] When rats are subjected to the standard FST, two swimming sessions are carried out 24 hours apart. The first one called “pre-test” has 15 minutes of duration

and is aimed to stress and sensitize the animals to develop immobility in the second session, the actual “test”, that lasts only 5 minutes (where the time animals spend immobile, swimming or climbing is measured).[33,37] Antidepressant drugs are typically administered 2-3x before the test swimming session (e.g. 24, 5 and 1 hour before the test session) and induce significant reduction in the time animals spend immobile in comparison to the vehicle control group (i.e., rats pursue more active escape strategy).[33,35] We decided to examine the effect of acute administration of ibogaine in the FST, since most of the previous pre-clinical studies using SUDs models were carried out with a single intra-peritoneal (i.p.) administration of 20 mg/kg (I_{20}) or 40 mg/kg (I_{40}).[13] Based on previous behavioral and pharmacokinetic (PK) studies using i.p. administration,[23,38-40] and our own previous work,[15,21] we selected two time points for the FST as 3 and 24 hours after ibogaine administration. At 3 hours, ibogaine and noribogaine are expected to have pharmacologically and behaviorally relevant concentrations in the rat brain, while the transient behavioral effects observed immediately after the i.p. administration of I_{40} (i.e. hypoactivity, tremor and piloerection), which could interfere with both the passive (immobility) and active (swimming and climbing) behaviors, are absent at this time point. In contrast, at 24 hours ibogaine would be no longer present in the brain and noribogaine would be at pharmacologically irrelevant concentrations, which would allow for examining potential long-lasting effects produced by both substances.

Figure 2A shows the effect of both doses of ibogaine in the FST evaluated 3 h after the acute administration. For immobility time, one-way ANOVA revealed a significant effect for treatment [$F_{(2,20)} = 4.11$, $P < 0.03$]. The Tukey post hoc test showed that only I_{40} was able to induce a significant decrease in the immobility time compared to the control group ($P < 0.05$; Fig. 2A). This can be interpreted as an antidepressant-like effect induced by I_{40}, and not due to nonspecific alterations on the animal motor activity since One-way ANOVA showed no significant changes [$F_{(2,15)} = 2.58$, $P = 0.11$] in the first 5 min of the animals motor activity evaluated 3 h after the drug i.p.

administration in the open field test (OFT, Fig. 2A,d). Remarkably, whereas a wide range of clinically efficacious antidepressants typically require two or more administrations to induce an antidepressant-like behavior in the rat FST, a single acute I_{40} administration elicited a statistically significant effect. In contrast, one-way ANOVA did not reveal a significant effect for treatment for the swimming [$F_{(2,20)} = 2.73$, $P = 0.08$] or climbing time [$F_{(2,20)} = 0.65$, $P = 0.53$]. Thus, neither active behavior was significantly altered by ibogaine administration, however there was an upward trend in the swimming readout. Figure 2B shows the effect of ibogaine in the FST evaluated 24 h after its i.p. administration. One-way ANOVA did not reveal a significant effect for treatment; neither for immobility [$F_{(2,17)} = 0.85$, $P = 0.44$], swimming [$F_{(2,17)} = 1.31$, $P = 0.29$] nor climbing time [$F_{(2,17)} = 0.53$, $P = 0.59$], although the swimming time showed a qualitative trend toward a dose-dependent increase.

In order to correlate the behavioral results and the in vivo concentrations of ibogaine and noribogaine, we carried out PK studies in brain (Figure 2C) and plasma (See Supporting Information, SI) for both drugs after I_{40} using the same route of administration, evaluation time, and rat strain.

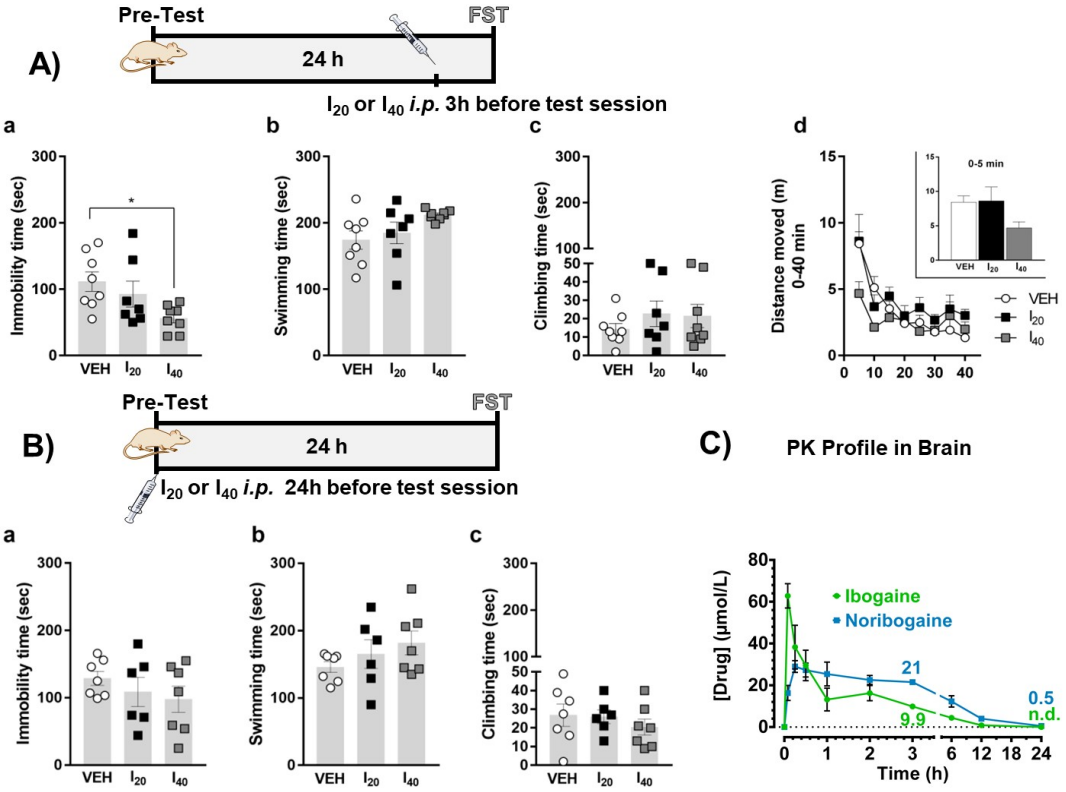

Figure 2. Ibogaine i.p. acute administration elicits dose-dependent and time-dependent antidepressant-like effects in rats. A/ Effect of ibogaine (i.p. 20 and 40 mg/kg) evaluated in the FST (a-c) 3 h after the acute administration. The locomotor activity was recorded in a separate group of animals in the Open Field Test (OFT, d). The inset graph shows the distance traveled by the animals in the first 5 min, in accordance with the test session period of the FST. Only I_{40} was able to induce a significant decrease in the immobility time compared to the control group ($P < 0.05$) without any significant change in locomotor activity. B/ Effect of Ibogaine (i.p. 20 and 40 mg/kg) evaluated in the FST 24 h after the administration. No significant antidepressant-like effect was found for either dose. C/ Pharmacokinetic profile showing ibogaine and noribogaine total concentrations in the rat brain after I_{40} i.p. administration (concentrations of ibogaine and noribogaine at FST relevant time points are highlighted). Error bars represent mean ± SEM. One-way ANOVA followed by Tukey's multiple comparisons test. * = $P < 0.05$ as compared to vehicle control. N = 7 - 8

Our results confirmed earlier PK reports in rats, regarding ibogaine´s fast metabolism to noribogaine (which becomes the relatively major species in plasma and

brain after ~ 45 min) and a high brain/plasma concentration ratio for both drugs (ibogaine ~7.7 and noribogaine ~7.9). Nevertheless there were some differences between our data and that reported by Baumman et al[23]: namely, our results showed ibogaine´s C_{max} being greater than the noribogaine's value, both in plasma (1.5 fold) and in brain (2.2 fold) (for PK details see Supporting Information), while the reversed scenario was found by the noted report. A possible explanation for this discrepancy can be related to differences between whole blood (Baumann) and plasma (our study) drug content and or a different strain of rats used.[41]

Correlation of the PK values and the behavioral outcome suggests that the response observed in the FST depends on the simultaneous presence of both ibogaine and noribogaine in the brain. At 3 hours where a significant reduction in the immobility was found, both drugs are circulating and evident in the brain tissue, where the mean total concentration of 21 μM was found for noribogaine and 9.9 μM for ibogaine (Figure 2C). In contrast, at 24 hours, where no effect was observed in the FST, ibogaine was not detectable while a residual amount of noribogaine (a mean value of 0.5 μM, ~2% of its C_{max}) was found in the brain. These results led to the next question of which compound is responsible of the reduction of the immobility time at 3 hours after the I_{40} treatment.

Norbogaine induces a dose-dependent antidepressant-like effect in rats but with a different time profile than ibogaine. In order to study the impact of noribogaine in the behavioral effect induced by ibogaine, we decided to study the acute effect of two doses of noribogaine, 20 mg/kg (N_{20}) and 40 mg/kg (N_{40}) in the FST. Since no previous complete pharmacokinetic profiles were found in the literature for noribogaine i.p. administered to rats, concentrations in plasma and brain were determined up to 24 hours for N_{40} (see Figure 3C) in order to select time points for the FST study. The pharmacokinetic data obtained is consistent with previous results in several species showing high bioavailability and high brain penetration of noribogaine

after direct noribogaine administration (for key parameters, see Supporting Information).[14,22,39,40,42] Guided by the PK results, we carried out the FST at 0.5 h after noribogaine i.p. administration, where the maximal brain concentration of noribogaine is achieved. Additionally, to compare the results to those obtained after 3 h of i.p. ibogaine administration, another experimental group was assayed at this time point after noribogaine administration.

Results showed a significant and robust effect for the noribogaine treatment on the time rats spent immobile after 0.5 hours of the i.p. administration [$F_{(2,18)} = 7.24$, $P < 0.01$] (Figure 3A). Tukey post-hoc analysis showed a significant decrease in the immobility time following N_{40} relative to vehicle-pretreated animals ($P < 0.01$) and the lower dose treatment ($P < 0.05$). In contrast, one-way ANOVA did not reveal significant changes for swimming [$F_{(2,18)} = 2.44$, $P = 0.11$] or climbing behaviors [$F_{(2,18)} = 1.85$, $P = 0.18$]. To evaluate potential behavioral confounds, the motor activity of animals was examined (Figure 3A). One-way ANOVA indicated that no significant changes in the animal locomotor performance were seen after N_{40} treatment [$F_{(2,12)} = 0.60$, $P = 0.56$]. In contrast, at 3 hours one-way ANOVA did not show significant changes [$F_{(2,14)} = 0.77$, $P = 0.48$] in the immobility time of noribogaine-treated animals (Figure 3B). Further, neither swimming [$F_{(2,14)} = 0.65$, $P = 0.53$] nor climbing behavior [$F_{(2,14)} = 0.48$, $P = 0.62$] were altered significantly by noribogaine (Figure 3B), although there was a qualitative upward trend in the swimming time.

The PK results showed that at 0.5 hours a mean total concentration of 144 μM of the drug was reached in the brain. Since N_{20} was not effective at significantly altering the behavioral patterns of the animals, considering a linear pharmacokinetic profile, we can suppose that ~ 72 μM of noribogaine assumed to be in the brain at 0.5 hours after N_{20} (half of 144 μM found for the C_{max} after N_{40} dose) was not sufficient to produce a statistically significant effect, although a clear trend was observed (Figure 3A).

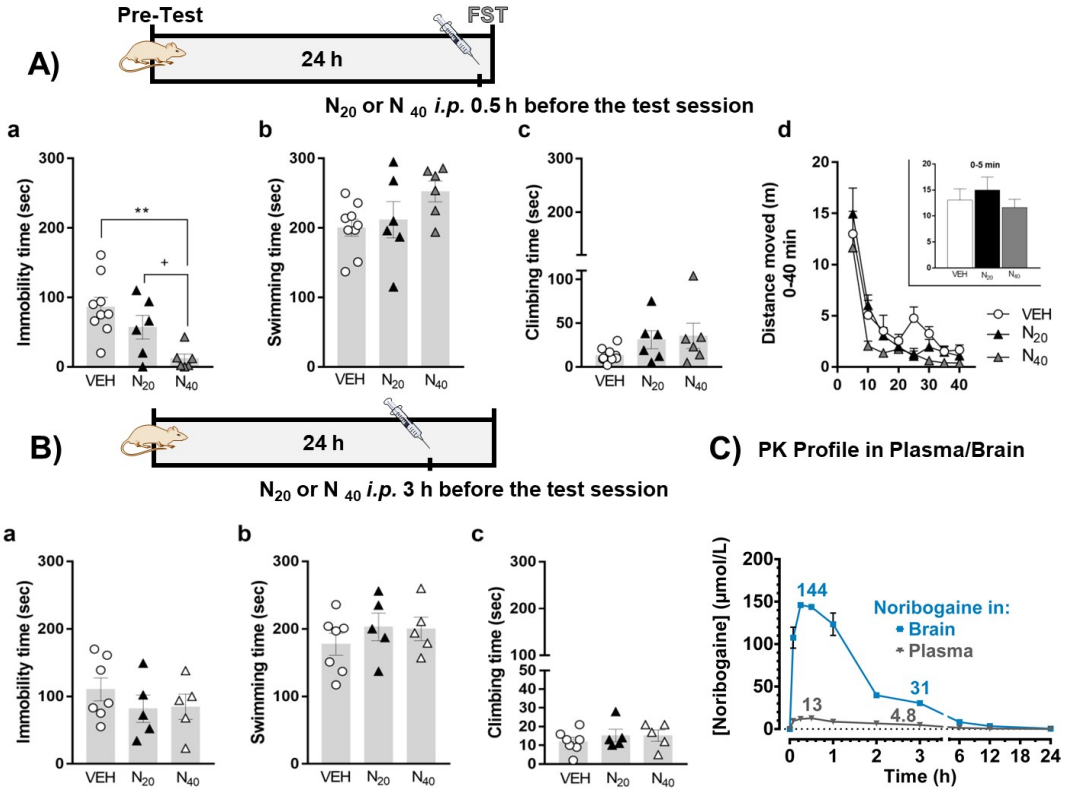

Figure 3. **Noribogaine i.p. acute administration elicits dose-dependent and time-dependent antidepressant-like effects in rats**. A/ Effect of noribogaine (20 and 40 mg/kg) evaluated in the FST 0.5 h after the acute administration. The locomotor activity was recorded in a separate group of animals in the Open Field Test (OFT, d). The inset graph shows the distance traveled by the animals in the first 5 min, in accordance with the test session period of the FST. N_{40} was able to induce a significant decrease in the immobility time relative to vehicle-pretreated animals ($P < 0.01$) and N_{20} ($P < 0.05$), without any significant change in the distance traveled by the animals in the OFT. B/ Effect of noribogaine (i.p. 20 and 40 mg/kg) evaluated in the FST 3 h after the acute administration. No antidepressant-like effect was found for either dose. C/ Pharmacokinetics profile showing noribogaine concentrations in rat brain and plasma after N_{40} i.p. administration (concentrations of noribogaine at FST relevant time points are highlighted). Error bars represent mean ± SEM. One-way ANOVA followed by Tukey's multiple comparisons test. ** = $P < 0.01$ as compared to vehicle control and + = $P < 0.05$ as compared to noribogaine. N = 5 - 9

Similarly, the mean total brain concentration of 31 μM of noribogaine determined at 3 hours is not enough to exert an antidepressant-like effect in the FST. Consequently, it is evident that noribogaine at concentrations found in the rat brain 3 hours after i.p. administration of ibogaine (I_{40}, 21 μM, compare concentration values at 3 hours in Figure 2C and 3C) cannot alone drive the antidepressant-like effect of ibogaine. Therefore, the next question was whether ibogaine itself may actuate the antidepressant-like effect at brain concentration at or above that found 3 hours post drug administration (9.9 μM, I_{40} treatment). In order to investigate this possible scenario, we examined the capacity of ibogaine to evoke an antidepressant-like effect in the FST under conditions where metabolism to noribogaine is minimized.

Ibogaine itself does not drive the antidepressant-like effect found in the FST after ibogaine i.p. administration. In this experiment, we administered ibogaine through the intravenous route (i.v.) and evaluated the behavioral response in the FST. As reported previously,[23] ibogaine i.v. administration greatly reduced its metabolism to noribogaine because of liver first-pass avoidance, producing maximal ibogaine concentration values one-minute post-injection, with only trace amounts of noribogaine detected in blood. An ibogaine dose of 10 mg/kg i.v. used in that study, produced significant transient behavioral impairments in rats, such as tremors, forepaw tapping, abnormal postures, body sway and staggering-type locomotion that lasted for 30 minutes after administration. In order to avoid these behavioral impairments that could interfere with the swimming or climbing performance, we tested the effect in the FST after 1 minute of the i.v. administration of 1 mg/kg (I_1) and 5 mg/kg (I_5) of ibogaine (Figure 4A). In addition, concentration profiles for ibogaine and noribogaine in plasma and brain were determined after the higher dose (Figure 4B).

For immobility time, one-way ANOVA did not reveal a significant effect for treatment [$F_{(2,20)} = 0.73$, $P = 0.49$]. In addition, neither swimming [$F_{(2,20)} = 1.81$, $P = 0.18$] nor climbing behavior [$F_{(2,20)} = 2.80$, $P = 0.08$] were altered significantly by either

ibogaine i.v. doses. Nevertheless, a trend for reduction in the immobility time can be observed for the I_5 treatment. While it is conceivable that ibogaine would show a reduction in the immobility time at higher concentrations, it is not feasible to examine higher doses due to the above-mentioned behavioral side effects that are not compatible with animal performance in the FST. Examining brain PK results we confirmed that total noribogaine concentrations at 1 minute after injection were minimal (~0.07 μM) and ibogaine total concentration was determined to be 23 μM. Therefore, this brain level of ibogaine by its own was not able to produce a robust antidepressant-like effect in the FST. Comparing this result with the concentration of ibogaine detected 3 hours after i.p. I_{40} administration (~ 10 μM), it seems that ibogaine itself cannot be responsible for reduction in the immobility time found in the FST during this experimental condition.

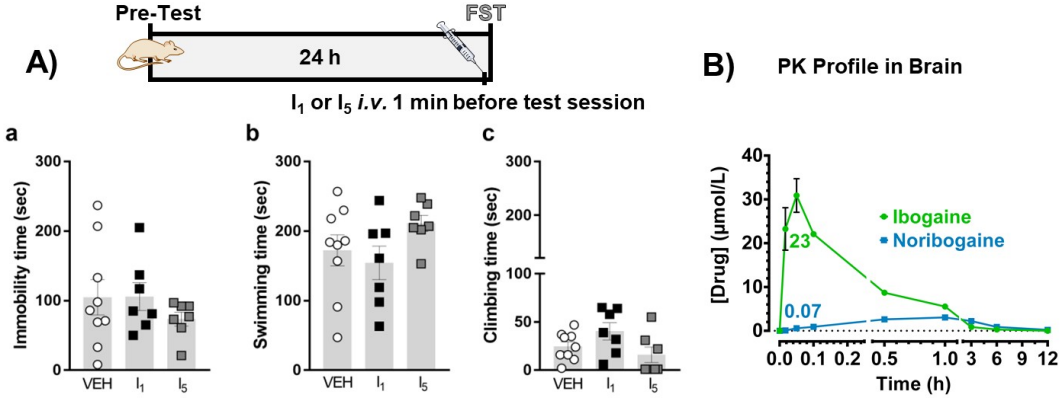

Figure 4. Ibogaine i.v. acute administration does not elicit an antidepressant-like effect in rats. A/ Effect of ibogaine (i.v. 1 and 5 mg/kg) evaluated in the FST 1 minute after the acute administration. No antidepressant-like effect was found at either dose. B/ Pharmacokinetics profile showing ibogaine and noribogaine total concentrations in brain after I_5 i.v. administration. Error bars represent mean ± SEM. One-way ANOVA followed by Tukey's multiple comparisons test. Non significance was found. N = 7 - 9

Interpretation: Both ibogaine and noribogaine are required for expression of antidepressant-like effect in the FST after ibogaine i.p. administration. Figure 5 A-

B summarizes the relationship between brain PK and behavioral data obtained in the FST described previously. As can be seen, 23 μM of ibogaine found in the rat brains at 1 minute after i.v. I_5 administration, or 31 μM of noribogaine found after 3 h of i.p. N_{40} administration, did not produce a statistically significant antidepressant-like response in the FST. In contrast, when both drugs are present in the brain together at even lower individual concentrations (9.9 μM ibogaine, 21 μM noribogaine) after 3 h i.p. I_{40} administration, a significant reduction in the immobility time was observed, suggesting a putative additive or synergistic effect produced by both drugs after I_{40} administration. Alternatively, these results could be rationalized by formation of an additional unknown metabolite of ibogaine (that would be responsible for the antidepressant-like effect), which would be formed after the i.p. but not intravenous administration.

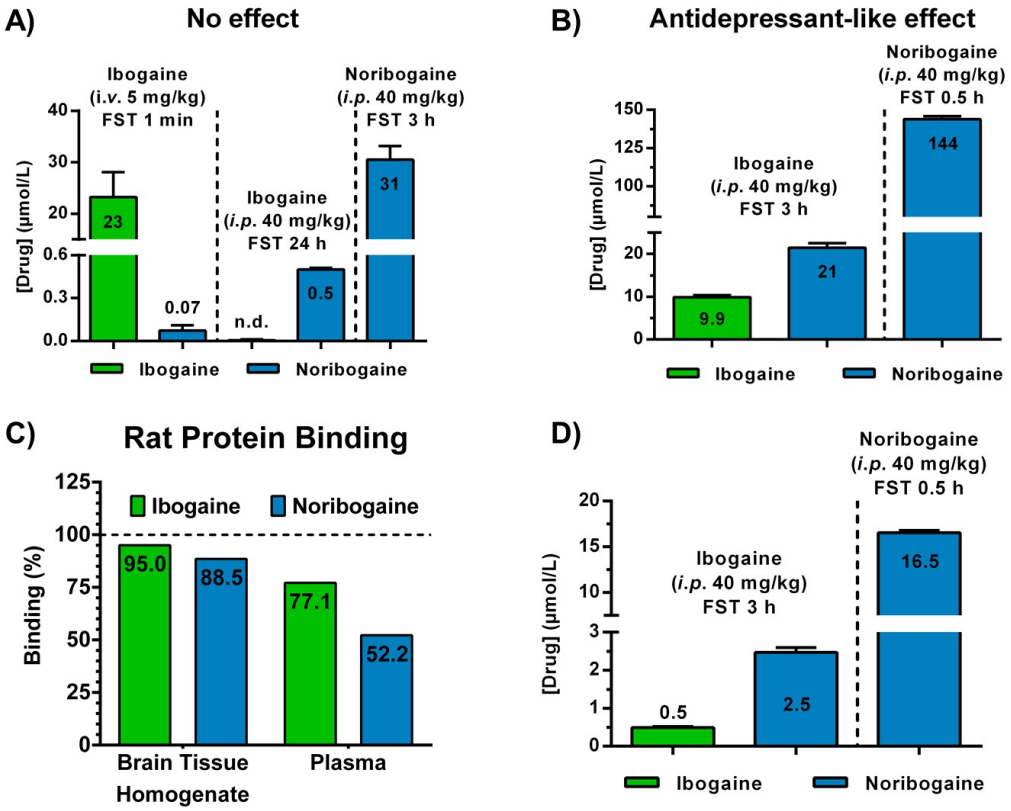

Figure 5. Correlation between pharmacokinetic data of ibogaine and noribogaine and the behavioral outcomes induced by both drugs in the FST at different times. Total concentrations of ibogaine and noribogaine found in rat brain after treatment A/ without effect

and B/ with antidepressant-like effect. C/ Binding of ibogaine and noribogaine to plasma proteins and brain tissue. D/ Estimated free brain concentrations of ibogaine and noribogaine found in rat brain that correlate with an antidepressant-like effect. Error bars represent mean ± SEM.

Regarding a possible neurochemical mechanism that explain the observed antidepressant-like effect, both drugs have been shown to inhibit SERT, noribogaine being approximately ten-times more potent than ibogaine (IC_{50} for noribogaine ~ 50-300 nM).[22-24] We determined the plasma protein binding (in rat and human plasma) and rat brain tissue binding for both ibogaine and noribogaine, which allows for estimation of free plasma and free brain concentrations (Figure 5C and 5D). We estimate that at 3 hours post I_{40} free brain concentration of ibogaine is ~ 0.5 μM and noribogaine 2.4 μM, which is within a range relevant for SERT modulation. Thus, both drugs could inhibit 5-HT reuptake and produce an enhancement in serotoninergic transmission. *In vivo* microdialysis studies in rats treated with ibogaine and noribogaine (1 and 10 mg/kg, i.v.),[23,25,26] showed that both drugs can induce significant increments in the extracellular 5-HT content in the nucleus accumbens (NAcc) within the same range (two to threefold compared to the control group, noribogaine being more potent), comparable to effects induced by SSRIs. Similar results were obtained evaluating the effect of ibogaine on 5-HT tone using in vivo microvoltammetric studies.[43] However, additional neurochemical studies should be done to investigate the *per se* effect of ibogaine or noribogaine and their combination at the same doses assayed here, on 5-HT extracellular levels in brain regions that are more relevant to depression and other mood disorders (e.g., prefrontal cortex and hippocampus).

Finally, it is well documented that antidepressant drugs that selectively inhibit noradrenaline uptake, reduce immobility and increase climbing behavior without affecting swimming, while antidepressants that inhibit serotonin uptake decrease immobility but increase swimming behaviour.[44] None of both active behaviors were

significantly modified by ibogaine and noribogaine treatments used in this study, although there were trends toward increasing the time rats spent swimming compared to the control groups. This may reflect the abovementioned enhancement in the serotoninergic transmission owing to SERT inhibition and 5-HT re-uptake attenuation. However, a sole modulation of SERT does not provide a comprehensive explanatory model as the estimated free brain concentration of > 8 μM is required for noribogaine to elicit significant FST effects when used alone: N_{20} was not significant, which is estimated to give ~ 72 μM of noribogaine total tissue concentration (½ of 144 μM measured at 30 minutes), which is approximately equivalent to ~ 8 μM of free noribogaine brain concentration). This estimated concentration range is > 20 greater than the in vitro SERT inhibitory potency of noribogaine, the more potent SERT inhibitor of the two compounds. The two-drug effect suggests additional target(s) at play. To further probe this hypothesis, we carried out an additional experiment to test whether fluoxetine (a classical SSRI) was able to induce an antidepressant-like effect in the FST after a single dose of 40 mg/kg.

Fluoxetine does not induce an antidepressant-like effect after a single dose treatment. To compare the efficacy of the iboga alkaloids used in this study to classical SSRIs, we examined the effect elicited by fluoxetine, the prototypical SSRI, following the same injection schedule and dose as those for noribogaine (Figure 6). In contrast to noribogaine, fluoxetine (40 mg/kg) did not modify the immobility (t_{10} = 0.62, P = 0.55), swimming (t_{10} = 0.32, P = 0.75) or climbing time (t_{10} = 0.28, P = 0.78), suggesting that noribogaine was more effective than fluoxetine at inducing an antidepressant-like effect in this model. In order to obtain a significant reduction in the immobility time, three doses of fluoxetine were required in our experimental setting (See supporting information, consistent with previous reports).[33-35] Fluoxetine is a potent SERT blocker (low nM range)[45] and produces significant increases in extracellular 5-HT as established by *in vivo* microdialysis studies.[46] Our results suggest substantial

mechanistical differences between the iboga alkaloids and classical SSRIs. Typical SSRIs inhibit SERT in a competitive manner,[47] while ibogaine acts non-competitively at the transporter but displays competitive binding towards SSRIs and stabilizes an inward-facing conformation.[24,48,49] The functional and biological consequences for these different interaction modes need further investigation. Alternatively, a polypharmacological mode of action for the iboga alkaloids with respect to antidepressant-like behavior represents a rational mechanistic model (e.g. NMDA-R non-competitive antagonism, which has been described for ibogaine, can contribute to its antidepressant-like effects). Efforts toward elucidating the neurochemical mechanisms behind the antidepressant-like effect found in this study for I_{40} and N_{40} are ongoing in our laboratories.

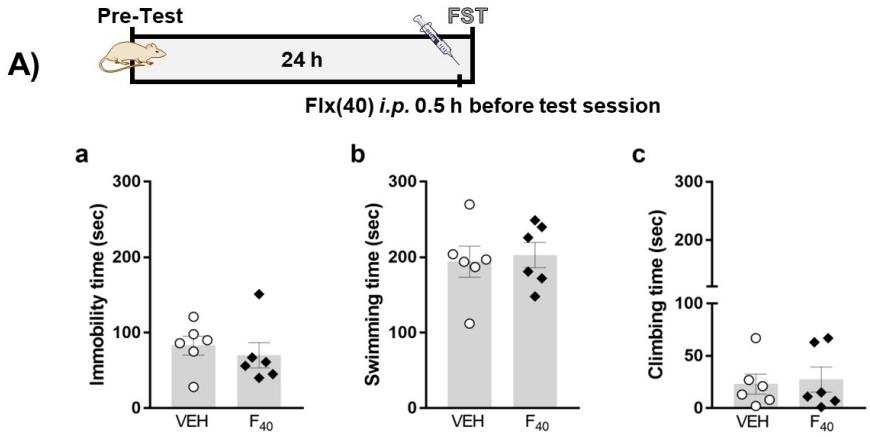

Figure 6. Fluoxetine 40 mg/kg i.p. acute administration does not elicit an antidepressant-like effect in rats. Acute effect of F_{40} administration in the FST 30 minutes after the i.p. administration. No antidepressant effect was found. Error bars represent mean ± SEM. Unpaired Student *t*-test. Non significance was found. N = 6

CONCLUSIONS

In the present study we demonstrated for the first time that an i.p. administration of ibogaine and noribogaine in rats produce an acute antidepressant-like effect in a dose- and time-dependent manner. Using a single dose of 40 mg/kg, the effect was observed after 3 hours of administration of ibogaine, while noribogaine had

a shorter duration (present 30 minutes after administration but lost at 3 hours). We demonstrated that the antidepressant-like response produced after ibogaine i.p. administration correlates with the presence of both ibogaine and noribogaine in rat brain at concentrations that cannot produce the same behavioral outcome on their own, indicating a potential additive/synergistic effect of both drugs or the existence of an unknown active metabolite. Moreover, the effect found using a single dose of ibogaine or noribogaine could not be reproduced by an equivalent dose of the classic SSRI fluoxetine. The utility of FST has been challenged owing to its poor translational potential (i.e. many compounds that show positive results in FST failed in clinical studies), however its pharmacological validity or reverse translational validity is excellent (i.e. many different classes of clinically efficacious antidepressants are active in rodent FST). Therefore, FST serves well for the purposes of this study where a clinical signal was reported for ibogaine and we aim to reverse engineer in animal models to gain mechanistic insights. In this context, our results indicate a potentially different neurochemical mechanism for the iboga alkaloids as compared to classical SSRIs, and constitute the first step toward identifying putative differences between classical antidepressant drugs (e.g., fluoxetine) and ibogaine or noribogaine as a potential new class of antidepressant drugs. Additional behavioral tests will be carried out in the future to examine the effect of ibogaine/noribogaine on other aspects of animal behavior that bear face validity to human disease such as motivational and anhedonic readouts.

Finally, our findings provide preclinical confirmation of the recently reported antidepressant effects of ibogaine in opioid-dependent subjects. It is therefore conceivable that the antidepressant efficacy may contribute to the anti-addictive property of ibogaine and noribogaine.

METHODS

Animals

Forced Swim Test and Open Field Test were carried out in the Instituto de Investigaciones Biológicas Clemente Estable (IIBCE, Uruguay) using adult male Wistar rats weighing 290-320 bred in the animal facility of the School of Medicine (Universidad de la República - Uruguay). All animals were acclimated in IIBCE facility for 5 days after they arrived. Animals were housed in groups of 4-5 in plastic cages (50 cm × 37.5 cm × 21 cm) and kept under controlled conditions (temperature 22 ± 2°C, 12-h day-night cycle, lights on at 7:00 am) with food and water available *ad libitum*. All procedures were carried out in accordance to the Bioethics Committee Guidelines of the IIBCE and following the "Guide for use and care of Animals in the Laboratory" of the National Institute of Health, (NIH Publications N° 8023, revised 1978) and current ethical regulations under animal experimentation law N° 18.611 (Montevideo Uruguay).

Pharmacokinetics studies were conducted at Sai Life Sciences Limited, Pune, India, with approval of Institutional Animal Ethics Committee (IAEC) in accordance with requirement of The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Healthy male Wistar rats (8-12 weeks old) weighing between 280 to 300 g were procured from Global, India. Three rats were housed in each cage. Temperature and humidity were maintained at 22 ± 3 ºC and 30-70 %, respectively and illumination was controlled to give a sequence of 12 h light and 12 h dark cycle. All the animals were provided laboratory rodent diet (Envigo Research private Ltd, Hyderabad). Reverse osmosis water treated with ultraviolet light was provided ad libitum.

In all the experiments involving animals, adequate measures were taken into account to minimize pain, discomfort or stress, and all efforts were made to use the minimal number of animals necessary to obtain reliable scientific data.

Drugs

Ibogaine·HCl

The ibogaine used in this study was chemically synthesized in the Laboratorio de Síntesis Orgánica, Facultad de Química – Universidad de la República, using voacangine as starting material, which was extracted from the root bark of *Voacanga africana* using a modification of a previously described procedure.[50] Briefly, 100 g of grounded root bark of *Voacanga africana* was extracted with a 1% aqueous solution of HCl (6 x 500 mL). The combined aqueous extracts were basified by adding concentrated NH_4OH until pH 10 - 11. A brown precipitate was separated by centrifugation and dried at 60°C for 24 h. This solid was taken in acetone and filtered to discard root impurities. The solvent was evaporated in vacuo to afford a total alkaloid extract of 3.5-4.0 g. Column chromatography (SiO_2, Hex:EtOAc:NH_4OH, 90:10:0.01) allowed to obtain 1 g of pure voacangine which was analyzed by ^{1}H and ^{13}C NMR (See supporting information). Voacangine was decarboxylated as follows. To a solution of voacangine in EtOH (0.45 M) in a double necked round bottomed flask, KOH in pellets (10 equivalents) was added. The solution was heated to reflux until consumption of the starting material was evident by thin layer chromatography (TLC) analysis. EtOH was removed under reduced pressure, and the residue was dissolved at 0 °C in a round bottomed flask using a 6% (v/v) aqueous solution of HCl (enough quantity to adjust pH to 1). The system was then heated to reflux for five minutes. Once the starting material consumption was evident by TLC analysis, the solution was carefully basified using 50% NaOH (pH 10 - 11). Precipitation of ibogaine as a white solid was observed. Ethyl acetate was added, and the resultant biphasic system was transferred into a separation funnel. The aqueous phase was extracted three times with EtOAc. The combined organic layers were dried under Na_2SO_4, and the solvent was removed *in vacuo*. Ibogaine free base was obtained with an 86% yield and was analyzed by ^{1}H and ^{13}C NMR (see supporting information). Crystallization from EtOH afforded a crystalline solid which was converted to the corresponding hydrochloride by treatment with diethyl ether saturated with HCl(g). Purity of ibogaine·HCl was determined by GC-MS analysis as 98.3 % (see supporting information). Dissolution of ibogaine-HCl to prepare the

samples for i.p. injection was carried out using warm saline/ethanol (9:1) vehicle that was previously degassed by nitrogen bubbling.

Noribogaine·HCl

Noribogaine·HCl used for the FST studies was prepared by demethylation of ibogaine in the Laboratorio de Síntesis Orgánica, Facultad de Química, Universidad de la República. Briefly, under Nitrogen atmosphere, aluminium (3.0 equivalents, previously washed with hexanes and dried overnight at 70 °C) was suspended in MeCN (enough volume for a 0.3 M suspension), and iodine (3.0 equivalents) was added. The suspension was heated under reflux until brownish color from iodine disappeared. The resulting suspension was cooled to reach room temperature where a solution of 0.2 M of ibogaine in MeCN (0.3 equivalents) was added. The suspension was heated under reflux for at least 3 hours, until analysis by TLC (hexanes: ethyl acetate, 1:1 + 1% NH_4OH) revealed complete consumption of ibogaine. After cooling to room temperature, the suspension was added to aqueous HCl 5 % to destroy excess of aluminium species. The resulting aqueous phase was basified by adding $NaHCO_3$, and further extracted exhaustively with ethyl acetate. Combined organic layers were dried using Na_2SO_4, and solvent distilled *under vacuo* to obtain a crude reaction mixture, which was purified using column chromatography (SiO_2, hexanes: ethyl acetate, 1:1 + 1% NH_4OH) to obtain noribogaine as a pure white solid with 76 % yield. The free base was converted to the corresponding hydrochloride by treatment with diethyl ether saturated with HCl (g). Purity of noribogaine·HCl was determined by GC-MS analysis as 95.2 % (see supporting information). Dissolution of noribogaine·HCl to prepare the samples for i.p. injection was carried out using warm saline/ethanol (95:5) vehicle that was previously degassed by nitrogen bubbling.

Noribogaine·HCl used for the PK studies was prepared at Columbia University by Vaclav Havel.

A solution of voacangine (0.15 M) in CH_2Cl_2 (anhydrous) was cooled in ice bath (0 °C) and treated with EtSH (4.5 equiv.) and BBr_3 (1M in CH_2Cl_2, 1.5 equiv., added dropwise over 5 min). After all reagents were added, reaction mixture was allowed to warm to room temperature. Progress of reaction (from small aliquots of reaction mixture quenched with saturated $NaHCO_3$ solution) was monitored by TLC (hexanes: ethyl acetate, 1:2), until no more starting material was observed (usually 1-2 h). Reaction was quenched with a small amount of CH_3OH (until oily precipitates completely dissolved) and poured to a saturated $NaHCO_3$ solution. The mixture was extracted 3x with CH_2Cl_2 (until no more extraction was observed by TLC), combined extracts were dried over Na_2SO_4 and concentrated *under vacuo*. Desmethyl-voacangine (typical yield >95%) is obtained as an off-white foamy solid. Material is of sufficient purity to use for the next step without further purification, if necessary, it can be purified by column chromatography (SiO_2, hexanes: ethyl acetate, gradient of 20 to 30%).

Desmethyl-voacangine and either KOH or $LiOH \cdot H_2O$ (5 equiv.) were combined in a mixture of EtOH and H_2O (2:1, 0.3 M). Reaction mixture was further heated to 80 °C under argon atmosphere in a closed reaction vessel (a dark brown solution is formed). After TLC indicated full conversion of starting material (hexanes: ethyl acetate, 1:2, <24 h) reaction mixture was cooled to room temperature and concentrated *under vacuo*. Residue was dissolved in a small amount of H_2O (~15 - 20 mL for 1g of starting material) and the solution was added to a hot 2M HCl (~30 mL for 1g of starting material, pH must be ~1 after the solutions are combined) and the mixture was further refluxed for 5 min under argon atmosphere. After cooling to RT, the mixture was partially neutralized with 50% NaOH solution (to pH 5 - 6) and final adjustment was done using excess solid $NaHCO_3$ (careful addition). Mixture was extracted repeatedly with CH_2Cl_2 (until no more material was extracting as evidenced by TLC, if needed CH_2Cl_2:iPrOH 9:1 mixture can be used to increase the speed of extraction). Combined organic extracts were dried over Na_2SO_4, concentrated *under vacuo* and the dark brown residue was pre-purified by a short column chromatography (SiO_2, hexanes:

ethyl acetate + 2% Et_3N, 1:2 to 1:1). Excess Et_3N was removed from isolated noribogaine by repeated evaporation from MeOH/H_2O mixture. The still slightly colored noribogaine free base was dissolved in CH_3OH (~10 - 15 mL for 1g of free base) and the solution was acidified using aq. HCl (36%) to pH (~2, estimated by a pH paper). Upon cooling to RT noribogaine·HCl started to crystallize from the solution. The suspension was further cooled in freezer (-20 °C), the solid was collected by filtration and washed repeatedly with cold CH_3OH to obtain an off-white (grey to light pink) crystalline noribogaine·HCl (average yield of 60 - 65%) and a less pure methanolic fraction. The crystalline noribogaine·HCl contains ~0.8 equiv. of CH_3OH bound in its crystal structure which is not removed even after extensive drying *under vacuum.*

Behavioral tests

Forced Swim Test (FST)

FST is one of the most commonly used assays for the study of depressive-like behavior in rodents, since examines certain aspects of despair and coping behavior in rodents[37]. Its sensitivity to a broad range of antidepressant drugs makes it a suitable screening test[38]. The procedure used for the FST was performed as previously described.[33,44,51] The apparatus consisted of a transparent cylindrical tank (50 cm height and 20 cm diameter) filled with water (24 - 25 °C) to a depth of 34 cm (in order to allow rats to swim or float without touching the bottom of the tank with their paws). Two experimental swimming sessions within an interval of 24 h were carried out as previously described.[51] Briefly, in the first pretest session, the animals were placed in the tank for 15 min, immediately dried and returned to their home cage. During the test session, the total time spent immobile (making only the movements necessary to remain afloat), swimming (horizontal movements throughout the cylinder) and climbing (vigorous and upward-directed movements of the forepaws along the cylinder wall)

were scored by a single, trained observer in real time during 5 min.[44,52] Sessions were recorded for later analysis or confirmation when necessary.

These experimental groups were independently used for the behavioral studies: 1) animals received an intraperitoneal (i.p.) injection of ibogaine at 20 mg/kg, 40 mg/kg, or vehicle (ethanol 5 % and saline; control group) and then exposed to the FST test session 24 h and 180 minutes after the administration; 2) animals received an i.p. injection of noribogaine at 20 or 40 mg/kg i.p. or vehicle (control group), and tested 30 or 180 min after the administration; 3) animals received an intravenously (i.v.) injection of ibogaine at 1 and 5 mg/kg, or vehicle (control group) and exposed to the FST test session immediately after the administration; 4) animals received an i.p. injection Fluoxetine at 40 mg/kg (equivalent dose of noribogaine) only once or vehicle (saline).

Open Field Test (OFT)

This behavioral test was carried out in an independent group of animals to discard that the behavioral changes in the FST had been associated to alterations in the animal motor activity. The OFT apparatus consists of a square arena (45 cm wide × 45 cm long × 40 cm high) with transparent plastic walls indirectly illuminated (35 luxes) to avoid reflection and shadows. Locomotor activity was recorded automatically by a camera located above the field connected to a computer equipped with the Ethovision XT 12.0 software (Noldus, The Netherlands). We measured the horizontal locomotor activity defined as the total distance moved in meters (m). After recording the animal behavior, the OF was cleaned with alcohol 30 % before placing the following rat .[53] In all the experiments rats were naive to the OF and were used only once. All the experiments were performed between 9:00 and 14:00 h.

Pharmacokinetic Experiments

These experiments were conducted at Sai Life Sciences Limited. Plasma pharmacokinetics and brain distribution of ibogaine and its metabolite noribogaine were

determined in three study groups of male Wistar rats following a single intravenous (5 mg/kg) and intraperitoneal (40 mg/kg) administration of ibogaine and intraperitoneal (40 mg/kg) administration of noribogaine. Solutions of nor/ibogaine were administered in 10% ethanol and 90% normal saline via intraperitoneal/intravenous route. The dosing volume administered was 5 mL/kg for intravenous and 10 mL/kg for intraperitoneal route. Blood samples (approximately 120 µL) were collected under light isoflurane anesthesia from retro orbital plexus from a set of three rats at specified time intervals. Samples were collected into labeled micro-tubes, containing K_2EDTA solution (10 µL of 20% K_2EDTA of solution per mL of blood) as an anticoagulant. Plasma was immediately harvested from the blood by centrifugation at 4000 rpm for 10 min at 4 ± 2 ºC and stored below -70 ± 10 ºC until bioanalysis. Immediately, after blood collection animals were euthanized with excess CO_2 and Brain was isolated at each time point. Brain rinsed three times in ice cold PBS (for 5 - 10 seconds/rinse using ~ 5 - 10 mL fresh PBS in disposable petri dish for each rinse) and dried on blotting paper. Brain samples were homogenized using ice-cold phosphate buffer saline (pH 7.4) and homogenates were stored below -70 ± 10 ºC until analysis. Total homogenate volume was three times the tissue weight. See Supporting Information for detailed results.

Statistical Analysis

One-way analysis of variance (ANOVA) followed by post hoc Tukey's multiple comparisons tests and Unpaired Student *t*-test were conducted to assess the main effects of treatments in the FST and OFT. Calculations were carried out using GraphPad Prism 7 software. All data are presented as mean ± SEM, and significance was determined at $P < 0.05$.

ASSOCIATED CONTENT

Supporting information contains analytical and NMR spectra of the drugs used administered to the animals, and details from the pharmacokinetic study and the FST. This information is available free of charge via the Internet at http://pubs.acs.org/.

AUTHOR INFORMATION

Corresponding authors

Dr. Ignacio Carrera. Departamento de Química Orgánica, Facultad de Química – Universidad de la República, Av. General Flores 2124, C.P. 11800, Phone: +59829247881 Fax: +59829241906, E-mail: icarrera@fq.edu.uy

Dra. Cecilia Scorza. Departamento de Neurofarmacología Experimental, Instituto de Investigaciones Biológicas Clemente Estable, Av. Italia 3318, C.P. 11600, Phone: +59824871616, E-mail: cscorza@iibce.edu.uy

Dr. Dalibor Sames. Department of Chemistry, Columbia University, 3000 Broadway MC3101, New York, NY 10027 USA, E-mail: sames@chem.columbia.edu

Author Contributions

Experiments were designed by P.R., D.S., C.S. and I.C. Experiments were performed by P.R., J.U., J.P.P., S.F. A.L.R. and C.S. Data analyses were performed by P.R. C.S. V.H. D.S. and I.C. Manuscript was written by P.R., V.H., D.S., C.S. and I.C.

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