ELSEVIER

Drug and Alcohol Dependence 54 (1999) 183–194

DRUG and ALCOHOL DEPENDENCE

Behavioral profile of constituents in ayahuasca, an Amazonian psychoactive plant mixture

Cory S. Freedland [1,a], Robert S. Mansbach [b,*]

[a] *Department of Psychology, Connecticut College, 270 Mohegan Avenue, New London, CT 06320, USA*
[b] *Department of Neuroscience, Pfizer Central Research, Eastern Point Road, Groton, CT 06340, USA*

Received 4 May 1998; accepted 18 August 1998

Abstract

Ayahuasca is a psychoactive plant mixture typically composed of the β-carboline-rich *Banisteriopsis caapi* vine and the hallucinogenic plant Psychotria viridis. Ayahuasca has long been used by aboriginal populations for its putative spiritual and medicinal benefits. Although the presumed primary chemical constituents of ayahuasca have been identified, little is known about the basic in vivo pharmacology of the extract. Two principal constituents of ayahuasca, the β-carboline harmine and *N*,*N*-dimethyltryptamine (DMT) were selected for detailed study in mice using the Functional Observational Battery (FOB). The *B. caapi* extract was then examined alone and in combination with DMT. Harmine and the *B. caapi* extract produced similar effects in the FOB, particularly in the open field. Clonic and tonic motor movements were augmented by DMT administration. Harmine and *B. caapi* decreased acoustic startle amplitude without significantly affecting prepulse inhibition. DMT appeared to attenuate startle-decreasing effects of harmine and *B. caapi*, although these effects fell just short of significance. These results suggest that the behavioral effects of *B. caapi* in mice may be attributed in large part to its principal alkaloid species, harmine, and related β-carbolines in the extract. Hence, the presence of the banisteriopsis vine in the admixture may directly contribute to the unique subjective effects of ayahuasca.

Keywords: Ayahuasca; Mice; Functional observational battery; Harmine; Dimethyltryptamine; Ethnopharmacology

1. Introduction

Ayahuasca is a hallucinogenic decoction of potent psychotropic plants indigenous to the Amazon basin of South America (Ott, 1994). It has been known under a number of different names including caapi, yage, mihi, natema, dapa, daime, vegetal and hoasca (Grob et al., 1996). Prior to European colonization of South America, the plant was used for purposes of divination, witchcraft and religious ritual (Dobkin de Rios, 1972). It is considered by some aboriginal populations to be an effective medicine and used to both diagnose and treat disease (Harner, 1973). Recently, the medicinal use of ayahuasca has been proposed as a possible treatment for cocaine addiction (Mabit, 1996).

The scientific study of ayahuasca was begun by Spruce (1908) who identified one of the primary ingredients in the decoction as a large woody vine that would later be assigned the botanical designation *Banisteriopsis caapi*. Spruce later concluded that the plant decoctions known in discrete regions of South America as yage, caapi and ayahuasca, were, in fact, all mixtures derived from the banisteriopsis vine (Gates, 1982). Early field research also revealed that other plants, including the tryptamine-rich Psychotria viridis, were often added to the banisteriopsis vine in the preparation of the decoction (McKenna and Towers, 1984; Ott, 1994).

* Corresponding author. Tel.: +1 860 4416452; fax: +1 860 4411854; e-mail: mansbach@pfizer.com

[1] Present address: Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Medical Center Blvd., Winston-Salem, NC 27101, USA.

0376-8716/99/$ - see front matter
PII: S0376-8716(98)00154-9

Rivier and Lindgren (1972) performed analytical studies on constituents of banisteriopsis and psychotria. Eight of nine samples contained the β-carboline harmine as the major alkaloid (22–62% of total alkaloid content) and *N*,*N*-dimethyltryptamine (DMT) as the second most important alkaloid (20–41% of total alkaloid content). It was concluded that a typical dose of the decoction from this region, 200 ml, would yield 25 mg of DMT and 40 mg of β-carbolines, primarily harmine (Rivier and Lindgren, 1972). McKenna et al. (1984) reported similar results using indigenous samples of ayahuasca. A recent pharmacokinetic study of orally administered ayahuasca reported peak plasma concentrations of DMT ranging from 12 to 26 ng/ml and harmine concentrations of 36–222 ng/ml (Callaway et al., 1996).

Most investigators believe that the unique properties of ayahuasca arise from the combination of harmine and DMT (McKenna, 1996; Strassman et al., 1996). In addition, it has been suggested that by virtue of their monoamine oxidase (MAO) inhibiting properties, harmine and related structures may increase the oral bioavailability of DMT, which is not well absorbed by this route of administration (Buckholtz and Boggan, 1977). However, little is known about the general pharmacology of these compounds in combination, and the issues of abuse liability or toxicity of ayahuasca are becoming increasingly important with the advent of religious groups such as the Union de Vegetal and Santo Daime which utilize the decoction as a ritual sacrament (Callaway et al., 1994; Grob et al., 1996). Moreover, the proposed application of ayahuasca as a pharmacotherapy for cocaine addiction by groups such as the Takiwasi treatment clinic in Peru (Mabit, 1996), suggests that the general pharmacology of the decoction should be investigated.

In animals, harmine, harmaline, and structurally related β-carbolines are known to produce characteristic effects on body temperature and motor function. Most notably, harmine dose-dependently increases tremor in the mouse (Kawanishi et al., 1994), an effect that has been suggested to involve serotonergic (Kelly and Nayler, 1974), noradrenergic (Yamazaki et al., 1979), and GABAergic (Costa et al., 1975) mechanisms.

The subjective and physiological effects of DMT in humans have been well documented and closely resemble those of the structurally related indolealkylamine LSD (Szara, 1956, 1957; Strassman and Qualls, 1994; Strassman et al., 1994, 1996). In vitro, DMT binds with nanomolar affinity to 5-HT2 receptors, as do LSD and phenylisopropylamine hallucinogens such as 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane (DOB) and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM). Behavioral effects of DMT and similar compounds in animals have been investigated in a number of paradigms and are consistent with those of known hallucinogens. For example, the DMT analog 5-MeODMT produced dose-dependent substitution in animals trained to discriminate DOM or LSD (Young et al., 1982; Glennon et al., 1983).

The purpose of this investigation was to evaluate the behavioral effects of *B. caapi* extract and compare the results to those obtained with its principal constituent, both alone and in combination with DMT, the active constituent in Psychotria viridis. To this end, the Functional Observational Battery (FOB; Moser et al., 1988; Tegeris and Balster, 1994) was used. The FOB is a standardized observational neurobehavioral assay consisting of a series of tests for rapidly assessing sensory, autonomic, and motor integrity. The tests are intended to evaluate global behavioral function and establish dose-response characteristics of unknown compounds. In the present study, the actions of DMT, harmine, and combinations of harmine and DMT were first assessed in the FOB. Following these studies, *B. caapi* extract and *B. caapi*/DMT combinations were studied in the FOB with the goal of evaluating the differences between the principal constituents of ayahuasca and an intact sample of the plant containing the alkaloid species.

2. Method

2.1. Subjects

Adult male CD-1 mice (Charles River Breeding Laboratories, North Wilmington, MA), weighing 27–44 g at the time of testing ($n = 10$ per dose for all groups), were group housed in $45 \times 21 \times 21$-cm plastic cages fitted with steel wire tops and containing wood chip bedding in a temperature-controlled room on a 12-h light/dark cycle (7:00–19:00 h). Water and food were available ad libitum. Testing was conducted during the light cycle.

2.2. Apparatus

The following equipment was used for the FOB. One $45 \times 21 \times 21$-cm plastic box was used for scoring home cage measures. Two $45 \times 21 \times 21$-cm plastic cage boxes were used for scoring stimulus reactivity and the open field measures. The grip strength measure utilized a grip strength meter (Coulbourn Instruments, Columbus, OH). A VWR rectal thermometer with small animal probe was used for the body temperature measurements. Startle studies were conducted using San Diego Instruments (San Diego, CA) startle chambers and interface equipment. Stimuli were delivered and data collected by an IBM-compatible computer. Acoustic stimuli were delivered via a Radio Shack Supertweeter located in the chamber's ceiling. Responses were de-

tected using a sealed accelerometer device. Data were collected as 100 1-ms voltage readings which began immediately following the onset of the startle-inducing stimuli. The average of the 100 readings was selected as the dependent measure.

2.3. Experimental procedure

All animals were brought into the experimental procedure room at least 30 min prior to drug administration. The battery of tests was administered to each mouse either 5 (harmine) or 10 min (DMT, DMT-harmine, *B. caapi* extract, extract-DMT) post-injection. Times were selected based on previous and pilot experiments, in order to assure detection of neurobehavioral effects. Injections of harmine and DMT were administered intraperitoneally in a volume of 10 ml/kg. Injections of the *B. caapi* extract preparation were administered orally in the same volume. Mice were randomly assigned to dose groups ($n = 10$/dose), and the number of mice tested at each dose was counterbalanced across test days. Mice were tested only once. The observer was always blind to the dose identification. All data were recorded on standardized sheets and later entered into a microcomputer for statistical analysis. Procedures were approved by the Pfizer IACUC.

2.4. Functional observational battery

The procedure was modified from the FOB described by Tegeris and Balster (1994). The principal modification was the substitution of an automated acoustic startle procedure (described below) for the observational method used in previous reports. Following pretreatment, each mouse was observed for 1 min in its home cage. The observer recorded each mouse's posture in its home cage, along with the presence, or absence of, clonic and tonic movements, vocalizations, and palpebral closure. Posture was scored according to the following criteria: 1, sitting or standing normally; 2, rearing; 3, asleep, lying on side, or curled up; 4, flattened, limbs may be spread out; 5, lying on side, limbs in air; 6, crouched over; and 7, head bobbing. The observer then removed the mouse from the home cage, rating the ease of removal and the animal's reactivity to being handled. Ease of removal was assessed using the following criteria: 1, very easy (sits quietly, allows observer to pick it up); 2, easy (some vocalizations, little resistance); 3, moderately difficult (rears, often follows observer's hand); 4, animal flinches (with or without vocalizations); 5, difficult (runs around cage, is hard to grab, with or without vocalizations); and 6, very difficult (tail and throat rattles, attempts to bite, with or without vocalizations). Reactivity was assessed using similar criteria: 1, low (no resistance, easy to handle); 2, moderately low (slight resistance, with or without vocalizations); 3, moderately high (may freeze, be tense, or rigid in hand, with or without vocalizations); and 4, high (squirming, twisting, or attempting to bite, with or without vocalizations). While transferring the mouse to the open field the observer recorded palpebral closure, lacrimation, salivation, piloerection, and eye observations. Eye observations were scored according to the following criteria: 1, normal; 2, chromodacryorrhea; 3, deposits around eye(s); 4, exophtalmus; 5, nystagmus-unusual, repetitive eye movements; and 6, opacity.

The mouse was then placed in a $45 \times 21 \times 21$-cm box for open field observation. The mouse was observed for a 2-min period during which the observer recorded rearing, clonic movements, and tonic movements. During this 2-min period, the gait and mobility of each mouse was assessed qualitatively and assigned a corresponding score. Gait was assessed using two scales, gait and gait score. Gait was scored according to the following criteria: 0, no abnormality observed; 1, ataxia, excessive sway, rocks or lurches; 2, hindlimbs show exaggerated or overcompensated movements, drag, or are splayed; 3, feet markedly point outwards from body; 4, forelimbs drag, are extended, or unable to support weight; 5, walks on tiptoes; 6, hunched or crouched body position; and 7, body drags or is flattened against surface. Gait score was assigned according to the following criteria: 1, normal; 2, slightly abnormal; 3, moderately abnormal; and 4, severely abnormal. Mobility score was assessed as follows: 1, normal; 2, slightly abnormal; 3, somewhat impaired; and 4, totally impaired. The mouse's level of arousal and respiration were also recorded. Arousal level was scored as follows: 1, very low (stupor, coma); 2, low (somewhat sluggish, some head or body movement); 3, somewhat low (slightly sluggish, some exploratory movements with periods of immobility); 4, normal; 5, somewhat high (slightly excited, tense, sudden darting or freezing); and 6, very high (hyperalert, excited, sudden bouts of running or body movements). Stereotypy, bizarre behavior, defecation and urination were also noted.

Following the 2-min observation period the stimulus reactivity portion of the FOB began. Animals were approached from the front with a pen, tapped with a pen on their hind quarter, gently pinched with large metal forceps, and their responses recorded. Grip strength was measured by passing the animals' forelimbs and hindlimbs over the grip strength meter three times. Scores were recorded and averages determined for fore- and hindlimb strength. Rectal temperature was taken with the mouse gently restrained by hand.

Acoustic startle sessions, which assessed reflex amplitude and prepulse inhibition (PPI), were conducted immediately following the stimulus reactivity portion of the FOB. The sessions contained four discrete trial

types, administered in a variable order in three blocks. Each block consisted of four presentations of a 40-ms, 120 dB stimulus alone, four presentations of this stimulus preceded by 100 ms by a 20-ms, 75 dB prepulse, two presentations of the prepulse alone, and two trials in which there were no changes in stimulus conditions but samples were recorded. The three blocks of trials were preceded by an initial 120-dB trial which was excluded from the data analysis. Results were analyzed for effects on startle amplitude and PPI. PPI was defined as the percentage of startle magnitude in trials preceded by a prepulse relative to trials where no prepulse was presented.

2.5. Chemicals

N,*N*-dimethyltryptamine (5, 10, 17 and 32 mg/kg; Sigma, St. Louis, MO) was dissolved in 0.9% saline. Harmine (5, 10, 17 and 32 mg/kg; Sigma, St. Louis, MO) was dissolved in distilled water. *B. caapi* extract (100, 300, 1000 and 1700 mg/kg; NP-25971, Pfizer Natural Products Division) was dissolved in a combination (5:5:90) of ethanol, emulphur, and 0.9% saline. The *B. caapi* extract used in this study was tested for the presence of alkaloids before being tested in the FOB. HPLC analysis revealed the presence of an alkaloid which appeared to be harmine. Mass spectrometry analysis confirmed the presence of harmine as the principal alkaloid in the extract, supporting previous findings (McKenna et al., 1984; Ott, 1994; Callaway et al., 1996).

2.6. Data analysis

All rank-order data were analyzed using the Kruskal–Wallis one-way analysis of variance. In the case of significant differences within groups, the Mann–Whitney *U*-test was applied between each experimental group and the control group to assess significant effects. Descriptive data were tested for significance using χ^2-analysis. Interval data were tested for significance from control using a two-tailed independent Student's *t*-test. Startle data were analyzed by ANOVA, with Dunnett's *t*-test applied to main effects with significant differences.

3. Results

3.1. Harmine, DMT and harmine/DMT interaction

3.1.1. Home cage measures

Most of the FOB results are summarized in Table 1. Harmine had marked effects on home cage posture at the highest dose ($P < 0.001$). When co-administered with 32 mg/kg DMT, however, significantly flattened posture was observed at the 5 and 32 mg/kg harmine doses. DMT alone produced no significant alterations in this measure.

All three treatments increased the ease with which the animals were removed from the home cage ($P < 0.02$). This effect was seen with only the highest dose of DMT ($P < 0.01$). Harmine increased ease of removal at doses of 17 and 32 mg/kg ($P = 0.002$). The increased ease of removal present with the harmine/DMT combination was observed at these same dose levels ($P < 0.001$). Harmine treatment significantly increased reactivity to being handled at doses of 17 ($P = 0.04$) and 32 ($P = 0.0007$) mg/kg.

DMT did not produce significant clonic or tonic motor movements. In contrast, both harmine ($P < 0.0001$) and the harmine/DMT combination ($P < 0.0001$) induced robust clonic and tonic motor movements in the home cage. The most characteristic feature of these movements was a marked tremor. The clonic effect of harmine alone was seen at the 10, 17 and 32 mg/kg doses ($P < 0.0001$; Fig. 1) while tonic effects were only observed at the 32 mg/kg level ($P < 0.0001$; Fig. 2). The clonic effects of the combination were seen at all doses tested ($P < 0.0002$; Fig. 1), while the tonic effects were present at the 17 ($P = 0.06$) and 32 mg/kg ($P = 0.0005$; Fig. 2) doses. Significant potentiation of harmine's clonic effects by DMT was observed at the 5 ($P = 0.0001$) and 17 mg/kg ($P < 0.05$) dose levels, as well as a trend in the 32 mg/kg ($P = 0.08$) group. No significant difference was seen when the tonic effects of harmine and the combination were compared; however, this lack of significant difference may reflect the absence of tonic effects at all but the highest dose in the harmine group and the two highest doses in the combination group. The fact that tonic motor movements were present in the 17 mg/kg combination group but not in the 17 mg/kg harmine group suggests potentiation of harmine's tonic effects by co-administration of DMT. Comparison of these two groups revealed a trend toward significance ($P = 0.06$; Fig. 2).

3.1.2. Open field measures

In the open field all three treatments decreased arousal levels ($P < 0.0001$; Table 1). Harmine-induced decreases in arousal were seen in the 10 ($P = 0.003$), 17 ($P = 0.002$) and 32 ($P = 0.002$) mg/kg dose groups. DMT produced reductions in arousal at the 17 ($P = 0.01$) and 32 ($P = 0.005$) mg/kg levels. The interaction group showed reductions in arousal at all four dose levels tested ($P < 0.001$). Mobility was also reduced in all three groups. Harmine alone produced decreases at the 17 and 32 mg/kg ($P < 0.05$) dose levels, while DMT produced decreases only at the highest dose tested ($P < 0.05$). The interaction group showed decreased mobility at the two highest doses tested ($P < 0.02$).

Table 1
Effects of harmine, DMT and *B. caapi* extract in various measures of the FOB

Treatment	Posture	Ease of removal	Reactivity to being handled	Clonic movements	Tonic movements	Gait/gait score	Mobility	Arousal	Reactivity to touch	Body temp.	Hindlimb strength
Harmine	*	↑**	↑**	↑**	↑*	↑**/↑**	↓**	↓**	—	↓**	↓*
DMT	—	↑*	—	—	—	—	↓*	↓**	—	↓**	—
Harmine/DMT	**	↑**	—	↑**	↑**	↑*/↑**	↓**	↓**	↑**	↓**	–
B. caapi	**	↑**	—	↑**	—	↑**/↑**	↓**	↓**	—	↓**	—
B. caapi/DMT	**	↑**	—	↑**	↑*	↑**/↑**	↓**	↓**	—	↓**	—

* Effects at the highest dose tested. ** Effects seen at multiple doses. Arrows refer to direction of effect, if applicable. Increases in gait/gait score reflect deficits in these two categories ($n = 10$/dose)

DMT had no effects on gait in the FOB. Harmine produced significant hindlimb splay and overcompensated movements at the 17 and 32 mg/kg dose levels ($P < 0.001$). The harmine/DMT combination produced the same effect on gait but only at the highest dose tested ($P < 0.01$). Both harmine and the harmine/DMT combination produced increases in gait score. This effect was seen in both groups at the 17 and 32 mg/kg levels ($P < 0.05$). In the open field the combination group showed increased reactivity to touch ($P < 0.05$). This effect was seen in the 17 and 32 mg/kg groups. Treatment with harmine significantly reduced hindlimb grip strength ($P = 0.01$). This effect was limited to the 32 mg/kg ($P = 0.001$) group. All three treatments significantly reduced body temperature at all doses tested ($P < 0.01$).

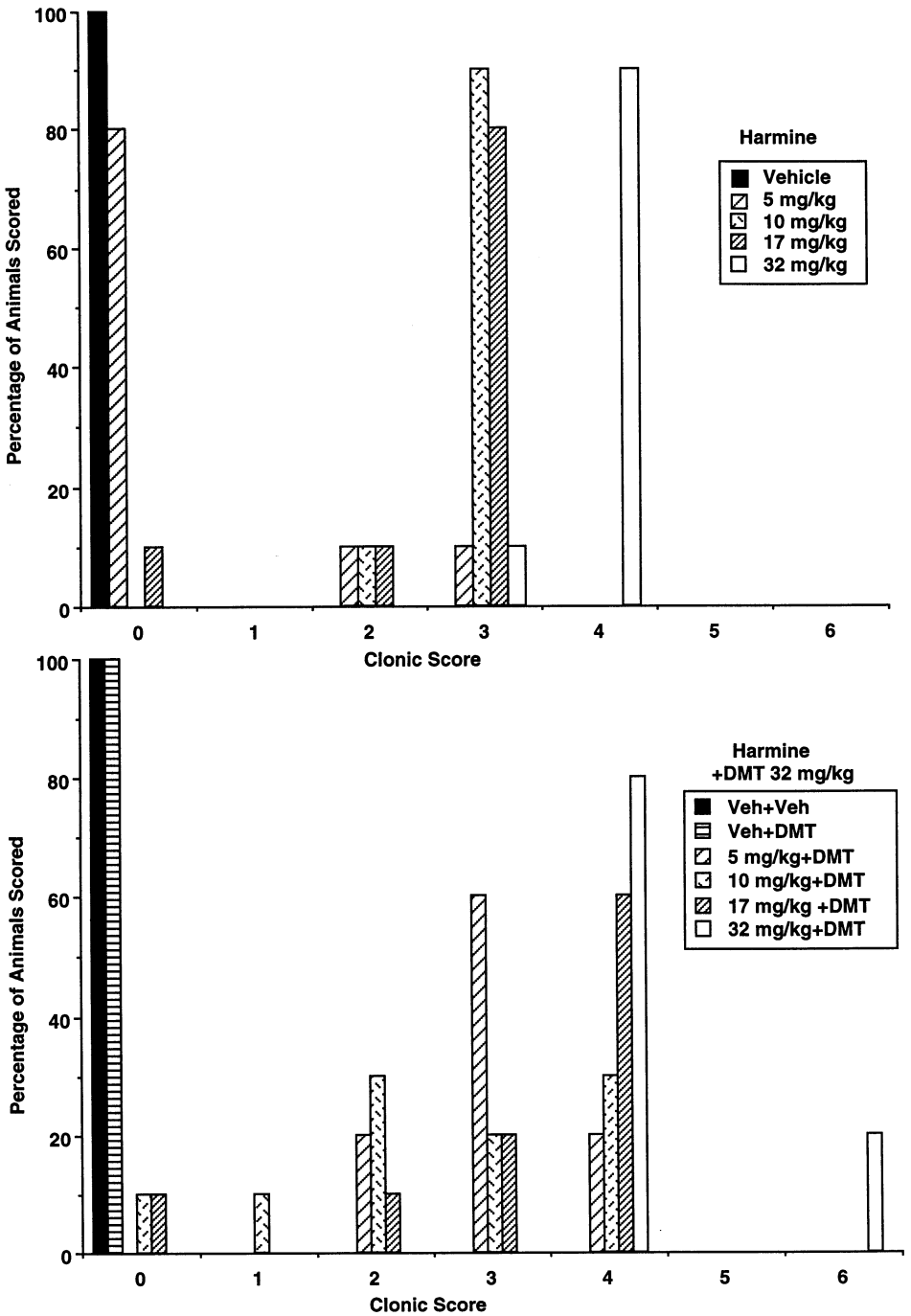

Fig. 1. Effects of harmine and harmine/DMT combinations on clonic motor movements. Scores refer to: 0, no clonic movements present; 1, repetitive movements of mouth and jaws; 2, quivers of limbs, ears, head or skin; 3, mild tremors; 4, severe tremors or whole-body tremors; 5, myoclonic jerks, twitching of a muscle or muscle groups; and 6, clonic convulsions ($n = 10$).

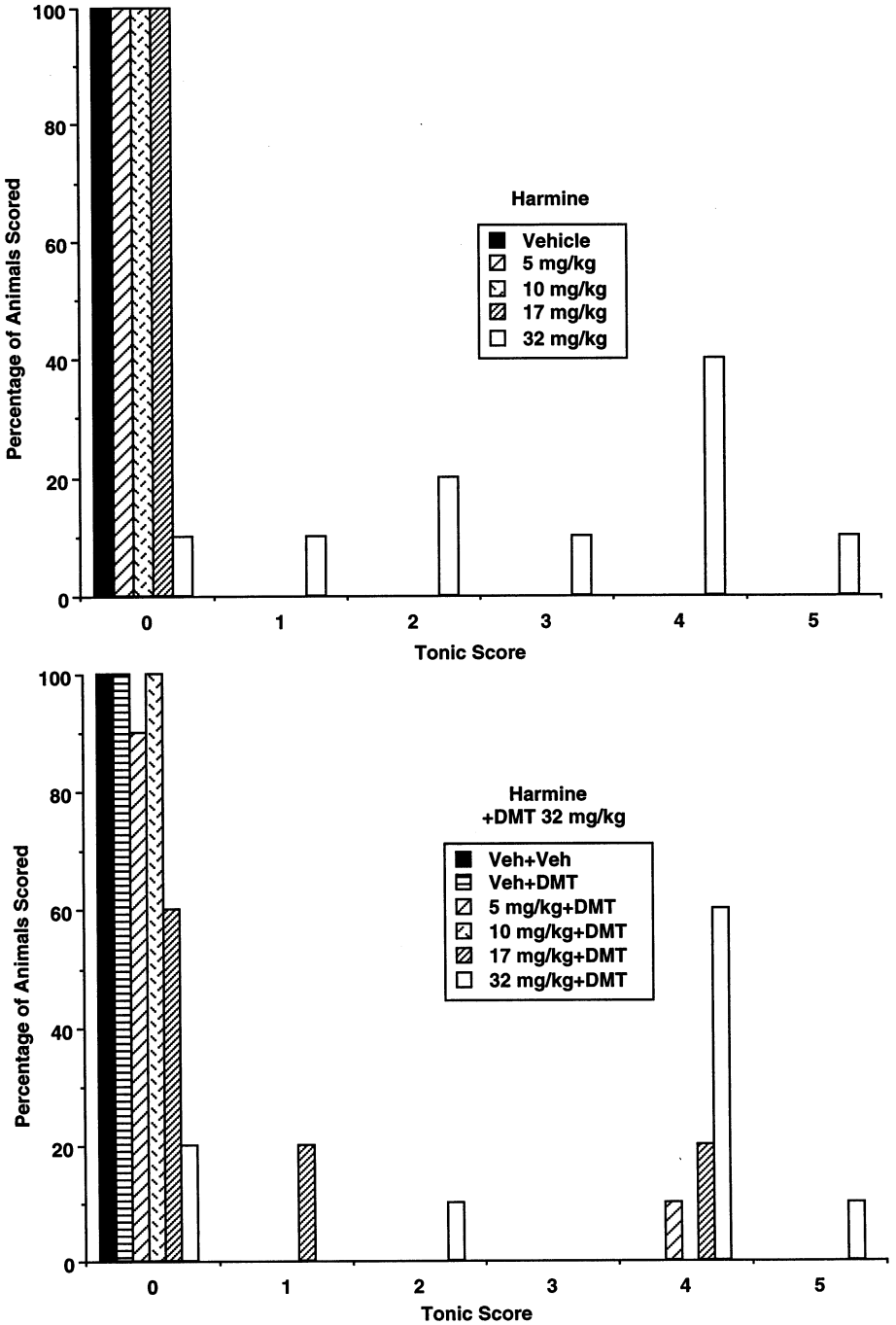

Fig. 2. Effects of harmine and harmine/DMT combinations on tonic motor movements. Scores refer to: 0, no tonic movements present; 1, tonic convulsions: persistent contraction of voluntary muscles; 2, opisthotonus: head and body rigidly arched backwards; 3, emprosthotonus: head and body rigidly extended forward; 4, jumping with all feet leaving surface; and 5, severe clonic and/or tonic convulsions resulting in dyspnea, postictal depression or death ($n = 10$).

3.2. B. caapi and B. caapi/DMT interaction

3.2.1. Home cage measures

In the home cage the extract alone and in combination with DMT significantly flattened posture ($P < 0.0001$; Table 1). This effect was observed in both treatments at the 560 and 1700 mg/kg doses of the extract. Both treatments increased ease of removal ($P < 0.01$). Again, this effect was seen with the two highest doses of the extract tested alone and in combination with DMT (Table 1). Treatment with the extract alone induced clonic motor movements in the 560 ($P < 0.05$) and 1700 ($P < 0.0001$) mg/kg groups (Fig. 3, top panel). Clonic motor movements were induced at

all doses tested when administered in combination with DMT ($P < 0.005$; Fig. 3, bottom panel). This leftward shift in the clonic dose response curve seen with co-administration of DMT was significant at the 100, 300 and 560 mg/kg dose levels ($P < 0.0006$). No tonic motor effects were observed when the extract was administered alone. However, in combination with DMT the extract produced significant tonic motor movements in the 1700 mg/kg group ($P = 0.0018$; Fig. 4).

3.2.2. Open field measures

In the open field the extract given alone or combined with DMT significantly decreased arousal ($P < 0.01$) and mobility. Decreased arousal and mobility were observed in the 560 and 1700 mg/kg dose groups of the extract alone. The effects on gait were characterized by hindlimb splay and exaggerated movements at the same dose levels. Decreases in mobility and arousal were seen in the 300, 560 and 1700 mg/kg groups when the extract

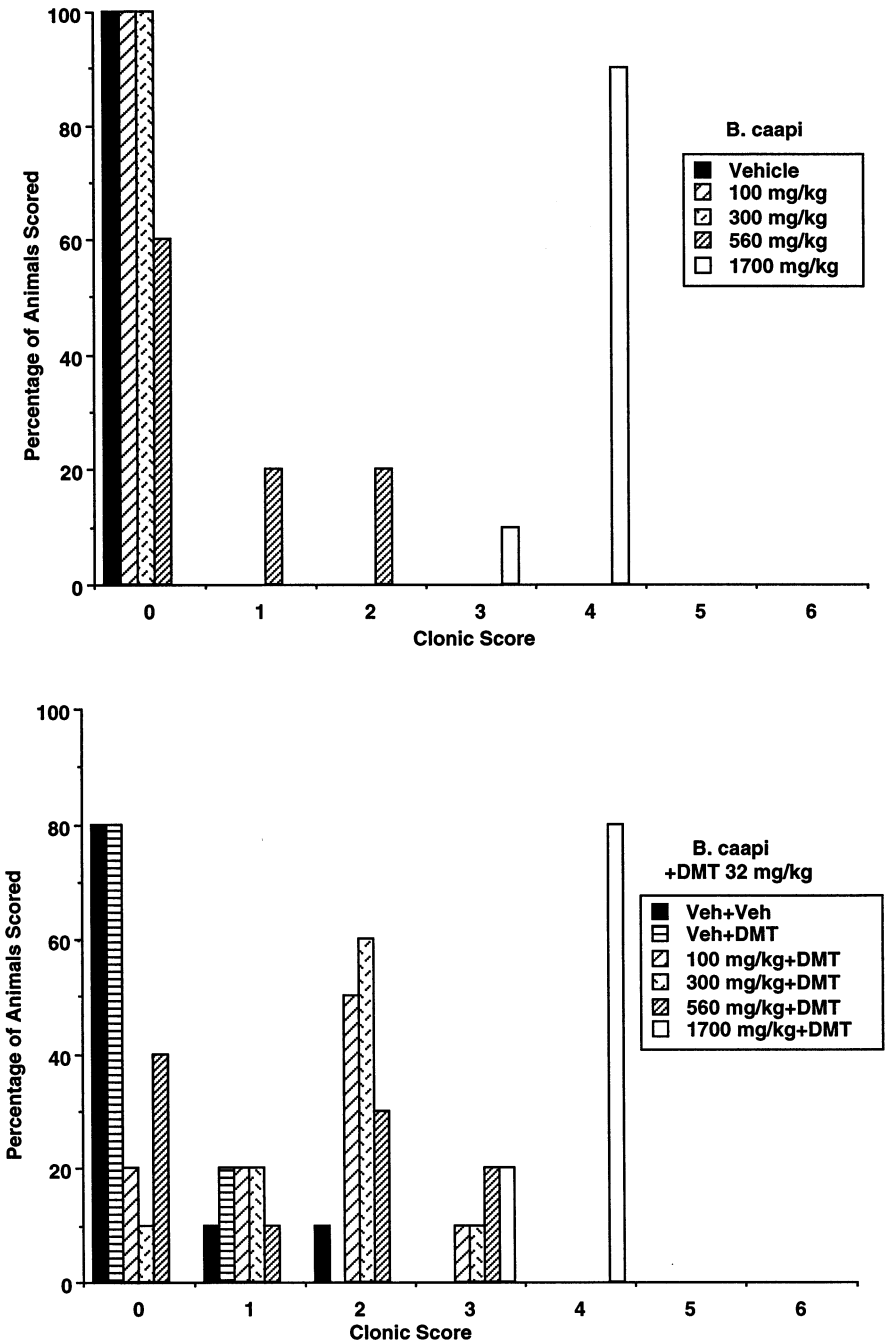

Fig. 3. Effects of *B. caapi* extract and *B. caapi*/DMT combinations on clonic motor movements. Scores refer to: 0, no clonic movements present; 1, repetitive movements of mouth and jaws; 2, quivers of limbs, ears, head or skin; 3, mild tremors; 4, severe tremors or whole-body tremors; 5, myoclonic jerks, twitching of a muscle or muscle groups; and 6, clonic convulsions ($n = 10$).

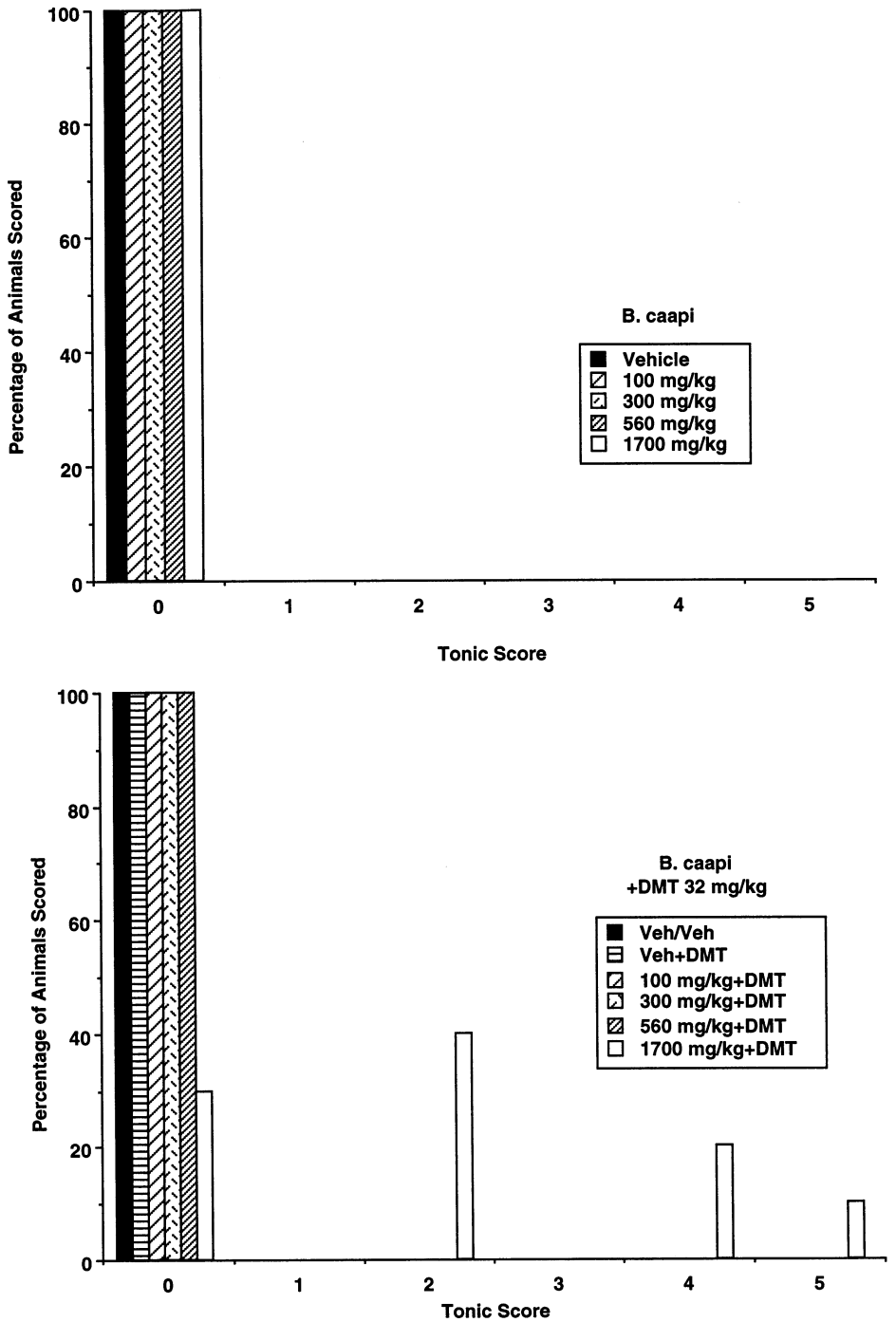

Fig. 4. Effects of *B. caapi* extract and *B. caapi*/DMT combinations on tonic motor movements. Scores refer to: 0, no tonic movements present; 1, tonic convulsions: persistent contraction of voluntary muscles; 2, opisthotonus: head and body rigidly arched backwards; 3, emprosthotonus: head and body rigidly extended forward; 4, jumping with all feet leaving surface; and 5, severe clonic and/or tonic convulsions resulting in dyspnea, postictal depression or death ($n = 10$).

was administered in combination with DMT. Increases in gait score and gait following treatment with the combination were only present at the 560 and 1700 mg/kg dose levels. Both treatments significantly reduced body temperature ($P < 0.01$) at all dose levels examined.

3.3. Startle amplitude and prepulse inhibition

3.3.1. Harmine, DMT, and harmine/DMT combination

Startle data are presented in Table 2 as absolute amplitude ('startle stimulus alone'), amplitude in the presence of the prepulse ('+ prepulse') and prepulse

inhibition, which is expressed as the percentage decrease in amplitude in the presence of the prepulse as compared to the startle stimulus alone. Significant prepulse inhibition was observed for all experiments in the control group. Treatment with harmine significantly decreased absolute startle amplitude at doses of 10 mg/kg and higher, and resulted in a trend toward increased percent prepulse inhibition that fell just short of significance ($P = 0.07$; Table 2). Administration of DMT had no effect on amplitude but resulted in a significant increase in percent prepulse inhibition at the 17 and 32 mg/kg dose levels ($P < 0.05$). The combination of harmine and DMT also had no effect on startle amplitude but produced a significant decrease in percent prepulse inhibition that was attributed to the effects of DMT ($P < 0.03$) and to an abnormally large amount of prepulse inhibition in the control group.

Table 2
Effects of drugs and banisteriopsis extract (BAN) on acoustic startle amplitude (arbitrary startle units) and PPI ($n = 9$–10)

Treatment (mg/kg)	Startle stimulus alone	+Prepulse	%PPI
Vehicle	67.5	30.7	56.0
Harmine 5	56.6	18.9	73.4
Harmine 10	30.7[a]	6.4[a]	82.0
Harmine 17	32.4[a]	9.5[a]	77.3
Harmine 32	16.7[a]	2.9[a]	73.2
Vehicle	84.2	43.8	48.8
DMT 5	71.6	27.2	65.6
DMT 10	65.3	29.0	55.7
DMT 17	71.6	22.1	71.1[b]
DMT 32	68.6	23.8	67.7[b]
Vehicle/vehicle	47.0	12.2	83.8
Vehicle/DMT 32	72.1	29.3	58.6
Harmine 5/DMT 32	51.1	9.1	80.0
Harmine 10/DMT 32	53.1	19.4	64.0
Harmine 17/DMT 32	35.2	16.4	49.5
Harmine 32/DMT 32	36.9	25.3	30.1[b]
Vehicle	64.4	25.5	66.7
BAN 100	69.3	25.5	71.2
BAN 300	56.7	10.5	83.9
BAN 560	52.9	9.2	84.5
BAN 1700	20.4[a]	5.9	52.0
Vehicle/vehicle	38.3	16.9	69.3
Vehicle/DMT 32	61.0	10.1	86.8
BAN 100/DMT 32	79.6	17.7	79.8
BAN 300/DMT 32	70.6	8.9	89.0
BAN 560/DMT 32	70.3	21.2	79.2
BAN 1700/DMT32	99.3	36.1	66.6

[a] Significant decrease in startle amplitude versus control ($P < 0.05$), Dunnett's t-test following significant main effect.[b] Significant change in PPI versus control ($P < 0.05$), Dunnett's t-test following significant main effect.

3.3.2. B. caapi and B. caapi/DMT combination

On the absolute startle measure, the extract produced a significant decrease at 1700 mg/kg. In contrast, administration of the *B. caapi*/DMT combination produced a dose-dependent increase in amplitude that fell narrowly short of significance ($P = 0.054$). Neither treatment had significant effects on percent prepulse inhibition, although a strong trend toward increased inhibition was noted in the combination experiment ($P = 0.08$).

3.4. Other measures

No significant effects were observed in any treatment groups for the following measures: palpebral closure, lacrimation, piloerection, respiration, stereotypy, defecation/urination, tail pinch response, approach response, and forelimb grip strength.

4. Discussion

The present investigation represents a preliminary evaluation of chemical constituents in ayahuasca, a plant decoction used extensively in portions of South America for both medicinal and spiritual purposes. Human studies with ayahuasca are suggestive that the plant mixture produces phenomenological effects similar to those of known hallucinogens. Specifically, Grob et al. (1996) reported that ayahuasca administration resulted in scores on the Hallucinogen Rating Scale (Strassman et al., 1994) consistent with those produced by modest doses of DMT, a known constituent of the plant mixture. Subjective reports on the effects of ayahuasca by members of a syncretist religious group centered around use of the decoction suggest insightful, dream-like experiences containing metaphorical images which guide users toward adaptive transformations in attitude and behavior (Andritzky, 1989; Grob et al., 1996). Accordingly, these users claim remissions of prior psychiatric disorders, including anxiety, depression, and dependence on alcohol and drugs (Grob et al., 1996).

While the presence of DMT may reasonably account for a major component of the unique subjective effects of ayahuasca, preparation of the banisteriopsis vine yields a considerable quantity of pharmacologically active β-carbolines, principally harmine and harmaline. Orally ingested β-carbolines function as MAO inhibitors which may increase the bioavailability of DMT, which is otherwise poorly absorbed by this route (Buckholtz and Boggan, 1977; Ott, 1994). Because β-

carbolines also produce actions in the CNS, these compounds may directly contribute to the effects of ayahuasca on behavior. Based in part on these findings, the present experiments examined constituents of ayahuasca in a neurobehavioral battery of tests which might yield some insight into the putatively synergistic effect of the plant components.

Harmine produced a variety of observable behavioral changes in the FOB. As expected, the effects of harmine and banisteriopsis extract were qualitatively similar, most notably in the clonic-tonic, gait, mobility, arousal and acoustic startle measures. Moreover, both treatments produced marked tremor at the higher doses, a finding consistent with that reported by Kawanishi et al. (1994) with harmine. The presence of clonic and tonic movements heavily influenced the other measures in the FOB, especially posture, reactivity, mobility and gait. Taken together, these behavioral alterations are consistent with some aspects of the serotonin syndrome (Sternbach, 1991). The robust tremor induced by harmine and banisteriopsis caused the majority of animals to remain relatively inactive in the open field. Both treatments also produced significant hypothermia, as Ho (1977) reported for harmine.

The behavioral effects of DMT seen in this investigation were generally consistent with previous reports (Wilkinson and Dourish, 1991). DMT did not affect the clonic and tonic measures of the FOB. The lack of tremor following DMT treatment is in contrast to the observations of Martin et al. (1985) who reported the induction of tremor following treatment with both 5-HT and the DMT analog 5-MeODMT. The mobility and arousal alterations associated with DMT were similar to those seen with harmine. In the open field animals were relatively inactive with little exploratory behavior observed.

The highly salient clonic effects of harmine were significantly potentiated by co-administration with DMT, which by itself had little effect on this measure. A similar potentiation was observed for the tonic measure. As with pure harmine, effects of the banisteriopsis extract on clonic movements were intensified by DMT. Unlike harmine, banisteriopsis had no effect on tonic movements even after doses as high as 1700 mg/kg. When combined with DMT, however, harmine-like tonic signs did emerge, further suggesting a synergistic effect of the two compounds. Because the interactions observed between DMT and the other treatments were all observed under conditions where the DMT was administered parenterally, it seems reasonable to suggest that the unique effects of ayahuasca may be due to factors in addition to an increase in bioavailability of orally ingested DMT. Moreover, the hallucinogen-like effects of ayahuasca reportedly can last for several hours or more (Grob et al., 1996), as compared with the ultra-short acting time course of experimentally administered DMT when injected intravenously (Strassman et al., 1996). The longer time-course of the decoction may be determined by slower absorption of DMT when given orally, its delayed metabolism due to depressed systemic MAO, and by the intrinsic psychoactive effects of its β-carboline constituents.

Harmine and the banisteriopsis extract produced similar effects in the acoustic startle experiments, decreasing amplitude at the higher doses while leaving PPI unaffected. Administration of DMT alone did not markedly alter startle amplitude, but did produce an unexpected increase in PPI at the 17 and 32 mg/kg doses. Previous studies in rats report disruption (decrease) of PPI with hallucinogens, findings which may be related to their schizophrenomimetic properties (Sipes and Geyer, 1997). The PPI increase with DMT was not observed in the harmine/DMT interaction experiment, possibly because of very high baseline PPI in that study, but did appear once more in the banisteriopsis/DMT experiment. These latter observations were not statistically significant. There was also some evidence that DMT administration prevented the amplitude decreases induced by harmine and banisteriopsis. In both interaction experiments, there were no significant effects on startle amplitude, and a strong trend toward increased amplitudes in the DMT/banisteriopsis study ($P = 0.054$).

The present investigation thus confirms that administration of extracts of the banisteriopsis vine produces effects similar to that of its known principal psychoactive constituent, harmine. Further, co-administration of either the extract or harmine with DMT, the active constituent of the other plant species in the ayahuasca mixture, produced interactive effects on some measures. Differences in the interactive effects with DMT of harmine and the extract, though subtle, could reflect the actions of other alkaloids known to be present in the banisteriopsis vine. These data represent a preliminary step in understanding the cellular basis for ayahuasca intoxication. Confirmation of its putatively therapeutic effects on psychopathology and addiction, however, as well as potentially dangerous interactions which could arise from co-administration of ayahuasca with psychiatric drugs (Callaway et al., 1996), await controlled studies.

Acknowledgements

This research was supported by Pfizer Central Research, Groton, CT. The authors thank Mark Guadliana and Jack Oscarson of the Pfizer Natural Products Group for chemical analysis of Banisteriopsis samples.

References

Andritzky, W., 1989. Sociopsychotherapeutic functions of ayahuasca healing in amazonia. J. Psychoact. Drugs 21, 77–89.

Buckholtz, N.S., Boggan, W.O., 1977. Monoamine oxidase inhibition in brain and liver produced by β-carbolines: structure activity relationships and substrate specificity. Biochem. Pharmacol. 26, 1991–1996.

Callaway, J.C., Airaksinen, M.M., McKenna, D.J., Brito, G.S., Grob, C.S., 1994. Platelet serotonin uptake sites increased in drinkers of ayahuasca. Psychopharmacology 116, 385–387.

Callaway, J.C., Raymon, L.P., Hearn, W.L., McKenna, D.J., Grob, C.S., Brito, G.S., Mash, D.C., 1996. Quantitation of *N*,*N*-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with ayahuasca. J. Anal. Toxicol. 20, 492–497.

Costa, E., Guidotti, A., Mao, C.C., Suria, A., 1975. New concepts of the mechanism of action of benzodiazepines. Life Sci. 17, 167–186.

Dobkin de Rios, M., 1972. Visionary Vine: Hallucinogenic Healing in the Peruvian Amazon. Chandler, San Francisco, CA, USA.

Gates, B., 1982. A monograph of Banisteriopsis and Diplopterys, Malpighaceae. Flora Neotropica 30, New York Botanical Garden, New York.

Glennon, R.A., Young, R., Rosecrans, J.A., 1983. Antagonism of the effects of the hallucinogen DOM and the purported 5-HT agonist quipazine by 5-HT2 antagonists. Eur. J. Pharmacol. 91, 189–196.

Grob, C.S., McKenna, D.J., Callaway, J.C., Brito, G.S., Neves, E.S., Oberlander, G., Saide, O.L., Labigalini, E., Talca, C., Miranda, C.T., Strassman, R.J., Boone, K.B., 1996. Human psychopharmacology of hoasca, a plant hallucinogen used in ritual context in Brazil. J. Nerv. Ment. Dis. 184, 86–94.

Harner, M.J., 1973. Hallucinogens and Shamanism. Oxford University Press, London.

Ho, B.T., 1977. Pharmacological and biochemical studies with β-carboline analogs. In: Essman, W.B., Vazelli, L. (Eds.), Current Developments in Psychopharmacolgy, vol. 4. Spectrum, New York, pp. 155–177.

Kawanishi, K., Eguchi, N., Hayashi, T., Hashimoto, Y., 1994. Relationship between occurance of tremor/convulsion and level of β-carbolines in the brain after administration of β-carbolines into mice. Pharm. Biochem. Behav. 47, 689–699.

Kelly, D.M., Nayler, R.J., 1974. Mechanisms of tremor induced by harmine. Eur. J. Pharmacol. 27, 14–24.

Mabit, M., 1996. Takiwasi: ayahuasca and shamanism in addiction therapy. MAPS Newslett. 6, 24–27.

Martin, P., Frances, H., Simon, P., 1985. Dissociation of head twitches and tremors during the study of interactions with 5-Hydroxytryptophan in mice. J. Pharmacol. Methods 13, 193–200.

McKenna, D.J., 1996. Plant hallucinogens: springboards for psychotherapeutic drug discovery. Behav. Brain Res. 73, 109–116.

McKenna, D.J., Towers, G.H.N., 1984. Biochemistry and pharmacology of tryptamines and β-carbolines: a minireview. J. Psychoact. Drugs 10, 195–223.

McKenna, D.J., Towers, G.H.N., Abbot, F., 1984. Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and β-carboline constituents of ayahuasca. J. Ethnopharmacol. 11, 189–206.

Moser, V.C., McCormick, J.P., Creason, J.P., MacPhail, R.C., 1988. Comparison of chlordimeform and carbaryl using a functional observational battery. Fundam. Appl. Toxic. 11, 189–206.

Ott, J., 1994. Ayahuasca Analogues. Natural Products, Kennewick, WA, USA.

Rivier, L., Lindgren, L., 1972. Ayahuasca, the South American hallucinogenic drink: an ethnobotanical and chemical investigation. Econ. Botany 26, 101–129.

Sipes, T.E., Geyer, M.A., 1997. DOI disrupts prepulse inhibition of startle in rats via 5-HT2A receptors in the ventral pallidum. Brain Res. 761, 97–104.

Spruce, R., 1908. Notes of a Botanist on the Amazon and Andes. MacMillan, London.

Sternbach, H., 1991. The serotonin syndrome. Am. J. Psychiatr. 148, 705–713.

Strassman, R.J., Qualls, C.R., 1994. Dose-response study of *N*,*N*-dimethyltryptamine in humans. I: neuroendocrine, autonomic, and cardiovascular effects. Arch. Gen. Psychiat. 51, 85–97.

Strassman, R.J., Qualls, C.R., Uhlenhuth, E.H., Kellner, R., 1994. Dose-response study of *N*,*N*-dimethyltryptamine in humans. II. Subjective effects and preliminary results of a new rating scale. Arch. Gen. Pharmacol. 51, 98–108.

Strassman, R.J., Qualls, C.R., Berg, L.M., 1996. Differential tolerance to biological and subjective effects of four closely spaced doses of *N*,*N*-dimethyltryptamine in humans. Biol. Psychiat. 39, 784–795.

Szara, S.I., 1956. Dimethyltryptamin: its metabolism in man; the relation of its psychotic effect to serotonin metabolism. Experientia 15, 441–442.

Szara, S.I., 1957. The comparison of the psychotic effect of tryptamine derivatives with the effects of mescaline and LSD-25 in self-experiments. In: Garattini, S., Ghetti, V. (Eds.), Psychotropic Drugs. Elsevier, Amsterdam, pp. 460–467.

Tegeris, J.S., Balster, R.L., 1994. A comparison to the acute behavioral effects of alkylbenzenes using a functional observational battery in mice. Fundam. Appl. Toxicol. 22, 240–250.

Wilkinson, L.O., Dourish, C.T., 1991. Serotonin and animal behavior. In: Peroutka, J. (Ed.), Serotonin Receptor Subtypes: Basic and Clinical Aspects. Wiley-Liss, pp. 147–210.

Yamazaki, M., Tanaka, C., Takaori, S., 1979. Significance of central noradrenergic system on harmaline induced tremor. Pharm. Biochem. Behav. 10, 421–429.

Young, R., Rosecrans, J.A., Glennon, R.A., 1982. Comparative discriminative stimulus effects of 5-methoxy-*N*,*N*-dimethyltryptamine and LSD. Life Sci. 30, 2057–2062.