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DOCTORAL THESIS

Ayahuasca: physiological and subjective effects, comparison with *d*-amphetamine, and repeated dose assessment

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Ayahuasca: physiological and subjective effects, comparison with *d*-amphetamine, and repeated dose assessment

Memòria presentada per

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Certifica:

Que la memòria presentada per Rafael Guimarães dos Santos amb el títol **"Ayahuasca: physiological and subjective effects, comparison with *d*-amphetamine, and repeated dose assessment"** ha estat realitzada sota la seva direcció. La memòria reuneix les condicions per ser presentada per optar al grau de Doctor.

Per a que consti als efectes oportuns signo el present certificat a Barcelona, 1 de Desembre de 2011.

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To Iara, Bete, and Adauto. With love.

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CONTENTS

INTRODUCTION

1. A brief overview of psychedelics

The use of psychedelic substances to produce modifications in perception, emotion, mood, cognition, and to achieve different states of consciousness has been documented in different human cultures all over the world (Harner, 1976a; Grinspoon and Bakalar, 1981; Schultes and Hofmann, 1992; Furst, 1994; Ripinsky-Naxon, 1995; Abraham et al., 1996; Spinella, 2001; Furst, 2004; Baker, 2005; Labate and Goulart, 2005). The motivations to use such substances have been therapeutic, religious, ritual, magical, aphrodisiac and for pleasure, for political decisions as well as for war purposes (Schultes and Hofmann, 1992; Furst, 1994).

The usage of these substances has been widely spread among human groups in Africa, Asia, Europe and the Americas, and there are archeological findings suggesting the use of such substances from time immemorial (Schultes, 1998; Furst, 2004). Samorini (2002) presents evidence of intentional use of psychedelic compounds by animals ranging from insects to primates, and proposes that some human groups learned how to use psychedelics like the *Amanita* mushroom and the roots of the iboga shrub observing animals that became "intoxicated" with such substances.

There is a wide variety of species that produce psychedelic compounds. They can be fungi (ergot, several species of *Psilocybe* mushrooms and correlated species), cacti (peyote, San Pedro), vines (ayahuasca, morning glories), trees (nutmeg, several species of the *Virola* genus) and shrubs (iboga, several species of the Solanaceae family). Psychedelic drugs can also be semi-synthetic, like LSD, and synthetic, like 4-Bromo-2,5-dimethoxyphenethylamine (2C-B).

The major psychedelic drugs can be divided into three groups: monoamine, cholinergic, and amino acid derivatives. The monoamine group can be subdivided into two categories: indole derivatives or indolealkylamines, and phenylalkylamines. The phenylalkylamine group can be further divided into phenylethylamines or phenethylamines and phenylisopropylamines or amphetamines (Grinspoon and Bakalar, 1981; Glennon, 1994; Abraham et al., 1996; Spinella, 2001).

Among the monoamines we find lysergic acid amides (also called ergolines or lysergamides; LSD, LSA or ergine), *β*-carbolines (harmala alkaloids), ibogaine, and tryptamines (psilocin, psilocybin, dimethyltryptamine [DMT]), all having the indole moiety in their structure. Mescaline is the most important representative of the phenethylamines, and 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDE), and many others, are among the phenylisopropylamines. The cholinergic psychedelics (also called *delirants* or *true hallucinogens*) are represented by the tropane alkaloids hyoscyamine, scopolamine or hyoscine, and atropine, and the amino acid derivatives include ibotenic acid, muscimol, muscazone and muscarine (Grinspoon and Bakalar, 1981; Glennon, 1994; Abraham et al., 1996; Spinella, 2001). There are also the *psychedelic* or *dissociative* anesthetics, which include the synthetic arylcyclohexylamines like phencyclidine (PCP) and ketamine (Vollenweider, 2001).

The ritual use of a large number of natural psychedelics is still present in many human groups. The mescaline-containing cacti peyote and San Pedro are used in Mexico and Peru, respectively, the iboga shrub in Gabon, the *jurema* tree in northeast Brazil, and ayahuasca in the western Amazon (Baker, 2005; Labate and Goulart, 2005). Scientific interest for these drugs initiated in the second half of the 19th century, but systematic psychological, psychiatric and pharmacological investigations began around 1940-1950, with the discovery of LSD by Albert Hofmann, and ended prematurely in the late 1960s, because of social hysteria associated with the widespread use of these substances among adepts of the hippie culture. Despite animal studies and some isolated human investigations, the next twenty-five years were marked by a silence in the scientific study of psychedelics (Grob, 1998; Grof, 2001; Bouso-Saiz and Gómez-Jarabo, 2007).

In the last decades, despite political and cultural resistance, some research has been done with these drugs. Psilocybin, LSD, ayahuasca, DMT, ketamine, and ibogaine are some of the compounds that are currently under pharmacological and, in some cases, even clinical-therapeutic investigations (see for a review Bouso-Saiz and Gómez-Jarabo, 2007, and also the website of the *Multidisciplinary Association for Psychedelic Studies* – MAPS, www.maps.org).

The past and current human investigations with *β*-carbolines, *N*,*N*-dimethyltryptamine (DMT) and ayahuasca are of special interest for the objectives of this thesis. *β*-Carboline and DMT research is motivated in part by the fact that they are the main alkaloids present in ayahuasca, a psychoactive preparation whose use has spread in the last two decades from the Amazon jungle to the urban centers of South and North America, Europe, Asia, and Africa (Balzer, 2004; Halpern, 2004; Labate, 2004; Labate and Araújo, 2004; Labate et al., 2009; Trichter et al., 2009). Ayahuasca has also motivated Europeans, North Americans and other foreigners to travel to the Amazon in what has been dubbed by some as "drug tourism" (Kristensen, 1998; Arrévalo, 2005; Winkelman, 2005; Razam, 2006).

Also, another interesting phenomenon is the use of alternative plant sources of *β*-carbolines and DMT to prepare ayahuasca analogues. Ott (1994, 1999) created the term *anahuasca* to designate these ayahuasca analogues (see also Callaway, 1995a). Ott (1994) provided the scientific names for over 120 plants capable of producing ayahuasca analogues in more than 4000 different combinations. The term *pharmahuasca*, on the other hand, refers to the combination of the alkaloids obtained by isolation or synthesis or even the combination of DMT plus a synthetic monoamine oxidase (MAO) inhibitor.

The pharmacology of the main active principles present in ayahuasca, namely DMT and the *β*-carbolines, is reviewed below before addressing the pharmacology of ayahuasca as a whole.

2. *N,N*-Dimethyltryptamine (DMT)

2.1. Sources and uses of DMT

DMT (see Figure 1) was synthesized in 1931 by the Canadian chemist Richard Manske, before it had ever been discovered in any mind-altering plant. It was first isolated by the Brazilian chemist O. Gonçalves de Lima in 1946 (Ott, 1994, 1999; Strassman, 2001, 2008a). DMT is known to occur in more than fifty plant species (Ott, 1994), and it has been proposed that it is also present endogenously in animals and humans based on two types of evidence: (1) the discovery in several species of mammals of an enzyme (INMT) that is capable of producing DMT; and (2) analytical studies that have directly detected DMT in animal tissues, blood and urine (Axelrod, 1961; Saavedra and Axelrod, 1972, 1973; Gillin et al., 1976; Thompson and Weinshilboum, 1998; Forsstrom et al., 2001; Karkkainen et al., 2005; see also Gillin et al., 1978; Barker et al., 1981; Callaway, 1995b; Wallach, 2009; Halberstadt, 2011).

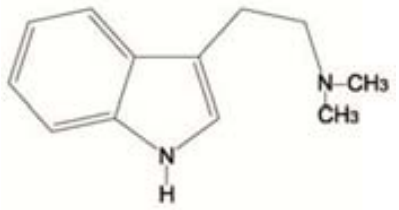

Figure 1: Chemical structure of *N,N*-dimethyltryptamine.

DMT is found in some of the main Latin American psychedelic preparations: snuffs, *jurema*, and ayahuasca (Holmstedt and Lindgren, 1967; McKenna et al., 1984a, 1984b; Schultes and Hofmann, 1992; Furst, 1994; Ott, 1994, 1999, 2001a, 2001b, 2004a).

Snuff can be prepared from the seeds of *Anadenanthera peregrina* and *A. colubrina* (Leguminosae), from the barks of several *Virola* species (Myristicaceae), and also from the leaves of *Justicia* species (Acanthaceae) (McKenna et al., 1984b; McKenna and Towers,

1985; Schultes, 1998; Ott, 2001a, 2001b, 2004a; Wright, 2005; see also Holmstedt and Lindgren, 1967; Schultes, 1967; Seitz, 1967). *Jurema* is made from the root bark of several species of the genera *Mimosa*, *Acacia* and *Pithecelobium* (Leguminosae) (Ott, 1994; Da Mota, 1996; Ott, 1999; Albuquerque, 2002; Camargo, 2002; Da Mota and Albuquerque, 2002; Da Mota and Barros, 2002; Grunewald, 2002; Pinto, 2002; Reesink, 2002; Ott, 2004a, 2004b; Da Mota, 2005), and *ayahuasca* is made from several species of the *Banisteriopsis* vine (Malpighiaceae) alone or in combination with plant admixtures that are added to the brew and may contain DMT, such as *Psychotria viridis* (Rubiaceae) and *Diplopterys cabrerana* (Malpighiaceae) (McKenna et al., 1984a; Schultes and Hofmann, 1992; Ott, 1994; McKenna et al., 1995; Callaway, 2003; Ott, 2004a; Callaway, 2005a, 2005b; Callaway et al., 2005; Luna, 2005; Gambelunghe et al., 2008; Pires et al., 2009; Moura et al., 2010; see for a review Ott, 1994; Riba, 2003).

These psychedelic preparations can be used as day-to-day stimulants, like the *yopo* snuff (Schultes, 1998), or in complex magico-religious rituals, like the ayahuasca ceremonies in Brazil (MacRae, 1992, 1999; Labate, 2004; Labate and Araújo, 2004; Labate and MacRae, 2006; Labate et al., 2009). In these contexts these drugs are often regarded as providers of access to the supernatural realm, where one can see the future, ask for advice from spirits and ancestors, and find out the cause of a disease and its remedy (Dobkin de Rios, 1972; Harner, 1976a; Furst, 1994; Labate and Goulart, 2005).

2.2. Pharmacology of DMT

2.2.1. Neurochemistry

N,N-Dimethyltryptamine (or simply DMT) is a tryptamine, like psilocin and psilocybin, and is among the monoamine psychedelics of the indole group, together with LSD, the *β*-carbolines and ibogaine (Grinspoon and Bakalar, 1981; Spinella, 2001), although it is 1000 times less potent than LSD (Smith et al., 1998).

DMT and the classical psychedelics in general display agonist activity at several serotonin receptors. Many studies report the 5-HT_{2A} receptors as the main target for these drugs (McKenna and Peroutka, 1989; McKenna et al., 1990; Strassman, 1992; Abraham et

al., 1996; Strassman, 1996; Grella et al., 1998; Vollenweider et al., 1998; Aghajanian and Marek, 1999; Vollenweider, 2001; Rabin et al., 2002; González-Maeso et al., 2003; Riba, 2003; Nichols, 2004; Carter et al., 2005; Fantegrossi et al., 2008; González-Maeso et al., 2007, 2008; Halberstadt et al., 2008; Geyer et al., 2009; González-Maeso and Sealfon, 2009a, 2009b; Keiser et al., 2009; Ray, 2010; Vollenweider and Kometer, 2010; Halberstadt and Geyer, 2011; Moreno et al., 2011). In fact, there is a significant correlation between psychedelic potency and 5-HT_2 receptor affinity for these compounds (Grella et al., 1998; Glennon et al., 2000).

Additionally, some studies have reported MAO inhibiting properties for DMT (Waldmeier and Maître, 1977; McKenna et al., 1984b; see for a review Barker et al., 1981; Riba, 2003). McKenna et al. (1984b) reported that DMT has MAO-inhibiting effects comparable in potency to those of THH. There is also evidence that besides serotonergic neurotransmission, DMT affects dopaminergic, noradrenergic, adrenergic and cholinergic transmission (Haubrich and Wang, 1977; Waldmeier and Maître, 1977; Jenner et al., 1978, 1980; Pierce and Peroutka, 1989, cited in Riba, 2003; see also Barker et al., 1981; Ray, 2010) and activates a trace amine-associated receptor (TAAR1) (Bunzow et al., 2001; see for a review Jacob and Presti, 2005; Burchett and Hicks, 2006; Wallach, 2009).

Recently, it was demonstrated that DMT is an agonist at the sigma-1 receptor, where it might act as an endogenous regulator (Fontanilla et al., 2009; Su et al., 2009). Finally, there is recent evidence that DMT and other psychedelic tryptamines are substrates of the serotonin uptake transporter (SERT) and the vesicle monoamine transporter (VMAT2) (Cozzi et al., 2009). However, the contribution of all these activities to the overall effects of pure DMT or of DMT-containing psychedelics like ayahuasca is unknown.

In vivo binding of radiolabeled DMT shows greatest accumulation in the cerebral cortex, caudate, putamen, and amygdala in the rat brain (Yanai et al., 1986). Also in rats, it was found to bind to the cortex, cerebellum, thalamus, basal ganglia and medulla following intraperitoneal (i.p.) injection (Barker et al., 2001). It has been demonstrated that, like with glucose and certain amino acids, the brain actively transports DMT across the blood-brain barrier (Yanai et al., 1986). ^{131}I-labeled DMT, injected into rabbits, was detected in urine

within 24 h after injection and remained in the brain: up to 0.1% of the injected dose was detected 7 days after injection in the olfactory bulb (Vitale et al., 2011).

2.2.2. Subjective effects

Despite being a potent psychoactive chemical, DMT was found to be psychologically inactive orally, intranasally and rectally in doses up to 1000 mg (13 mg/kg), 20 mg (0.28 mg/kg) and 125 mg (1.7 mg/kg), respectively (Ott, 1999; see for a review Riba, 2003). In the 1950s, Hungarian chemist, psychiatrist and psychopharmacologist Stephen Szára started the systematic study of the human pharmacology of DMT (Szára, 1956, 1957, 2007). His investigations (see for a review Szára, 2007) as well as subsequent studies (e.g., Gillin et al., 1976; Strassman and Qualls, 1994; Strassman et al., 1994; Strassman et al., 1996) led to postulate that the subjective effects of DMT were qualitatively similar to those elicited by better-known psychedelics, like mescaline and LSD. Perceptual, emotional, and cognitive modifications were commonly reported (for the subjective and visionary experiences of pure DMT, see Szára, 1957; Strassman, 2001; Rodriguez, 2007; Cott and Rock, 2008; Strassman, 2008b).

DMT is rapidly metabolized and its effects short-lived (Gillin et al., 1976; see also Barker et al., 1981). Although DMT has been found to be inactive orally in doses as high as 1 g, it has been found to be psychoactive after intramuscular (i.m.) administration (0.25-2.00 mg/kg), when inhaled as vaporized free-base (0.2-0.7 mg/kg) and after intravenous (i.v.) administration (0.2-0.4 mg/kg) (Szára, 1956, 1957; Gillin et al., 1976; Strassman and Qualls, 1994; Strassman et al., 1994; Ott, 1999; see for a review Riba, 2003). The i.m. route often produces an experience that initiates around 3-5 minutes and ends after 1 hour (Szára, 1956, 1957; Gillin et al., 1976). With i.v. injections or with smoked DMT, the subjective effects initiate almost instantaneously (around 30 seconds) and end after 20-30 minutes (Strassman and Qualls, 1994; Strassman et al., 1994; see for a review Riba, 2003).

2.2.3. Pharmacokinetics

Szára (1956) identified 3-indoleacetic acid (IAA) in urine as a metabolite of DMT following its i.m. administration. IAA recovery ranged from 8.3 to 24.9% of the

administered dose. This study could not demonstrate unchanged DMT in urine. Gillin et al. (1976) reported that peak concentrations of DMT, which averaged approximately 100 ng/ml, were reached after 10-15 min following an i.m. injection of a 0.7 mg/kg dose, and then fell rapidly to baseline levels. After about 45-120 min, DMT levels were undetectable. By the i.v. route, mean peak value at 2 min after a 0.4 mg/kg dose was approximately 90 ng/ml; plasma levels could be measured up to 30 min after injection and had virtually disappeared at 60 min for all doses (0.05, 0.1, 0.2 and 0.4 mg/kg) (Strassman and Qualls, 1994; see for a review Riba, 2003).

2.2.4. Neuroendocrine effects

DMT increases serum levels of prolactin, growth hormone (GH) and cortisol in humans, although no effect of DMT on follicle stimulating hormone, thyroid stimulating hormone (TSH) or luteinizing hormone secretion was observed (Meltzer et al., 1982). In subsequent studies, DMT produced dose-dependent increases in the levels of prolactin, cortisol, β-endorphin and corticotropin (adrenocorticotropic hormone, ACTH). GH levels also rose in response to DMT, and melatonin levels were unaffected (Strassman and Qualls, 1994; Strassman et al., 1996).

Regarding the effects of DMT in the above cited neuroendocrine measures, there could be several physiological explanations. Psychedelics similar to DMT produce neuroendocrine changes as well. LSD increases GH levels, but does not alter prolactin (see for a review Passie et al., 2008; Hintzen and Passie, 2010), while psilocybin increases TSH, prolactin, cortisol, and ACTH levels (Hasler et al., 2004). These effects could be mediated by 5-$HT_{1A/2A}$ receptors. Administration of a 5-HT_{1A} agonist induces increases in GH, ACTH, cortisol and prolactin levels (Seletti et al., 1995; Pitchot et al., 2002; see also Nichols and Nichols, 2008). Moreover, Strassman (1996) found reduced prolactin response in a DMT study after a pre-treatment with a 5-HT_{1A} antagonist, which provides evidence supporting a stimulatory role for the 5-HT_{1A} site in human prolactin secretion. Also, serotonin is implicated in the prolactin-secretory response, since $5HT_{1A}$, $5HT_{2A}$, and $5HT_{2C}$ serotonin receptor agonists increase plasma prolactin *in vivo* (Freeman et al., 2000).

2.2.5. Cardiovascular and autonomic effects

DMT produces increases in blood pressure, heart rate, pupillary diameter and rectal temperature (Szára, 1956; Gillin et al., 1976; Strassman and Qualls, 1994; Strassman et al., 1996).

These effects could be mediated by the 5-HT_{2A} receptor, whose activation causes rises in blood pressure and generalized sympathetic activation (Ramage and Villalón, 2008). The effects after DMT are also observed for analogous drugs such as LSD, which produces pupillary dilation, increases in heart rate, blood pressure, and in body temperature, while respiration remains generally unchanged (see for a review Passie et al., 2008; Hintzen and Passie, 2010). Also, psilocybin elicits significant increases of blood pressure, heart rate, pupillary diameter, and body temperature (Gouzoulis-Mayfrank et al., 1999; Hasler et al., 2004; Griffiths et al., 2006; Moreno et al., 2006; Griffiths et al., 2011; Grob et al., 2011; see for a review Passie et al., 2002).

It is unlikely that the sympathomimetic effects of DMT are mediated by the 5-HT_{1A} receptors, since 5-HT_{1A} agonists induce decreases in temperature and do not produce significant elevations in blood pressure or heart rate (Seletti et al., 1995; Pitchot et al., 2002). On the contrary, 5-HT_{1A} agonists have been found to produce significant reductions in blood pressure (Fanciullacci et al., 1995; Koudas et al., 2009), similar to that produced by 5-HT_2 antagonists (Koudas et al., 2009).

The administration of 5-HT_{1A} agonists and 5-HT_2 antagonists induces miosis (Fanciullacci et al., 1995; Koudas et al., 2009). As DMT has been found to produce increases in pupil size, it is reasonable to assume that this effect may be mediated by 5-HT_2 receptors. Regarding body temperature, 5-HT_7 antagonists block 5-HT-induced hypothermia, and in 5-HT_7 receptor knockout mice, 5-HT and 5-HT_7 receptor agonists fail to produce hypothermia (see Nichols and Nichols, 2008). There is evidence that DMT binds to 5-HT_7 receptors (Keiser et al., 2009; Ray, 2010; Halberstadt and Geyer, 2011), which could explain the hyperthermic effects of DMT.

2.2.6. Immunological effects

To our knowledge, there are no published studies evaluating the effects of DMT on immunological parameters. Regarding other psychedelics, there is evidence that LSD can cause elevated or decreased leucocyte counts in animals – decreased eosinophil and lymphocyte counts, and elevated neutrophil counts (see for a review Hintzen and Passie, 2010). *In vitro* studies reported suppressed proliferation of B lymphocytes, inhibited production of the cytokines interleukin (IL)-2, IL-4, and IL-6, as well as inhibited induction of cytotoxic T lymphocytes. *In vitro* studies reported contradictory findings regarding natural killer (NK) cells, depending on dosage levels (in doses not considered clinically relevant), with low doses enhancing both basal and IL-2 augmented NK cells, and higher doses suppressing NK cells (see for a review Hintzen and Passie, 2010). Experiments on mice showed that selective stimulation of 5-HT_{2A} receptors with DOI led to suppression of the immune response and reduction of the spleen and peripheral blood CD8+ T cell counts with the cytotoxic/suppressor function (Davydova et al., 2010).

The potential immunomodulatory effects of DMT have not been assessed in humans. LSD, on the other hand, has been found to modify leucocyte counts in humans – elevation of neutrophils and decrease of eosinophils (see for a review Hintzen and Passie, 2010). Human leucocytes have been found to be temporarily reduced in number between the second and fourth hours after psilocybin administration (see for a review Passie et al., 2002).

2.2.7. Adverse effects

Besides the physiological effects mentioned above, which include potentially dangerous elevations of cardiovascular parameters, acute DMT administration has been associated with unpleasant psychological reactions. Anxiety has been observed in clinical settings (Strassman et al., 1994); and depression and reexperimentation of acute symptoms have been reported days after study participation (Strassman, 1994, 1995).

2.2.8. Tolerance and sensitization

Classical psychedelics produce tolerance in humans (Isbell et al., 1956; Rosenberg et al., 1964; see for a review Wyatt et al., 1976; Nichols, 2004; Fantegrossi et al., 2008). It is also important to observe that besides tolerance, cross-tolerance also occurs between the classic psychedelics (i.e., among LSD, mescaline, and psilocybin) (Wyatt et al., 1976; Jaffe, 1990; Spinella, 2001; Nichols, 2004).

This tolerance is possibly explained by the down-regulation and desensitization of 5-HT_{2A} receptors (see for a review Riba, 2003; Nichols, 2004; Fantegrossi et al., 2008). Smith et al. (1999) reported that behavioral tolerance to DOI reflects down-regulation of 5-HT_{2A} receptors. Aloyo et al. (2001) reported 5-HT_{2A} receptor down-regulation associated with chronic psychedelic (DOI) administration. Gresch et al. (2005) demonstrated that LSD tolerance was associated with decreased 5-HT_{2A} receptor signaling. Dougherty and Aloyo (2011) reported tolerance to chronic DOI administration and down-regulation of 5-HT_{2A} receptors. Roth et al. (1995) reported that brief (1-hr) and prolonged (24-hr) exposure to DOI leads to desensitization of 5-HT_{2A} receptors without down-regulating these receptors. Moreover, these authors provided evidence that phosphoinositide (PI) hydrolysis and protein kinase C (PKC) are involved in this process. Romano et al. (2010) reported that chronic intrahippocampal LSD administration desensitizes the 5-HT_{2A} receptor. Gray et al. (2003) reported that two serine residues of the rat 5-HT_{2A} receptor, S421 in the C terminus and S188 in intracellular loop 2, are essential to the agonist-induced desensitization process.

Nevertheless, it is difficult to elicit tolerance to DMT in animals or humans (Cole and Pieper, 1973; Gillin et al., 1973, 1976; Kovacic and Domino, 1976; see for a review Gillin et al., 1978; Barker et al., 1981; Riba, 2003). Little or no cross-tolerance occurs between DMT and LSD, and LSD-tolerant individuals show undiminished responses to DMT (Rosenberg et al., 1964; Kovacic and Domino, 1976). Some researchers have speculated that one possible explanation for the lack of tolerance to DMT is that it is rapidly metabolized (Gillin et al., 1976; see also Barker et al., 1981). In addition to affinity for the 5-HT_{2A} receptor, DMT has also affinity for the 5-HT_{2C} receptor, as shown by Smith

and co-workers (1998). These authors showed that DMT induces tolerance at the 5-HT_{2C} receptor, but not at the 5-HT_{2A}.

In a clinical study, Strassman et al. (1996) did not find tolerance to the subjective effects or blood pressure increases after a dosing regimen involving four doses of 0.3 mg/kg DMT i.v. administered at 30 min intervals. On the other hand, neuroendocrine responses (ACTH, prolactin and cortisol) and heart rate decreased from the first to the fourth administered dose. This study strongly suggests that tolerance to the subjective effects of DMT does not develop easily in humans, and the inexistence of tolerance in the 5-HT_{2A} receptor described by Smith et al. (1998) further suggests that these subjective effects are mediated by this receptor.

3. The *β*-carbolines or harmala alkaloids

3.1. Sources of *β*-carbolines

β-Carbolines are alkaloids found in many plants, fungi, and animals (Allen and Holmstedt, 1980; McKenna and Towers, 1984; McKenna et al., 1984a, 1984b; Schultes and Hofmann, 1992; Ott, 1994, 2004a; Pfau and Skog, 2004; Cao et al., 2007), including many commercial cereal grains, fruits, vegetables, alcoholic beverages, soft drinks, sauces, yeast, animal protein, and related products (Adachi et al., 1991; Herraiz and Galisteo, 2003; Herraiz and Chaparro, 2006a, 2006b; Herraiz, 2007; see for a review Herraiz, 2000a; Pfau and Skog, 2004; Cao et al., 2007). There are presently 64 known *β*-carboline alkaloids dispersed throughout at least eight plant families (Schultes and Hofmann, 1980, cited in Lotsof, 1997; see also Allen and Holmstedt, 1980).

Several species of the *Banisteriopsis* genus (Malpighiaceae) used to produce ayahuasca are rich in *β*-carbolines (McKenna et al., 1984a; Ott, 1994; Callaway, 2005a; Callaway et al., 2005; see for a review Ott, 1994; Riba, 2003). The more commonly used of these species, *B. caapi*, is rich in harmine and tetrahydroharmine (THH); lower quantities of harmaline and traces of harmol, harmalol and other substances related or not to *β*-carbolines are also present (Hochstein and Paradies, 1957; Hashimoto and Kawanishi, 1975, 1976; Kawanishi et al., 1982; McKenna et al., 1984a; Aquino et al., 1991; Callaway et al., 1996; Pomilio et al., 2003; Callaway, 2005a; Callaway et al., 2005; Gambelunghe et al., 2008; McIlhenny et al., 2009; Pires et al., 2009; Wang et al., 2010; McIlhenny et al., 2011a, 2011b; see for a review Ott, 1994; Riba, 2003; see Figures 2-4).

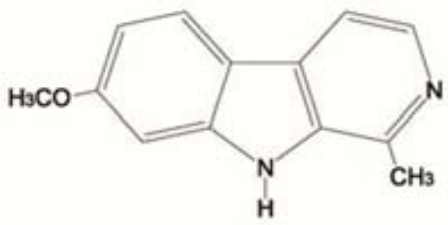

Figure 2: Chemical structure of harmine.

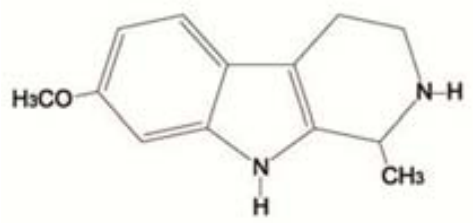

Figure 3: Chemical structure of THH.

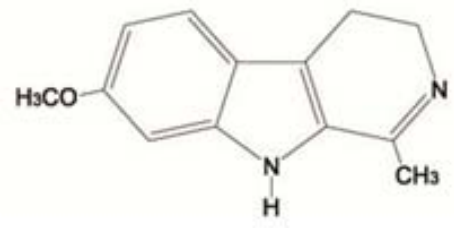

Figure 4: Chemical structure of harmaline.

The *β*-carbolines harmine (previously called telepathine, yajeine and banisterine), harmaline, THH, harmalol, harmol, and harman (also called harmane) are found in the seeds and roots of Syrian rue (*Peganum harmala*, Zygophyllaceae), a plant indigenous to North Africa, the Mediterranean Basin, the Middle East, Pakistan, India and Central Asia, and naturalized in North America, Europe, and some areas of South Africa and Australia (Abdel-Fattah et al., 1995; Mahmoudian et al., 2002; Frison et al., 2008; Herraiz et al., 2010).

P. harmala is also commonly used for the preparation of ayahuasca analogues (Ott, 1994). Nevertheless, the *β*-carboline content in the seeds of *P. harmala* is much higher than in the *B. caapi* plant. Alkaloids are found at a concentration of 2-7% dry weight in *P. harmala* seeds (Hemmateenejad et al., 2006, cited in Frison et al., 2008; see also Herraiz et

al., 2010), whereas quantitative analyses of *Banisteriopsis* have shown the stems – which are the main part of the plant used to prepare ayahuasca – to contain an average of 0.4% dry weight of alkaloids, ranging from 0.05% to 1.36% (see for a review Riba, 2003). Higher values were found by Rivier and Lindgren (1972), who reported a concentration of 1.95% in the roots of one specimen of *B. caapi* and of 1.9% in the leaves of another. However, even these concentrations are lower than those found in *P. harmala* seeds.

Harmaline was first isolated from *P. harmala* seeds and roots by Goegel in 1841, while harmine was first isolated from *P. harmala* seeds by Fritsche in 1847 (see Ott, 1994; Lotsof, 1997). In 1909, Gunn proposed that harmaline could be used to treat malaria, and similar assertions were subsequently made for harmine in 1911 (see Lotsof, 1997). In the 1920s and early 1930s, harmine from *B. caapi*, then called banisterine, was used in animals and patients with symptoms similar to Parkinson's disease. Some authors proposed the use of banisterine to treat postencephalitic parkinsonism, and in 1928 Beringer used banisterine to treat this disease (see for a review Sanchez-Ramos, 1991). Costa and Faria (1936) reported improvements in patients suffering from Parkinson's disease, muscular rigidity, epilepsy, depression, memory deficits, apathy, phobias, fatigue, and attention problems. Lewin and Schuster (1929, cited in Holmstedt, 1967) used 0.02-0.04 g of banisterine to treat Parkinson's patients and observed improvements in swallowing, chewing, speech, eating, arm movement and walking. They also found a lessening of muscular rigidity. Interest in harmine died quickly, but recently, reports have described the use of *B. caapi* extracts again in Parkinson's patients (Schwarz et al., 2003). In particular, a double-blind, randomized, placebo-controlled trial demonstrated that extracts prepared from the *Banisteriopsis* vine improved motor function in these patients (Serrano-Dueñas et al., 2001). The effects of harmaline in psychotherapy were evaluated by Naranjo (1969, 1975, both cited in Lotsof, 1997).

β-Carbolines are also found in some *Virola* species, a group of plants used for the preparation of psychedelic snuffs (*paricá*, *epéna*) and some oral preparations in the Amazon (Allen and Holmstedt, 1980; McKenna et al., 1984b; McKenna and Towers, 1985; Ott, 2001a, 2001b, 2004a). Trace amounts of *β*-carbolines were found in Myristicaceous (*Virola* spp.) preparations (McKenna et al., 1984b; McKenna and Towers, 1985; see also Holmstedt and Lindgren, 1967).

McKenna et al. (1984b) reported that a *β*-carboline was isolated from the seeds of *Anadenanthera peregrina* (Leguminosae), a plant also used in the preparation of psychedelic snuffs in the Amazon, such as *ñopo* or *yopo* (Schultes and Hofmann, 1992; Schultes, 1998; Ott, 2001a, 2004a; see also Schultes, 1967). Some of these same snuffs, commonly rich in tryptamines, also contain *β*-carbolines such as harmine, usually from powdered *B. caapi* (Ott, 1999, 2001a; Rodd, 2002, 2008; see also Holmstedt and Lindgren, 1967). The Piaroa indigenous group of Venezuela consume *β*-carboline-rich *B. caapi* before using their snuffs (Ott, 2001a; Rodd, 2002, 2008). Some admixture plants of *jurema* – an indigenous preparation made from the root bark of several DMT-rich species used in northeast Brazil – like *Passiflora sp.* (Passifloraceae), have *β*-carbolines as chemical constituents (Allen and Holmstedt, 1980). Some species of *Acacia* (Leguminosae) also contain *β*-carbolines (Ott, 1994), as well as coffee, chocolate, cocoa, tobacco, cigarettes and cigarette smoke (Totsuka et al., 1999, cited in Moura et al., 2007; Herraiz, 2000b; Herraiz and Chaparro, 2005, 2006a, 2006b; see for a review Pfau and Skog, 2004). Norharman (also called norharmane) and harman have also been detected in the basic fraction of marijuana smoke condensate (Kettenes-van den Bosch and Salemik, 1977, cited in Pfau and Skog, 2004).

Some *β*-carbolines have MAO inhibiting properties, and in the case of some snuffs and *jurema* preparations, the combination of *β*-carboline-rich plants with tryptamine-rich plants may produce a "formula" similar to ayahuasca and its analogues (Ott, 1994, 1999, 2001, 2004a, 2004b).

β-Carbolines are also endogenous compounds in several species of mammals (Zheng et al., 2000; Louis et al., 2002; Fekkes et al., 2004; Parker et al., 2004; see for a review McKenna and Towers, 1984; Pfau and Skog, 2004; Cao et al., 2007). *β*-Carbolines are also present in the human body (Adachi et al., 1991; Breyer-Pfaff et al., 1996; Fekkes et al., 1996; Pari et al., 2000; Zheng et al., 2000; Fekkes et al., 2001a, 2001b; Louis et al., 2002; Spijkerman et al., 2002; Louis et al., 2005, 2007, 2008a, 2008b, 2010; Louis and Zheng, 2010; see for a review McKenna and Towers, 1984), but their function is uncertain. Tetrahydro-*β*-carbolines (THBCs) have been discovered in mammalian brain and other tissues, leading to speculations about the possible role of these substances in human

psychopathology (Nielsen et al., 1982). 6-methoxy-tetrahydro-*β*-carboline (6-MeO-THBC), also known as 6-methoxy-tetrahydro-norharman, has been found in humans and has been named pinoline (Callaway, 1988).

3.2. Pharmacology of *β*-carbolines

3.2.1. Neurochemistry

β-Carbolines interact with several neurotransmitter systems in the brain. Nevertheless, since the effects of these substances as inhibitors of the MAO enzyme are the most important regarding ayahuasca's mechanism of action, only these effects will be discussed.

The harmala alkaloids are the most abundant compounds in ayahuasca potions (Callaway et al., 1999; Riba, 2003; Callaway, 2005a). Harmine, THH and harmaline, ayahuasca's main *β*-carbolines, are potent natural, selective, reversible, and competitive inhibitors of the MAO enzyme, especially of the MAO-A subtype, the form for which norepinephrine, serotonin, and presumably other tryptamines including DMT are the preferred substrates (Buckholtz and Boggan, 1977a; McKenna et al., 1984a; Kim et al., 1997; Miralles et al., 2005; Herraiz et al., 2010; Samoylenko et al., 2010; Wang et al., 2010; see also McKenna et al., 1998). *β*-Carbolines also have a relatively low affinity for liver MAO compared to brain MAO (McKenna et al., 1984a). Ayahuasca was found to be an extremely effective inhibitor of MAO *in vitro* and the degree of inhibition was directly correlated with the concentration of MAO-inhibiting *β*-carbolines (McKenna et al., 1984a).

There is some evidence, however, that THH, the second most abundant *β*-carboline in ayahuasca, acts as a selective inhibitor of serotonin reuptake as well as a MAO inhibitor (Buckholtz and Boggan, 1977b). The inhibition of both systems – MAO and serotonin reuptake – by the *β*-carbolines in ayahuasca may result in elevated levels of brain serotonin and other monoamines.

β-carbolines are compounds structurally similar to serotonin. Research has shown that these substances, especially harmine, have modest to high affinity for serotonin

receptors, especially the 5-HT_2 subtype (5-$HT_{2A/C}$), with potential for agonist, antagonist and partial agonist actions. In a drug discrimination test with rats, some *β*-carbolines (tetrahydro-*β*-carboline [THBC], 6-methoxy-tetrahydro-*β*-carboline [6-MeO-THBC], harmaline and harman) substituted partially for LSD (Nielsen et al., 1982). Also in rats, harmaline and 6-methoxyharmalan, but not harmine, showed similar effects as the phenylakylamine psychedelic DOM, but not for LSD, in tests of stimulus generalization, although these results were not very consistent (Glennon et al., 1983, cited in Glennon et al., 2000). Another study demonstrated that harman, but not 6-methoxyharmalan, harmaline, or THBC, showed only intermediate substitution for LSD (Helsley et al., 1998; for a review of this topic, see Riba, 2003). Despite partial results in stimulus generalization studies, *β*-carbolines have not been found to stimulate phosphoinositide hydrolysis, an effect produced by the psychedelic phenylalkylamines and indolealkylamines (Grella et al., 1998; Glennon et al., 2000; Spinella, 2001; Grella et al., 2003; see for a review Riba, 2003). Harmaline causes an enhancement of the serotonin concentrations in the brain, and harmine strongly inhibits the storage of norepinephrine in the adrenal vesicles (McKenna et al., 1984a).

3.2.2. Subjective effects

Unfortunately, there is not much scientific literature regarding the effects of *β*-carbolines in humans. Harmaline and harmine produced behaviors in dogs and cats that were interpreted by the investigators to be consistent with hallucinations (Gunn, 1935; Lewin, 1928, both cited in Sanchez-Ramos, 1991). Villablanca and Riobó (1970) reported that cats under the effect of harmaline would “catch some imaginary object with his paw or jump as it to avoid something”.

The ability of *β*-carbolines to elicit full-blown psychedelic effects in humans has been repeatedly contested (e.g., Ott, 1994; McKenna et al., 1998; Ott, 1999, 2004a). Nevertheless, there are some reports that ayahuasca preparations made exclusively with *Banisteriopsis* species can produce psychedelic experiences (Davis, 1997; Serrano-Dueñas et al., 2001; Rodd, 2008).

There are some human studies with β-carbolines. In 1930, Halpern (cited in Sanchez-Ramos, 1991) took up to 40 mg of harmine orally and 30 mg subcutaneously (s.c.) in self-experiments and reported excitement and other psychoactive effects. In 1957, Pennes and Hoch examined the oral, s.c. and i.v. effects of harmine in 28 psychiatric patients (mainly schizophrenics). The total doses ranged from 20 to 960 mg, and they used LSD and mescaline for comparison. Harmine was described to be psychedelic above 150-200 mg i.v., but inactive after oral administration (up to 960 mg). The compound was also inactive after s.c. administration. In 1970, Slotkin and collaborators administered i.v. harmine (0.5 mg/kg, 35-45 mg) to five healthy male volunteers but did not observe psychedelic effects.

Naranjo (1959) compared the effects of harmine, LSD, and mescaline, in animals and also in humans. In humans, the drug was administered orally (20-50 mg) and also by the i.m. route (10-20 mg). Oral harmine started to produce psychedelic effects after 20-30 minutes, produced its maximal effects at 30-60 minutes, and lasted 6-8 hours. I.m. harmine started to produce psychedelic effects at 5-10 minutes, produced its maximal effects at 30 minutes, and lasted 3-5 hours. The author reported that the effects of these drugs in humans were qualitatively similar, but quantitatively different, where LSD elicited more psychedelic and emotional effects, followed by mescaline and lastly by harmine. Harmine produced more “horrible” experiences, like being chased by demons and predatory animals. Harmine also produced aggression, in animals and in humans, and the harmine experience was characterized as “paranoid”. This author also reported that all drugs produced rapid tolerance, and that some cross-tolerance between the drugs also developed. Finally, harmine also produced mydriasis, hyperthermia, hyperglycemia, piloerection, higher respiratory amplitude and frequency, hypotension, salivation and lacrimation.

Naranjo (1967) found harmaline to be psychedelic at doses above 1 mg/kg i.v. or 4 mg/kg by mouth, “which is about one half the threshold level for harmine”. The onset of effects for these compounds was “about one hour after ingestion by mouth, but almost instantaneous after intravenous injection”. He also found harmaline “to be hallucinogenic at doses greater than 1 mg/kg i.v.”. Racemic THH, up to the amount of 300 mg, was administered to a volunteer who reported that, at this dosage level, “there were subjective effects similar to those which he experienced with 100 mg of harmaline”. According to

Naranjo, racemic THH "is about one-third as active as harmaline". Naranjo reported that the 6-substituted analogue of harmaline, 6-MeO-harmalan (or 6-Methoxyharmalan), produced threshold psychedelic activity at 1.5 mg/kg orally. Comparable levels (1.5 mg/kg) of 6-Methoxytetrahydroharman gave "milder effects", and is one-third as potent as 6-Methoxyharmalan.

Naranjo (1976, 1987) reported on some features of the experience with harmaline as reported by 35 volunteers in the University of Chile's Center for the Study in Medical Anthropology, who took it either orally or by the i.v. route, in different dosage levels and in some cases more than once. Naranjo reported psychedelic effects for this compound, and argued that the experimental subjects ingested either mescaline or LSD on a different occasion, and they all agree that their reactions to these drugs were very different from those brought about by harmaline. He argued that this difference partly lies in that harmaline induces a dream-like trance.

Seeing images with eyes closed, a mental state similar to dreaming, psychological and physical disturbances (e.g., nausea and vomiting), serenity, enhanced intelligence, visions of one's own death, feelings of floating, visions of geometric forms and of some specific animals – snakes, reptiles, birds and felines – were among the subjective experiences reported by Naranjo. He reported that harmaline induces "mythopoetic" activity and life-death consciousness. Despite all these effects, the studies by Naranjo have serious methodological limitations and should be considered with caution. Nevertheless, they are the primary source on the potential psychedelic effects of β-carbolines.

Based on self-experiments with harmine and also based on the literature, Shulgin and Shulgin (1997) reported euphoria (25-75 mg s.c.), bradycardia, tingling, hypotension, cold extremities and light-headedness (35-40 mg i.v.), excitement (40 mg orally), sedation (140 mg orally), visual hallucinations, bradycardia and hypotension (150-200 mg i.v.), relaxation (300 mg sublingually), psychotic symptoms (300-400 mg orally), dizziness, nausea and ataxia (750 mg sublingually) and, possibly, visual hallucinations (up to 900 mg orally). For harmaline they reported experiencing no effects (100 mg orally), numbness (150 mg orally), some perceptive (visual, auditory) modifications (175 mg orally), visual images with eyes closed or other visual modifications (200-500 mg orally), irritability and

disorientation (300 mg orally) and nausea and collapse of motor co-ordination (500 mg orally).

On the other hand, Ott (1999) maintains that the *β*-carbolines in ayahuasca preparations have merely "sedative, Valium-like psychoactivity" and that "plants rich in *β*-carbolines have found world-wide use as sedatives". Ott reported that an aqueous infusion of 15 g of *P. harmala* seeds without additives "acted as a sedative, with no visionary effect". It must be recognized, as already mentioned, that the *β*-carboline content in *P. harmala* is much higher than in *B. caapi*.

According to Ott (1999), the total amount of *β*-carbolines in ayahuasca ("just over two mg/kg", see for a review Riba, 2003) would not be responsible for the powerful psychoactive effects of the brew. In a self-experiment, Ott (1999) found that 1.5 mg/kg harmine (120 mg), the threshold dose capable of rendering DMT orally active, ingested in the absence of DMT, exerted "barely perceptible sedative effects". This author also reported another self-experiment in which 175 mg harmaline hydrochloride (146 mg base; 2.25 mg/kg) acted as a "mild sedative". Ott (1994, 2004a) also reported sedative, Valium-like effects for ayahuasca preparations lacking DMT-rich admixture plants.

Ott (2001b) reported that insufflating (intranasally) 7.5 mg of harmaline (as base-equivalent) "provoked no appreciable effects", although this same dose sublingually "elicited quite appreciable effects", "neither stimulating nor sedating". The same dose of harmine, sublingually, "was without effect". This author comments on another experiment in which insufflation of 0.5 mg/kg harmine free-base produced no notable effects.

3.2.3. Pharmacokinetics

Slotkin and DiStefano (1970) determinated levels of harmine metabolites after its i.v. administration to rats. More than 99% of the dose was recovered, and nearly 70% was excreted in the first 4 hours. 77% was excreted as harmol sulfate, 21% as harmol glocuronide and 1% as harmine and harmol. In another study, Slotkin et al. (1970) quantified blood and urinary levels of harmine and its urinary metabolites in humans and rats after i.v. administration. Within two min after dosing, less than 10% of the dose

remained in the blood of either species; after 4 h it was less than 1%. Urinary harmine and harmol concentrations were insignificant. Harmol sulfate was the primary conjugate in rats, while harmol glucuronide excretion predominated in humans.

In a study designed to model the kinetics of [^{11}C]-harmine binding to MAO-A in the human brain after i.v. administration and using positron emission tomography (PET), Ginovart et al. (2006) reported that the fraction of unmetabolized [^{11}C]-harmine in plasma decreased rapidly throughout the study: from 90% at 5 min to 11% at 90 min.

Yu et al. (2003) studied the involvement of the different cytochrome P450 isoenzymes in the biotransformation of harmine and harmaline. The authors reported that harmine and harmaline were metabolized to hydroxylated hamine and harmaline as well as their respective *O*-demethylated metabolites, i.e. harmol and harmalol. CYP2D6, CYP1A1 and CYP3A4 were responsible for these reactions (Riba et al., 2003; Yu et al., 2003; Callaway, 2005b; Riba and Barbanoj, 2005; Wu et al., 2009; Zhao et al., 2011).

3.2.4. Neuroendocrine effects

There are currently no data in the scientific literature regarding the neuroendocrine effects of harmine, THH or harmaline in humans or animals.

3.2.5. Cardiovascular and autonomic effects

P. harmala extract, as well as isolated harmine and harmaline, produced dose-dependent hypothermia in rats (Abdel-Fattah et al., 1995). Bradycardia and hypotension were among the most frequent symptoms reported in the studies by Pennes and Hoch (1957) and by Slotkin et al. (1970) involving the i.v. administration of harmine to humans. There are no studies on the cardiovascular effects of THH or harmaline in humans.

3.2.6. Adverse effects

Pennes and Hoch (1957) reported nausea, vomiting, tremor and body numbness after i.v. administration of harmine to humans. Naranjo found that this drug induced

hyperthermia and piloerection (Naranjo, 1959). Harmine has also been shown to induce severe tremor in humans (Naranjo, 1959; Wilms et al., 1999, cited in Al Deeb et al., 2002). Trouble focusing the eyes, tingling, cold extremities and light-headedness were among the most frequent symptoms reported in the study by Slotkin et al. (1970). Finally, Naranjo (1967) reported that harmaline caused paresthesias and numbness, physical discomfort, nausea, intense vomiting and dizziness.

3.2.7. Tolerance and sensitization

There are currently no controlled studies regarding the possible occurrence of tolerance or sensitization to harmine, THH and harmaline in humans. Naranjo (1959) compared the effects of harmine, LSD, and mescaline, and reported that all drugs produced rapid tolerance and that some cross-tolerance between drugs also developed. There is a report on the regular use of harmine in doses of 0.02-0.04 g to treat Parkinson patients (Lewin and Schuster, 1929, cited in Holmstedt, 1967). Some of the patients had been on medication continually for more than a year, without decrease in drug effect.

Harmaline-induced tremor exhibits pronounced tolerance in the rat. The tremor gradually decreased across four treatment days (Wang and Fowler, 2001). These authors proposed that this tolerance may occur at the level of the inferior olive due to a reduction in the synchronous activation of the inferior olivary neurons.

4. Ayahuasca

4.1. Plant sources and traditional uses

Ayahuasca is a Quechua term that has the following etymology: Aya – means "soul" or "dead spirit"; and Waska – "rope" or "vine". Thus Ayahuasca can be translated as "vine of the souls" or "vine of the dead". The term makes reference to *B. caapi*, the vine used as the main ingredient in the elaboration of a psychoactive beverage currently used by more than 70 different indigenous groups of the Amazon pertaining to 20 different language families and spread throughout Brazil, Colombia, Peru, Venezuela, Bolivia and Ecuador (Dobkin de Rios, 1972; Luna, 1986; Schultes, 1986; Narby, 1998; Luz, 2004; Schultes and Raffauf, 2004; Luna, 2005, 2011).

The word *ayahuasca* can describe the vine itself (*B. caapi* and related species), the beverage prepared from it, or the spiritual force present in the beverage, which can also contain many other plants, mainly *P. viridis* (Rubiaceae) or *D. cabrerana* (Malpighiaceae) (Schultes, 1986; Ott, 1994; McKenna et al., 1995; Davis, 1997; Callaway, 2003; Ott, 2004a; Schultes and Raffauf, 2004; Callaway, 2005a, 2005b; Luna, 2005, 2011).

The beverage is used for magico-religious purposes. These uses include acquiring protective spirits, determining the causes and cures of diseases, prophesizing the future, determining if wives are unfaithful, discovering enemies and their plans, preparing for war or hunting expeditions, finding game, contacting distant relatives, establishing and maintaining relations between villages, learning myths, art, chants and dances, gaining direction and guidance throughout life, consolidating group identity, performing rites of passage, obtaining pleasure and as an aphrodisiac (Dobkin de Rios, 1972; Lamb, 1974; Dobkin de Rios, 1976; Harner, 1976a, 1976b, 1976c; Kensinger, 1976; Siskind, 1976; Weiss, 1976; Arévalo Valera, 1986; Luna, 1986; Schultes and Hofmann, 1992; Ripinsky-Naxon, 1995; Lagrou, 1996; Davis, 1997; Fericgla, 1997; Keifenheim, 2004; Langdon, 2004; Luz, 2004; Zuluaga, 2004; Luna, 2005, 2011; see also Instituto Indigenista Interamericano, 1986).

Since the beginning of the twentieth century, ayahuasca has been used by syncretic religious groups originating in the Amazonian Brazilian states of Acre and Rondônia. These groups, the most prominent being the Santo Daime, Barquinha and União do Vegetal (UDV), use ayahuasca as a healing tool, for spiritual development, and as a vehicle to access the divine realm. In the Santo Daime and UDV rites, members consume ayahuasca usually twice a month, and in Barquinha it is not unusual to consume the brew four times per week (for information on the ayahuasca religions see: MacRae, 1992; Araújo, 1999; Groisman, 1999; MacRae, 1999; Labate, 2004; Labate and Araújo, 2004; Labate and MacRae, 2006; Labate et al., 2009).

4.2. Chemistry and DMT-*β*-carboline interaction

Chemical analyses have shown that the main components of ayahuasca are several alkaloids with *β*-carboline structure plus *N*,*N*-dimethyltryptamine (DMT) (McKenna et al., 1984a; Callaway et al., 1999; Callaway, 2005a, 2005b; Callaway et al., 2005).

The main *β*-carbolines in ayahuasca are harmine and THH, with harmaline generally found in lower amounts. Harmol, harmalol plus other substances related or not to *β*-carbolines are also present (Hochstein and Paradies, 1957; Hashimoto and Kawanishi, 1975, 1976; Kawanishi et al., 1982; McKenna et al., 1984a; Aquino et al., 1991; Callaway et al., 1996; Pomilio et al., 2003; Callaway, 2005a; Callaway et al., 2005; Gambelunghe et al., 2008; McIlhenny et al., 2009; Pires et al., 2009; Wang et al., 2010; McIlhenny et al., 2011a, 2011b; see for a review Ott, 1994; Riba, 2003). Callaway et al. (2005) reported the following mean overall alkaloid composition for *B. caapi* (dry weight): 4.83 mg/g harmine; 1.00 mg/g THH; 0.46 mg/g harmaline. Quantitative analyses of *Banisteriopsis* have shown the stems to contain an average of 0.4% dry weight of alkaloids (Riba, 2003). *β*-Carboline concentrations in assayed ayahuasca brews have ranged from 0.07 to 22.85 mg/ml for harmine; from 0.05 to 23.80 mg/ml for THH; and from 0.00 to 1.72 mg/ml for harmaline (Riba, 2003; Callaway, 2005a; McIlhenny et al., 2009; Pires et al., 2009).

The DMT present in ayahuasca is extracted into the brew from various plants that are used as admixtures to the tea. As mentioned above, these plants include *P. viridis* and *D. cabrerana*. Callaway et al. (2005) reported a mean overall alkaloid content for *P. viridis*

leaves of 7.50 mg/g DMT (i.e., 0.75%). The leaves of *D. cabrerana* contain an average of 0.7% of alkaloids (Riba, 2003). DMT concentrations in assayed ayahuasca brews have been shown to range between 0.00 and 14.15 mg/ml (Riba, 2003; Callaway, 2005a; McIlhenny et al., 2009; Pires et al., 2009).

The *β*-carbolines present in ayahuasca are potent natural, selective, reversible, and competitive inhibitors of MAO (Buckholtz and Boggan, 1977a; McKenna et al., 1984a; Kim et al., 1997; Miralles et al., 2005; Herraiz et al., 2010; Samoylenko et al., 2010; Wang et al., 2010; see also McKenna et al., 1998). THH also acts as a selective inhibitor of serotonin reuptake (Buckholtz and Boggan, 1977b).

When DMT is combined with MAO inhibitors such as the *β*-carbolines present in ayahuasca, it can reach systemic circulation and the central nervous system, thus producing its effects (McKenna et al., 1984a, 1984b; Ott, 1994, 1999; Riba and Barbanoj, 2005). In a series of self-experiments involving the oral ingestion of harmine plus DMT, Ott (1999) established the threshold dose of harmine necessary to render DMT orally active at 1.5 mg/kg (120 mg), confirming the harmine-DMT interaction. Ott (1999) also reported that doses of harmaline starting at 70 mg (1-1.2 mg/kg) could activate tryptamines.

4.3. Human pharmacology

4.3.1. Subjective effects

Published research has described intricate eyes-closed visual imagery, complex thought processes and a general state of heightened awareness following ayahuasca. Overall perceptual, cognitive, and affective processes are significantly modified in the presence of a clear sensorium. Despite altered perceptions and cognition, users remain aware of their surroundings and are able to communicate coherently (for the subjective and visionary experiences of ayahuasca, see Luna and Amaringo, 1999; Shanon, 2002; Villaescusa, 2002, 2007; Kjellgren et al., 2009; Trichter et al., 2009).

Callaway et al (1999) measured subjective effects using the Hallucinogen Rating Scale (HRS) – an instrument developed by Strassman et al. (1994) for the

phenomenological assessment of i.v. DMT. Scores on all the clinical clusters of the HRS were in the mild end of the spectrum compared with i.v. DMT. The clusters were comparable to an i.v. DMT dose of 0.1-0.2 mg/kg, and in one cluster (somaesthesia) scores were below the lowest dose of DMT measured by the scale, i.e., 0.05 mg/kg (Grob et al., 1996).

Pomilio et al. (1999, 2003) administered a 100 ml dose of ayahuasca to naïve subjects and also to experienced members of the União do Vegetal. The study included a control group. These researchers measured perceptual effects prior and after ayahuasca administration with the Hoffer & Osmond Test (HOD test). No effects were reported up to 35 min after ayahuasca administration. At this time point some volunteers reported marked perception alterations, mainly visual. Auditive hallucinations were never reported, while others experienced only slight time-space disorientation and all evidenced mood changes with "unmotivated laughing". Emotional modifications were sometimes present together with the perceptual changes.

Research into the pharmacology of ayahuasca in healthy volunteers has been conducted in a systematic way by our group since 1999. Subjective effects have been assessed by means of self-report instruments such as visual analogue scales (VAS), the Addiction Research Center Inventory (ARCI) and the Hallucinogen Rating Scale (HRS). The HRS was adapted into Spanish and its psychometric characteristics assessed by our group (Riba et al., 2001a). The assessment of the Spanish version included the administration of the questionnaire to 75 European users of ayahuasca after drug intake when acute effects had resolved. The study highlighted the reliability and convergent-discriminant validity of the instrument and showed that ayahuasca induces increases in all six subscales into which the HRS is divided.

Following this initial "field evaluation" study, our group assessed the subjective effects and tolerability of ayahuasca in a single-blind placebo-controlled clinical study in which three increasing doses of encapsulated freeze-dried ayahuasca (0.5, 0.75, and 1 mg DMT/kg body weight) were administered to six healthy male volunteers with prior experience in the use of the brew (Riba et al., 2001b). Given the specific prior experience of this group of volunteers with ayahuasca, the doses to be used in future studies were

decided based on their reports. Participants indicated that the 1.0 mg DMT/kg dose was exceedingly high. It was thus determined that the two doses to be used in the other studies would lie between 0.5 and 0.75 mg/kg (low dose) and between 0.75 and 1.0 mg/kg (high dose).

Ayahuasca produced significant dose-dependent increases in five of the six subscales of the HRS, in the LSD, MBG, and A scales of the ARCI, and in the "liking", "good effects" and "high" visual analogue scales. The psychological effects were first noted after 30-60 min, peaked between 60-120 min, and were resolved by 240 min.

Modified physical sensations and nausea were the most frequently reported somatic-dysphoric effects. At no time of the study did any of the volunteers lost consciousness or contact with reality. Volunteers reported a certain degree of anxiety or fear at the initial stage, tending to decrease thereafter, and the overall experience was regarded as pleasant and satisfactory by five of the six volunteers, which were able to interact with the experimenter and the environment without major problems at all three doses. One volunteer experienced an intensely dysphoric reaction with transient disorientation and anxiety at the medium dose and voluntarily withdrew from the study. According to the authors, it is noteworthy that this volunteer had the least amount of experience with ayahuasca, having consumed it prior to the study on only two occasions. Verbal support was sufficient to get him through this temporary crisis.

A larger study followed after the initial study by Riba et al. (2001b) involving the participation of 18 healthy volunteers and a double-blind crossover placebo-controlled design. Two doses (one low and one high) of encapsulated freeze-dried ayahuasca, equivalent to 0.6 and 0.85 mg DMT/kg body weight, were administered. The study also evaluated the subjective effects of ayahuasca, as had been done in the previous single-blind study. Subjective effects were analogous to those reported earlier (Riba et al., 2001b). However, scores on some measures at the 0.6 and 0.85 mg of DMT/kg doses tended to be lower than those obtained after 0.5 and 0.75 mg of DMT/kg doses, respectively. Riba et al. (2003) argued that sample size, study design, and prior exposure to ayahuasca could account for these differences.

4.3.2. Pharmacokinetics

Callaway et al. (1999) studied alkaloid pharmacokinetics in fifteen long-term users of the brew – members of the ayahuasca religion União do Vegetal. A standard dose of ayahuasca of 2 ml/kg body weight was used throughout the study.

Only 12 volunteers had sufficient DMT concentrations for pharmacokinetic calculations: C_{max} 15.8 ± 4.4 ng/ml, T_{max} 107.5 ± 32.5 min. Pharmacokinetic values for harmine and THH were determined for 14 volunteers (C_{max} 114.8 ± 61.7 ng/ml, T_{max} 102.0 ± 58.3 min [harmine]; C_{max} 91.0 ± 22.0 ng/ml, T_{max} of 174.0 ± 39.6 min [THH]), and only for 5 volunteers for harmaline (C_{max} 6.3 ± 3.1 ng/ml, T_{max} 145.0 ± 66.9 min). Plasma alkaloid concentrations for harmine, THH and DMT were determinated for 14 volunteers. Only THH was detected, at low levels, in three volunteers at the 24 h time point.

Pomilio et al. (1999, 2003) collected urine samples for DMT determination. After ayahuasca, both naïve subjects and experienced consumers showed DMT in their urine, which, according to the authors, was positively correlated with the perceptual alterations.

Yritia and co-workers (2002) quantified in plasma the four main alkaloids present in ayahuasca (harmine, harmaline, THH and DMT) plus two major putative metabolites (harmol and harmalol, the *O*-demethylation products of harmine and harmaline, respectively) after oral administration. Yritia et al. (2002) and Riba et al. (2003) found a time curve of DMT plasma levels which fit with the overall duration and peak values of subjective effects. Additionally, Yritia et al. (2002) measured the concentrations of harmol and harmalol, two alkaloids present in trace amounts in ayahuasca. These compounds were found in considerable amounts in human plasma following ingestion of the tea. Harmol and harmalol had not been assessed previously in plasma following oral dosing with ayahuasca.

Although MAO inhibition is generally acknowledged as the mechanism facilitating the absorption of DMT in the gastrointestinal tract, there is no direct proof that this enzymatic inhibition actually takes place in humans after the ingestion of ayahuasca (Riba and Barbanoj, 2005). Riba et al. (2003) investigated the MAO-inhibiting effects of

ayahuasca *in vivo* by measuring urine monoamine metabolite excretion. The study found increased urinary excretion of normetanephrine, a methylated breakdown product of norepinephrine. The increase in normetanephrine is in line with MAO inhibition. Nevertheless, Riba et al. (2003) found a non-significantly increased excretion of deaminated metabolites, which is not in line with a MAO-inhibiting profile, and so it was not possible to conclude if there was a systemic inhibition of MAO. This and the negligible harmine plasma levels found suggest a predominantly peripheral (gastrointestinal and liver) rather than cerebral site of action for harmine, as this compound did not even reach systemic circulation.

Riba et al. (2003) reported quantifiable plasma levels for DMT and THH. C_{max} values for DMT after the low and high ayahuasca doses were 12.14 ng/ml and 17.44 ng/ml, respectively. T_{max} (median) was observed at 1.5 h after both doses. The T_{max} for DMT coincided with the peak of subjective effects. T_{max} values for DMT and THH were similar to those reported by Callaway et al. (1999). These researchers reported a C_{max} (mean ± SD) for DMT of 15.8 ± 4.4 ng/ml, and a T_{max} (mean ± SD) of 107.5 ± 32.5 min. The apparent volumes of distribution (V_Z/F) and clearance values (CL/F) calculated for DMT decreased with dose, suggesting a nonlinear increment of DMT levels following the administration of larger doses of ayahuasca. According to the authors, these findings could be explained by the action of the higher amounts of β-carbolines administered. The calculated *V*z/F values were similar to the study of Callaway et al. (1999), although this group reported higher $t_{1/2}$ and lower CL/F values. In the case of DMT, according to the authors, these differences may be associated with the lower levels of harmala alkaloids present in the ayahuasca used, which could have led to a lower degree of MAO inhibition.

Based in the absence of measurable levels of harmine in plasma in the study by Riba et al. (2003), compared with the high levels in the study by Callaway et al. (1999), Riba and Barbanoj (2005) speculated that individual differences in the metabolism of harmine could perhaps be an explanation, and suggested that phenotyping or genotyping volunteers for the CYP2D6 and CYP1A1 involved in harmine *O*-demethylation could shed some light on these differences (see also Yu et al., 2003; Callaway, 2005b; Wu et al., 2009; Zhao et al., 2011). CYP2D6 also metabolizes harmaline (Riba et al., 2003; Yu et al., 2003;

Callaway, 2005b; Wu et al., 2009; Zhao et al., 2011). Zhao et al. (2011) also reported that harmine exhibited noncompetitive inhibition on the activity of CYP3A4.

4.3.3. Neuroendocrine effects

Callaway and co-workers (1999) found that plasma levels of prolactin, cortisol, and GH showed increases over basal values from 60 (cortisol) to 90 (GH) and 120 min (prolactin) after ayahuasca ingestion. According to the authors, the observed response is typical of serotonergic agonists, and is comparable to the values reported by Strassman and Qualls (1994) in response to i.v. DMT. But in this study the neuroendocrine response to oral DMT was delayed by a factor of 4 or 5 compared with the almost immediate (<15 min) response to injected DMT.

Pomilio et al. (1999, 2003) assessed cortisol, prolactin and serotonin blood levels before and 60 and 120 min after ayahuasca administration. These researchers described increases in blood cortisol and prolactin ± 60 min after ayahuasca administration. These increases were in agreement with the beginning of the psychological effects of ayahuasca, and, for naïve subjects, the increased neuroendocrine response correlated with a pronounced perceptual response. For the experienced consumers, a decrease in blood serotonin levels was observed. This observation is interesting, since the MAO-inhibiting properties of the β-carbolines in ayahuasca are expected to increase the levels of this neurotransmitter.

Nevertheless, the investigations by Pomilio et al. have many limitations, and show some inconsistencies. The authors reported that neuroendocrine and heart rate responses significantly decreased across administrations, suggesting the appearance of tolerance, while psychological tolerance was not observed. However, it is not clear if their conclusions referred to data from their own study, as this would have required the administration of more than one ayahuasca dose. While they a mention a second dose 60 min after the first administration, they do not provide any information on the experimental procedure. Finally, no information is provided regarding the statistical analysis employed.

4.3.4. Cardiovascular and autonomic effects

In the study by Callaway et al. (1999), heart rate increased from 71.9 bpm at baseline to a maximum of 79.3 bpm by 20 min, decreased to 64.5 bpm by 120 min, then gradually returned toward baseline levels by 240 min. There was a concomitant increase in blood pressure: both systolic and diastolic pressure increased to a maximum at 40 min (137.3 and 92.0 mm Hg, respectively) over baseline values (126.3 and 82.7 mm Hg, respectively) and returned to baseline values by 180 min.

Pupillary diameter increased over baseline values and pupils remained dilated after the last measurement at 240 min. Pupil size typically returned to normal after approximately 6 h at this dosage. Respiration rate increased slightly over baseline values, and fluctuated throughout the study, showing an overall increase after 240 min. Oral temperature increased slightly over baseline measures (37.0 ± 0.1°C), reaching a maximum of 37.3 ± 0.1°C by 240 min. But it should be noted that ambient room temperature also increased (33-38°C) throughout each day as the study sessions progressed from morning to afternoon (from 10:00 to 16:00 h).

Riba et al. (2001b) observed moderate increases in systolic blood pressure, diastolic blood pressure and heart rate, which led the authors to conclude that ayahuasca poses only a moderate risk from the cardiovascular point of view when administered to healthy volunteers. All three doses produced increases in systolic and diastolic blood pressure when compared with placebo, but changes were not statistically significant, although a robust trend toward significance was observed for systolic blood pressure at the high dose. Heart rate was affected very little by ayahuasca. Increases above baseline values were only seen for the medium and high doses at 45 min after drug administration. At no point did systolic blood pressure reach 140 mm Hg, nor did heart rate reach 100 bpm for any individual volunteer. On the other hand, two volunteers showed sporadic diastolic blood pressure values between 91-93 mm Hg after the medium and high doses, which lasted between 15 and 30 min.

The eighteen-volunteer double-blind study also evaluated the cardiovascular effects of ayahuasca, as had been done in the previous single-blind study. In the single-blind

study, Riba et al. (2001b) failed to observe statistically significant modifications of cardiovascular parameters in a six-subject sample. Evaluating this time a larger sample, Riba et al. (2003) reported that ayahuasca produced only moderate elevations of cardiovascular parameters. Statistically significant changes relative to placebo were only found for diastolic blood pressure at the high dose (9 mm Hg at 75 min), whereas systolic blood pressure and heart rate were moderately and non-significantly increased.

4.3.5. Immunological effects

There are currently no data in the scientific literature regarding the acute effects of ayahuasca on immunological function.

4.3.6. Adverse effects

Nausea, vomiting and diarrhea are extremely common in ayahuasca rituals and ceremonies (Callaway et al., 1999; Callaway, 2005b). These effects appear to vary between individuals and also depending on dosage and alkaloid composition of the tea (Callaway et al., 1999). Callaway et al. (1999) proposed that these effects may be caused by increased levels of unmetabolized serotonin, a consequence of MAO-A inhibition by both harmine and harmaline. According to these researches, vomiting results from increased vagal stimulation by central serotonin, whereas increased peripheral serotonin could stimulate intestinal motility to the point of diarrhea (Callaway et al., 1999; Callaway, 2005b).

Callaway and co-workers also reported a fine transient tremor and nystagmus in some cases. They argued that this could be due to receptor mediated interactions of harmala alkaloids on tryptamine binding receptors. THH, the second most abundant *β*-carboline in ayahuasca, acts as a selective inhibitor of serotonin reuptake (Buckholtz and Boggan, 1977b). In this regard it is worth noting that tremor is considered the second most common neurological adverse effect of SSRIs (Edwards and Anderson, 1999, cited in Arshaduddin et al., 2004). Based on preclinical data, it is reasonable to speculate that the pro-serotoninergic effects of the ayahuasca *β*-carbolines (inhibition of MAO and of serotonin reuptake) (Buckholtz and Boggan, 1977a, 1977b; McKenna et al., 1984a; Tariq et al., 2002; Mehta et al., 2003; Arshaduddin et al., 2004, 2005; Miralles et al., 2005) may

be responsible at least in part for the tremor observed in humans after consumption of ayahuasca, and that these three compounds may potentiate the actions of one another. Nevertheless, these manifestations seem to be short-lived, and there is no indication that they are related to neurological problems.

The most distressing event reported in the studies by Riba et al. was experienced by one volunteer during the pilot study after receiving the medium 0.75 mg/kg dose (Riba et al., 2001b). The participant experienced an intensely dysphoric reaction with transient disorientation and anxiety. Verbal support was sufficient to get him through this state, which lasted around 20 minutes. His displeasure with the experience led him to voluntarily withdraw from the study. According to the authors, it is noteworthy that this volunteer had the least amount of experience with ayahuasca, having consumed it prior to the study on only two occasions.

Riba and Barbanoj (2006) commented on a case of a volunteer who reported feelings of suspiciousness and menace after taking ayahuasca in the laboratory setting. According to the authors, all these signs disappeared completely after the expected time of action of ayahuasca (4-6 h), and no medical intervention was needed.

Adverse reactions have a greater probability to occur in individuals with no previous experience with psychedelics, although investigations with individuals consuming ayahuasca for the first time did not report serious adverse reactions (Barbosa and Dalgalarrondo, 2003; Barbosa et al., 2005). In controlled and supervised settings, anxiety, fear and panic episodes are generally transient and resolve without major complications (Strassman, 1984; Griffiths et al., 2006; Frecska, 2007; Griffiths et al., 2008; Johnson et al., 2008; Studerus et al., 2011; Griffiths et al., 2011). These observations are in line with those by Riba and collaborators.

Regarding laboratory determinations, blood analyses were conducted after each experimental session in the course of the pilot study conducted by Riba et al. (2001b) without any evidence of clinically relevant alterations in hematological indices or biochemical indicators of liver function or other standard analytical parameters (cellular counts, plasma bilirubin, and hepatic enzymes).

Riba and Barbanoj (2005) reported that in their pilot and final study combined, two volunteers showed systolic blood pressure values above 140 mm Hg at some point and four showed diastolic blood pressure values above 90 mm Hg, the diagnostic criteria for hypertension. One volunteer showed heart rate values above 100 bpm, the diagnostic criterion of tachycardia. The maximum values recorded at any time point were 146 mm Hg for systolic blood pressure, 96 mm Hg for diastolic blood pressure and 101 bpm for heart rate.

In view of these moderate cardiovascular effects, the authors concluded that ayahuasca seems relatively safe from a cardiovascular point of view, but they also stressed that the results refer only to single dose administrations in young healthy volunteers and were recorded in the absence of any physical exercise. They suggest that the cardiovascular picture could be different following repeated dose administration, while performing physical exercise such as dancing, or if ayahuasca were ingested by older individuals or by those with cardiovascular conditions.

Repeated administration, dancing, and older people taking ayahuasca is a very common practice in ayahuasca religions. Nevertheless, there are no published data describing clinically relevant cardiovascular alterations associated with acute or even long-term ayahuasca consumption.

The possibility of ingesting a lethal dose of ayahuasca is remote (Gable, 2007). According to some investigators, it is difficult to imagine a lethal dose when the effective dose is so near the point of emesis (Callaway, 2005b; Gable, 2007). However, some authors recommend that caution be taken regarding ayahuasca and, especially, “home made” ayahuasca analogues (Ott, 1996).

There are some contraindications to the use of MAO inhibitors such as liver and kidney impairment, severe or frequent headaches, uncontrolled hypertension, cardiovascular and cerebrovascular diseases (Savinelli and Halpern, 1995). There is also the potential risk of a serotonin syndrome, which may include mental status changes, agitation, tremor, diarrhea, autonomic instability, hyperthermia, sweating, muscle spasms,

rhabdomyolysis and death (Callaway, 1994; Hilton et al., 1997; Callaway and Grob, 1998; Callaway, 2002; Boyer and Shannon, 2005). The combination of MAO inhibitors such as some *β*-carbolines with selective serotonin reuptake inhibitors, with the amino acid tryptophan, or with other monoaminergic and serotoninergic substances such as antidepressives in general, could potentially produce this syndrome (Callaway, 1994, 1995a; Savinelli and Halpern, 1995; Hilton et al., 1997; Callaway, 2002; Boyer and Shannon, 2005; Frecska, 2007).

Individuals who have recently used ginseng, St. John's wort, dextromethorphan, amphetamine or 3,4-methylenedioxymethamphetamine (MDMA: "ecstasy") could suffer a potentially dangerous interaction when using ayahuasca (Bonson, 1994; Savinelli and Halpern, 1995; Vuori et al., 2003; Boyer and Shannon, 2005; Frecska, 2007; Silins et al., 2007; Pilgrim et al., 2011). Also important is the observation that harmine is a selective inhibitor of the human cytochrome P450 isozyme 2D6 (CYP2D6), which also metabolizes harmaline (Riba et al., 2003; Yu et al., 2003; Callaway, 2005b; Wu et al., 2009; Zhao et al., 2011). The addition of drugs that inhibit cytochrome isoform CYP2D6 to the therapeutic use of selective serotonin reuptake inhibitors has been associated with the serotonin syndrome, so extra caution should be observed if the person is taking a combination of ayahuasca (or related preparations) with this class of drugs (Boyer and Shannon, 2005).

Nevertheless, it must be noted that serotonin syndrome is not commonly reported in the context of sacramental ayahuasca use, and anecdotal evidence suggests that many people use antidepressives, including selective serotonin reuptake inhibitors, in combination with ayahuasca, without presenting any toxic reaction (*Mukasey v. CHLQ*, 2009a, 2009b). One possible explanation is that the ayahuasca *β*-carbolines are reversible inhibitors of MAO, and quite specific for MAO-A (Buckholtz and Boggan, 1977a; McKenna et al., 1984a; Kim et al., 1997; Miralles et al., 2005; Herraiz et al., 2010; Samoylenko et al., 2010; Wang et al., 2010; see also McKenna et al., 1998). The use of reversible MAO-A inhibitors alone or in combination with selective serotonin reuptake inhibitors appears to be safer (Hilton et al., 1997). Another possibility is that the significant up-regulation in the density of the serotonin transporter in blood platelets of ayahuasca long-term drinkers reported by Callaway et al. (1994) could enhance the uptake of

serotonin. This might reduce the chance of accumulation of this neurotransmitter, even in the presence of a pro-serotoninergic substance.

The combination of foods containing tyramine and MAO inhibitors can potentially lead to hypertension (Callaway, 1993, 1995a; Savinelli and Halpern, 1995; Yamada and Yasuhara, 2004; Youdim and Weinstock, 2004; Burchett and Hicks, 2006; Zucchi et al., 2006; Frecska, 2007). Nevertheless, the ayahuasca *β*-carbolines produce a reversible inhibition of MAO-A, and hypertensive crisis are much less likely with this kind of compounds (Yamada and Yasuhara, 2004; Youdim and Weinstock, 2004). As with the serotonin syndrome, it must be noted that tyramine-produced hypertension is not commonly reported in the context of sacramental ayahuasca use (*Mukasey v. CHLQ*, 2009a).

Psychedelics like psilocybin and mescaline can also produce interactions with ayahuasca and related preparations (Ott, 1994; Callaway, 1995a; Savinelli and Halpern, 1995; Ott, 1996), although no actual intoxications have been reported.

Despite its reputed safety, several fatalities have recently been reported associated with ayahuasca. An 18-year-old young man died some hours after an ayahuasca ritual (Neto, 2009; UOL Notícias, 2009; Gomes, 2010). In another case an 18-year-old man drowned some hours after an ayahuasca ritual (Gomes, 2010; iG Notícias, 2010) and a 33-year-old man died in an ayahuasca ritual in Colombia after experiencing convulsions (CityTv, 2010; La FM, 2010; Vera, 2010). Recently, a 40-year-old Swedish man was hospitalized several days after taking ayahuasca in Peru (El Comercio, 2010; La Republica, 2010; RPP Noticias, 2010; Cárdenas, 2011; La Gaceta, 2011), a 43-year-old French woman died in an ayahuasca ritual in the same country (Perú21, 2011) and two men (29 and 37-year-old) died in a ritual in Colombia (Colombia Reports, 2011; El Espectador, 2011; Vanguardia, 2011).

4.3.7. Neurophysiological effects

Riba and co-workers also evaluated the cerebral bioavailability and time-course of effects of ayahuasca by means of topographic quantitative-pharmaco-

electroencephalography (q-EEG), a technique that provides an objective measure of drug-induced central effects. The results showed that ayahuasca causes significant and dose-dependent modifications of brain electrical activity, which are more intense and longer lasting as the dose increases. After the low dose, statistically significant differences with placebo were obtained only at isolated electrode locations, but after the high dose, EEG changes were found over extensive scalp areas. Absolute power decreased in all frequency bands, most prominently in the theta band. Relative power decreased in the delta and theta bands, and increased in the beta band. These effects first attained statistical significance at 1 h, showed a peak between 1.5 and 2 h and gradually decreased thereafter, to disappear at 6-8 h, a time pattern similar to that of subjective effects (Riba et al., 2002a). Ayahuasca effects on the individual EEG variables were similar to those of psychostimulants (such as amphetamine and methylphenidate), serotonin releasers (such as fenfluramine), tricyclic antidepressants, antidepressants showing MAO inhibiting properties, and the selective serotonin reuptake inhibitor fluoxetine.

The data also showed that ayahuasca had contrary properties as those observed in drugs with a mixed anti-D_2 and anti-5-HT_2 profile, such as risperidone and ketanserin, suggesting the role of 5-HT_2 and dopamine D_2-receptor agonism in the effects of ayahuasca.

Riba et al. (2002b) explored the possibility that ayahuasca could temporally disrupt inhibitory neural mechanisms thought to intervene in the normal filtering of information. They investigated the acute effects of ayahuasca on sensory and sensorimotor gating as measured by P50 suppression and prepulse inhibition of the startle reflex (PPI), respectively. Ayahuasca produced significant dose-dependent reductions of P50 suppression, but no significant effects were found on the startle response, its habituation rate, or on PPI at any of the prepulse-to-pulse intervals studied. These findings would indicate, at the doses tested, a decremental effect of ayahuasca on sensory gating, as measured by P50 suppression, and no distinct effects on sensorimotor gating, as measured by PPI.

Frecska et al. (2003) investigated whether ayahuasca had any effect on binocular rivalry and explored if these eventual actions of ayahuasca could shed some light into the

concept of interhemispheric fusion. During binocular rivalry, two incompatible images are presented to each eye and these monocular stimuli compete for perceptual dominance, with one pattern temporarily suppressed from awareness. Ten individuals who were participating in ayahuasca ceremonies were requested to volunteer for binocular rivalry tests in the absence of ayahuasca and after drinking the brew. One of the inclusion criteria for experimental subjects was to ingest ayahuasca volume of at least 50 ml at the beginning of the experimental session. The alkaloid concentrations were as follows: harmine 1.36 mg/ml, THH 1.05 mg/ml, and DMT 0.73 mg/ml. Volunteers performed the rivalry test between 90 and 150 min after ayahuasca ingestion and on a separate day without ayahuasca (comparison condition). According to the authors, ayahuasca led to a decrease of rivalry alternation rates, increased length of one percept and there was evidence of phenomenal fusion, which was interpreted as indicating interhemispheric fusion.

Frecska et al. (2004), working with the same individuals and a similar methodology of their previous study (Frecska et al., 2003), also investigated whether binocular rivalry could illuminate common mechanisms in schizophrenia and 5-HT_{2A} mediated "hallucinosis" induced by ayahuasca. One variant of stimulus presentation in binocular rivalry experiments is dichoptic stimulus alternation (DSA), where stimuli are applied to the eyes in rapid alterations. In contrast with comparison subjects, schizophrenic patients can maintain slow perceptual alternations even with very high DSA rates, which may reflect impairment in visual information processing. Subjects on ayahuasca were able to maintain much longer horizontal or vertical dominance periods than without it despite the fact that the stimuli were alternating at rates up to almost 2 orders of magnitude faster than their endogenous rivalry rate. This finding is similar to what was reported in schizophrenic patients, and is in line with other studies reporting similarities between "psychedelic experiences" and acute schizophrenia (see for a review Vollenweider, 2001; Vollenweider and Geyer, 2001; Geyer and Vollenweider, 2008; Geyer et al., 2009; González-Maeso and Sealfon, 2009a).

Nevertheless, while this study targeted some common mechanisms in schizophrenia and ayahuasca-induced "hallucinosis", according to current views, psychosis and ayahuasca inebriation are not equivalent conditions, and this argument is valid for other psychedelics too. Psychosis is neither voluntary nor wished for; it is a disorderly mental

state over which the subject has no control. The gradual and inexorable progression of a symptom complex that includes disturbed thought process, depersonalization and auditory hallucinations, evolving into a generalized functional incapacitation, is characteristic of schizophrenia (Grinspoon and Bakalar, 1981; Grob, 1998).

In contrast, Frecska et al. (2004) postulate that when ayahuasca is taken in a traditional-ritual manner, it induces a state of being characterized by enhanced internal order. Furthermore, the authors also report that experienced ayahuasca drinkers have significant control over what is happening to them under the effects of the brew (Shanon, 2002).

Riba et al. (2004) assessed the differential involvement of cortical brain regions in the electroencephalographic effects of acute ayahuasca administration by means of low-resolution electromagnetic tomography (LORETA). Electroencephalography recordings were obtained at regular time intervals after placebo and the low and high ayahuasca doses. As indicated above, topographic EEG analysis showed an overall reduction in absolute power in the classical frequency bands, more pronounced in the slow delta and theta bands, and an increase in the relative weight of the higher frequency beta bands (Riba et al., 2002a). It is important to note that, at the doses administered, ayahuasca did not induce full-blown psychotic symptoms and none of the participants lost awareness of the drug-induced nature of the psychological effects experienced.

The LORETA analysis showed that ayahuasca decreased power density predominantly over the temporo-parieto-occipital junction, temporomedial cortex and in frontomedial regions. These areas comprise the somatosensory, auditory and visual association cortices, the temporo-parietal association cortex, and also paralimbic structures, with relevant roles in emotion and memory processes (Riba et al., 2004). The authors hypothesized that the sensory association cortex may have played a role in the perceptual modifications associated with ayahuasca. Additionally, the temporo-parietal and frontomedial association cortex, and the cingulate and the temporomedial cortices could account for more complex cognitive modifications elicited by ayahuasca, because these areas play relevant roles in the neurobiology of attention, emotion and memory (Riba et al., 2004).

Barbanoj and co-workers (2008) investigated the effects of acute daytime ayahuasca administration on sleep parameters. These included subjectively perceived sleep quality, polysomnography (PSG), and spectral EEG analysis. In this study, eighteen male volunteers received a single oral dose of encapsulated freeze-dried ayahuasca equivalent to 1.0 mg DMT/kg body weight, placebo, or 20 mg *d*-amphetamine in a randomized double-blind clinical trial. Barbanoj et al. reported that ayahuasca did not induce any subjectively perceived deterioration of sleep quality or PSG-measured disruptions of sleep initiation or maintenance, in contrast with *d*-amphetamine, a positive control used in the study. PSG analysis also showed that similarly to *d*-amphetamine, ayahuasca inhibits REM sleep, decreasing its duration. Also, whereas slow-wave sleep (SWS) power was decreased by *d*-amphetamine, ayahuasca enhanced power in this frequency band.

4.3.8. Neuroimaging studies

Exploring the previous psychotropic profile of ayahuasca, which highlighted enhanced introspective attention, altered somatic perceptions and intense emotional modifications, frequently accompanied by visual imagery, and reporting that despite recent advances in the study of ayahuasca pharmacology the neural correlates of acute ayahuasca effects remain largely unknown, Riba et al. (2006) conducted a neuroimaging study in which changes in blood flow were investigated following acute ayahuasca administration. These changes were measured by means of single photon emission tomography (SPECT) following a 1.0 mg DMT/kg body weight dose of ayahuasca.

This dose led to significant activation of frontal and paralimbic brain regions. Increased blood perfusion was observed bilaterally in the anterior insula/inferior frontal gyrus, with greater intensity in the right hemisphere, and in the anterior cingulate/frontomedial cortex of the right hemisphere, areas previously implicated in somatic awareness, subjective feeling states, and emotional arousal. A smaller cluster was found in the ventral anterior cingulate/subcallosal gyrus. Additional increases were observed in the left amygdala/parahippocampal gyrus, a structure also involved in emotional arousal. No significant decreases in regional cerebral blood flow were observed anywhere in the brain (Riba et al., 2006). According to Riba et al. (2006), bilateral insular

and cingulate activation are related to interoceptive attention, with the right anterior insula specifically subserving explicit awareness of bodily processes. The authors concluded that ayahuasca interacts with neural systems that are central to interoception and emotional processing, pointing to a modulatory role of serotonergic neurotransmission in these processes.

Recently, other neuroimaging experiments were performed involving healthy volunteers (Pinto et al., 2008; Prado et al., 2009; Pinto, 2010). In one of these studies, cerebral blood flow (CBF) changes were investigated using SPECT in 10 volunteers naïve to psychedelic use. Experiments were done in two sessions, at least one week apart, with patients at rest. In one of the sessions, volunteers were given two 100 ml doses of ayahuasca, the first one 30 min after arrival at the laboratory and the second one 45 min after the first. In the other study, 10 regular ayahuasca users underwent functional magnetic resonance imaging (fMRI) scans during the performance of a verbal fluency task. The researchers administered the test twice in the same day, and administered 150 ml of ayahuasca in one of the sessions. The ayahuasca used was analyzed and presented 0.18 mg/ml harmine and 0.08 mg/ml DMT (harmaline levels were not detected). According to the authors, behavioral changes were similar in the two experiments, with perceptual and thought alterations, and mood/affect elevation, with no loss of contact with reality. SPECT showed activation of the frontal and temporal cortex and limbic areas (parahippocampal gyrus/cingulate/frontomedial cortex). CBF was found to be decreased in a region of the right cerebellar hemisphere and in temporal and parietal lobe areas. fMRI data – independently from the task – showed activation of the same areas as reported in the SPECT study. Task-dependent fMRI data showed decreased activation of areas involved in language processing (Broca's area/regions of the frontal, temporal, occipital and parietal lobes/limbic areas/thalamus), and also activation in other areas (cingulate/temporal lobe).

Finally, a recent study using fMRI explored the neural basis of the visual imagery produced by ayahuasca (de Araújo et al., 2011). Nine frequent ayahuasca users participated in the study using an imagery task, where each subject drank 120-200 ml of ayahuasca (2.2 ml/kg of body weight; 0.8 mg/ml DMT and 0.21 mg/ml harmine). Ayahuasca produced increases in the activation of several occipital, temporal and frontal areas. In the primary visual area, the effect was comparable in magnitude to the activation levels of natural

image with the eyes open, and was specifically correlated with the occurrence of individual perceptual changes measured by psychiatric scales. The activity of ayahuasca was shown in areas involved in episodic and working memory, the processing of contextual associations, intentional prospective imagination, and the processing of information from internal sources.

4.3.9. Tolerance and sensitization

There are currently no data in the scientific literature regarding the possible occurrence of tolerance or sensitization after repeated ayahuasca administration.

HYPOTHESES

By the time when the present investigation was conceived, all data available on the human pharmacology of ayahuasca had been obtained in single-dose administration studies. These studies were either uncontrolled or placebo-controlled, but had never included active comparators. Autonomic and neuroendocrine data were available from an uncontrolled open-label study only. Acute effects of ayahuasca on the immune system had not been assessed. Finally, the pharmacology of ayahuasca and the potential occurrence of tolerance or sensitization following two repeated doses had not been evaluated.

Based on the knowledge of the pharmacology of DMT and ayahuasca at the time, the following hypotheses were postulated:

Study 1

a) In addition to its characteristic psychedelic effects, ayahuasca will induce general activation effects on the CNS analogous to those by a prototypical psychostimulant (*d*-amphetamine). This shared stimulatory pattern will be evidenced by self-report subjective rating scales.

b) Despite commonalities at the subjective level, given the different mechanism of action, the administration of ayahuasca will lead to a different pattern of neurophysiological (electroencephalographic) effects when compared with *d*-amphetamine. A relative power increase is postulated for ayahuasca in the EEG beta band.

c) The administration of ayahuasca will induce sympathomimetic effects analogous to those induced by *d*-amphetamine. This will be evidenced by analogous changes in autonomic measures.

d) Given the serotonergic mechanism of ayahuasca, the neuroendocrine response after ayahuasca will include elevations in prolactin and growth hormone, absent after *d*-amphetamine. Both ayahuasca and *d*-amphetamine will enhance cortisol secretion.

e) An unspecific modulation of cell immunity caused by sympathomimetic activation and cortisol secretion will lead to a redistribution of lymphocyte subpopulations that will be

similar between ayahuasca and *d*-amphetamine. A potentially specific effect of ayahuasca on the cell immune response will be evidenced by differential variations in lymphocyte subpopulations between ayahuasca and *d*-amphetamine.

Study 2

a) Tolerability of ayahuasca will be poorer after the second of two consecutive doses. This will be evidenced by a higher incidence of undesirable effects as compared to a single dose.

b) The administration of two consecutive doses of ayahuasca will lead to a disproportionate (non-linear) increase in plasma DMT concentrations after the second dose as compared to a single dose.

c) The administration of two consecutive doses of ayahuasca will lead to increased subjective and neurophysiological effects and to equal or lower cardiovascular, autonomic, neuroendocrine and immunomodulatory effects after the second dose as compared to a single dose.

d) The study of DMT plasma concentrations after the second dose will reveal a linear relationship between DMT levels and the intensity of subjective and neurophysiological effects. Neither tolerance nor sensitization are postulated for these variables.

e) The study of DMT plasma concentrations after the second dose will reveal a non-linear relationship between the intensity of cardiovascular, autonomic, neuroendocrine and immunomodulatory effects and DMT. The occurrence of tolerance is postulated for these variables.

AIMS OF THE STUDY

The present investigation aimed at expanding our knowledge of the pharmacology of ayahuasca in humans. The acute impact of ayahuasca in terms of psychological and physiological modifications was studied: a) in relation to another centrally active drug (the sympathomimetic psychostimulant *d*-amphetamine); and b) following two repeated doses.

The specific main objectives were:

Study 1

a) Study the profile of subjective effects induced by a single dose of ayahuasca in comparison to that by *d*-amphetamine, a sympathomimetic psychostimulant.

b) Assess the pattern of neurophysiological effects induced by ayahuasca as compared with that by *d*-amphetamine. Specifically measure drug-induced changes in relative power in the beta band of the electroencephalogram.

c) Assess the effects of ayahuasca on autonomic variables and establish to what extend these correspond to those after *d*-amphetamine, the sympathomimetic drug used as active comparator.

d) Measure the neuroendocrine response after ayahuasca as compared to that after *d*-amphetamine. Specifically measure the levels of prolactin, growth hormone and cortisol.

e) Assess the changes induced by ayahuasca in the distribution of lymphocyte subpopulations as compared with that induced by *d*-amphetamine.

Study 2

a) Assess the tolerability of ayahuasca when administered in two consecutive doses as compared to a single dose.

b) Assess the intensity of subjective, neurophysiological, cardiovascular, autonomic, neuroendocrine and immunomodulatory effects after the second of two consecutive doses as compared to a single dose.

c) Determine whether a linear or non-linear relationship exists between the intensity of subjective, neurophysiological, cardiovascular, autonomic, neuroendocrine and immunomodulatory effects and DMT plasma levels and assess the occurrence of tolerance or sensitization to these effects.

SUMMARY OF THE EXPERIMENTAL DESIGN

This thesis consists of two independent clinical studies. Study one involved the administration of a single ayahuasca dose, a placebo and an active comparator (*d*-amphetamine). Study two involved the administration of two repeated doses of ayahuasca. Below is the summary of the experimental design of each study.

Study 1

Participants: 10 male volunteers with prior experience in the use of psychedelics.

Design: Double-blind, randomized, crossover, controlled with placebo (lactose) and an active comparator (*d*-amphetamine). The doses used were 1.0 mg DMT/kg body weight ayahuasca and 20 mg *d*-amphetamine.

Variables: HRS, ARCI.
EEG.
Body temperature, pupillary diameter, pupillary light reflex, respiration rate. Prolactin, cortisol, growth hormone.
CD8 T cells, CD4 T cells, CD3 T cells, CD19 B cells and natural killer (NK) cells.
Pharmacokinetics.

Results in: ***Autonomic, neuroendocrine and immunological effects of Ayahuasca. A comparative study with d-amphetamine.***

Study 2

Participants: 17 male volunteers with prior experience in the use of psychedelics.

Design: Double-blind, randomized, crossover, placebo-controlled. The following treatment combinations were administered: (a) a lactose placebo and then, 4 h later, an ayahuasca dose; and (b) two ayahuasca doses 4 h apart. Ayahuasca doses were 0.75 mg DMT/kg body weight.

Variables: VAS, HRS, ARCI.
EEG.
SBP, DBP, HR.
Body temperature, pupillary diameter.
Prolactin, cortisol, growth hormone.
CD8 T cells, CD4 T cells, CD3 T cells, CD19 B cells and natural killer (NK) cells.
Pharmacokinetics.

Results in: ***Pharmacology of ayahuasca administered in two repeated doses.***

Abbreviations

VAS: Visual Analogue Scales

ARCI: Addiction Research Center Inventory

HRS: Hallucinogen Rating Scale

EEG: Electroencephalography

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

HR: Heart rate

RESULTS

Original publications

Autonomic, neuroendocrine and immunological effects of ayahuasca. A comparative study with d-amphetamine.

J Clin Psychopharmacol 2011; 31:717-726.

ORIGINAL CONTRIBUTION

Autonomic, Neuroendocrine, and Immunological Effects of Ayahuasca

A Comparative Study With D-Amphetamine

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Abstract: Ayahuasca is an Amazonian psychotropic plant tea combining the 5-HT_{2A} agonist *N*,*N*-dimethyltryptamine (DMT) and monoamine oxidase–inhibiting β-carboline alkaloids that render DMT orally active. The tea, obtained from *Banisteriopsis caapi* and *Psychotria viridis*, has traditionally been used for religious, ritual, and medicinal purposes by the indigenous peoples of the region. More recently, the syncretistic religious use of ayahuasca has expanded to the United States and Europe. Here we conducted a double-blind randomized crossover clinical trial to investigate the physiological impact of ayahuasca in terms of autonomic, neuroendocrine, and immunomodulatory effects. An oral dose of encapsulated freeze-dried ayahuasca (1.0 mg DMT/kg body weight) was compared versus a placebo and versus a positive control (20 mg D-amphetamine) in a group of 10 healthy volunteers. Ayahuasca led to measurable DMT plasma levels and distinct subjective and neurophysiological effects that were absent after amphetamine. Both drugs increased pupillary diameter, with ayahuasca showing milder effects. Prolactin levels were significantly increased by ayahuasca but not by amphetamine, and cortisol was increased by both, with ayahuasca leading to the higher peak values. Ayahuasca and amphetamine induced similar time-dependent modifications in lymphocyte subpopulations. Percent CD4 and CD3 were decreased, whereas natural killer cells were increased. Maximum changes occurred around 2 hours, returning to baseline levels at 24 hours. In conclusion, ayahuasca displayed moderate sympathomimetic effects, significant neuroendocrine stimulation, and a time-dependent modulatory effect on cell-mediated immunity. Future studies on the health impact of long-term ayahuasca consumption should consider the assessment of immunological status in regular users.

Key Words: ayahuasca, autonomic, neuroendocrine, immunity

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Ayahuasca is a psychoactive beverage consumed throughout the Amazon Basin as the aqueous infusion of *Banisteriopsis caapi* and *Psychotria viridis*, 2 plants endemic to the region.[1,2] The tea is a central element of Amazonian shamanism, being used for magico-religious, ceremonial, and medicinal purposes.[2] Chemical analyses have shown that *P. viridis* contains the orally labile serotonergic agonist and monoamine oxidase (MAO) substrate *N*,*N*-dimethyltryptamine (DMT), whereas *B. caapi* contains several alkaloids with β-carboline structure (harmine, harmaline, and tetrahydroharmine) showing MAO inhibiting properties.[3] The β-carbolines present in ayahuasca reversibly block visceral MAO-A,[4,5] allowing the access of DMT to systemic circulation and the central nervous system (CNS). At the molecular level, DMT binds at 5-HT_{2A}, 5-HT_{1A}, and 5-HT_{2C} receptor sites,[6–8] eliciting psychedelic effects in humans.[9,10]

In recent years, ayahuasca use has spread from the indigenous to the general population not only in the Amazon but also to countries around the world. A relevant factor in this expansion is the beverage's use in syncretic religions. Several religious groups originating from Brazil and using ayahuasca as a sacrament in a ceremonial context have expanded their activities to Europe and North America. These groups typically ingest ayahuasca over extended periods on a bimonthly basis.[11,12] Based on the traditional uses of ayahuasca and both anecdotal and empirical data on potential health benefits derived from ayahuasca use, some authors have proposed its therapeutic use, especially in the field of drug addiction.[13] Two studies among Brazilian regular users of ayahuasca have found a decrease in illicit drug consumption after initiation of regular ayahuasca use.[14,15] However, despite its potential health benefits, data on the impact of ayahuasca on human physiology are still limited and warrant further investigation.

Previous clinical research has shown that ayahuasca displays distinct physiological and psychotropic effects. At doses equivalent to 0.6 to 0.8 mg DMT/kg body weight, ayahuasca leads to moderate increases in cardiovascular measures, with only diastolic blood pressure reaching statistical significance.[10] The effects of the drug on the CNS have been demonstrated by subjective effects measures and neurophysiological recordings. The analysis of self-report questionnaires has shown significant increases in scales measuring psychostimulant-like subjective activation and modifications of perception and thought processes.[10,16] Electroencephalographic (EEG) effects include an increase in relative power in the faster β band.[17] Central nervous system effects after ayahuasca administration have also been demonstrated by means of neuroimaging techniques (single-photon emission computed tomography)[18] and sleep recordings.[19] The time course of subjective effects and EEG measures runs parallel to DMT concentrations in blood.[3] Increases in diastolic blood pressure and normetanephrine excretion[10] suggest that ayahuasca exerts general sympathomimetic effects, together with

more specific serotonergic effects such as the aforementioned increases in EEG relative β[17] and rapid eye movement sleep suppression.[19]

To date, no controlled study has assessed the impact of acute ayahuasca on neuroendocrine measures and the immune function. In a noncontrolled study, researchers found increases in cortisol levels above preadministration values following a single ayahuasca dose.[20] Cortisol release is known to have an impact on cell immunity, leading to lymphocyte redistribution.[21] Lymphocytes are the main cellular components of the immune system. Lymphocyte deficiencies may predispose to infectious diseases. Some viral infections, such as that caused by the human immunodeficiency virus, may cause sustained and marked decreases in some T-cell subpopulations (CD4 lymphopenia). Substance use, such as alcohol intake and cigarette smoking, has shown detrimental effects on lymphocyte subpopulations.[22,23] Regarding psychedelics, a recent study found decreases in CD8 lymphocytes following the administration of 4-iodo-2, 5-dimethoxyphenyl-isopropylamine (DOI, a 5-HT_{2A} receptor agonist) to mice. This effect was antagonized by the 5-HT_{2A} antagonist ketanserin.[24]

In view of the expanding use of ayahuasca worldwide, in the present study we aimed to explore (*a*) the physiological impact of acute ayahuasca administration in terms of autonomic and neuroendocrine effects and (*b*) the potential effects of ayahuasca on cell-mediated immunity. As described below, autonomic variables, hormone levels, and distribution of lymphocyte subpopulations were evaluated. Cortisol was assessed for its direct role in lymphocyte regulation,[21] and prolactin and growth hormone (GH) were selected as measures of serotonergic stimulation.[25,26] Cell-mediated immunity changes were analyzed assessing the most relevant lymphocyte subpopulations: T lymphocytes (CD3, CD4, CD8), natural killer (NK) cells, and B lymphocytes (CD19). In addition, to verify alkaloid absorption and CNS effects, we also measured DMT plasma levels, subjective effects, and relative EEG β power. To gain greater insight into the specificity or generality of ayahuasca effects, D-amphetamine, a standard sympathomimetic drug, was used as an active comparator.

MATERIALS AND METHODS

Volunteers

Ten young healthy male volunteers were recruited. Mean age was 29.0 years (range, 20–38 years); mean weight was 67.0 kg (range, 60–85 kg); and mean height was 1.77 m (range, 1.69–1.96 m). Volunteers underwent a structured psychiatric interview (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*). Exclusion criteria included presence or history of Axis I disorders and alcohol or other substance dependence. Eligibility criteria included prior use of psychedelics on at least 10 occasions without sequelae derived thereof, that is, psychedelic-related disorders as described in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*. A medical examination and laboratory tests were performed before study initiation to rule out any medical condition, allergies, and intolerances.

Participants had used psychedelics from 10 to 100 times. The most commonly used psychedelics were psilocybian mushrooms (10/10) and lysergic acid diethylamide (LSD) (9/10). Less commonly used were ketamine (5/10), peyote (4/10), and mescaline (1/10). None of the participants had used ayahuasca. Besides psychedelics, volunteers had consumed cannabis (10/10), cocaine (10/10), 3,4-methylenedioxymethamphetamine (MDMA) (8/10), and amphetamine (9/10). They reported moderate consumption of alcohol (7 drinks per week), cigarettes (fewer than 10 per day), and caffeinated drinks (<3 per day). Volunteers were in good health, confirmed by medical history, laboratory tests, and electrocardiogram. Prestudy examinations also included drug screening and serological testing (for hepatitis B and C and human immunodeficiency virus). The study was conducted in accordance with the Declarations of Helsinki and Tokyo concerning experimentation on humans and was approved by the hospital's ethics committee and the Spanish Ministry of Health. The volunteers received detailed information on the nature of ayahuasca, the general psychological effects of psychedelics, and their possible adverse effects, as reported in the psychiatric literature. All volunteers gave their written informed consent to participate.

Drugs

The administered drugs were a placebo (lactose), 20 mg D-amphetamine, and a freeze-dried encapsulated formulation of ayahuasca equivalent to 1 mg DMT/kg body weight. The freeze-dried material was obtained from a Brazilian batch of ayahuasca and contained 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline, and 11.36 mg tetrahydroharmine per gram. The ayahuasca dose administered was chosen based on an earlier work in which it had been proven to elicit full-blown psychotropic effects.[16] The administration of the placebo and the 2 active treatments in capsules allowed for the adequate masking of drug taste.

Study Design

The study was conducted according to a randomized, double-blind, placebo-controlled, crossover design and involved the participation on 3 experimental sessions at least 1 week apart. Volunteers were requested to abstain from any medication or illicit drug use in the 2 weeks before the experimental sessions and until study completion. Volunteers also abstained from alcohol, tobacco, and caffeinated drinks in the 24 hours before each experimental day. Urinalysis for illicit drug use was performed for each experimental session. On each experimental day, volunteers had a light breakfast before 10:00 AM, and at noon, they received capsules containing 1 of the 3 treatments. During measurements, the volunteers remained seated in a comfortable reclining chair in a quiet dimly lit room. All volunteers remained overnight in the laboratory and were discharged at noon of the following day.

Study Methods

Subjective Effects Measures

Subjective effects were measured by means of 2 self-report questionnaires: the Hallucinogen Rating Scale (HRS) and the Addiction Research Center Inventory (ARCI).

The HRS[9] measures psychedelic-induced subjective effects and includes 6 scales: *somaesthesia*, reflecting somatic effects; *affect*, sensitive to emotional and affective responses; *volition*, indicating the volunteer's capacity to willfully interact with his/her "self" and/or the environment; *cognition*, describing modifications in thought processes or content; *perception*, measuring visual, auditory, gustatory, and olfactory experiences; and finally *intensity*, which reflects the strength of the overall experience. In the present study, a Spanish adaptation of the questionnaire was used.[27] The range of scores for all HRS scales is 0 to 4.

The short version of the ARCI[28] consists of 5 scales or groups: MBG, morphine-benzedrine group, measuring euphoria and positive mood; PCAG, pentobarbital-chlorpromazine-alcohol group, measuring sedation; LSD scale, measuring

somatic-dysphoric effects; BG, the benzedrine group, measuring intellectual energy and efficiency; and the A scale, an empirically derived scale measuring amphetamine-like effects. The range of scores is 0 to 16 for MBG, −4 to 11 for PCAG, −4 to 10 for LSD, −4 to 9 for BG, and 0 to 11 for A. The questionnaire had been translated into Spanish and validated by Lamas and coworkers.[29] Volunteers answered the ARCI immediately before drug administration and 4 hours after drug intake, whereas the HRS was answered only at 4 hours after administration.

EEG Measures

Spontaneous brain electrical activity was recorded, preprocessed, and quantified following standard procedures as previously described.[17] In brief, recordings were obtained at 19 scalp locations according to the international 10/20 system by means of a Neuroscan SYNAMPS amplifier. Three-minute EEGs with eyes closed were obtained at 0 (baseline) and 30 minutes and at 1, 1.5, 2, and 2.5 hours after administration. The EEG signal was recorded using high-pass and low-pass filters of 0.3 and 30 Hz, respectively, and digitized online with a sampling frequency of 100 Hz. Following ocular artifact rejection and correction steps, spectral analysis was performed using the fast Fourier transform. The target variable: relative power (expressed as percentage) in the β (13–30 Hz) frequency band was calculated from the spectral density curves at each electrode and time point. An average of relative β power in all 19 leads was used in the subsequent statistical analysis.

Autonomic Measures

Body temperature (°C) was measured by means of a mercury thermometer placed in the participant's armpit. Measurements were conducted at −15 (baseline 1), 0 (baseline 2), and 30 minutes and at 1, 1.5, 2, 4.5, 6, 8, and 10 hours after administration.

Pupillary diameter and pupillary light reflex (PLR) were determined using a Compact Integrated Pupillometer (AMTech GmbH, Weinheim, Germany), tested in darkness after a 5-minute dark adaptation period. Participants were instructed to fix their gaze on a target point located on the wall of the examination room at a distance of about 3 m to prevent pupillary near response or accommodation adjustments. Pupillary light reflexes were elicited by standardized light stimuli (whole-field stimulation) from a light-emitting diode with a duration of 200 milliseconds and using 3 increasing intensities (2.35×10^3, 4.7×10^3, and 9.4×10^3 cd/m^2), stimulus intensity being measured at the source. Changes in pupillary diameter were recorded for 2 seconds with a sampling rate of 250 Hz and stored on a personal computer.[30]

Three consecutive measures were made for each level of intensity, and the mean value was obtained. The different intensities were administered at 2-minute intervals. The target variables were as follows: initial pupillary diameter (in millimeters) and latency (in milliseconds) and amplitude (in millimeters) of the miotic light reflex response. The initial pupillary diameter is the diameter obtained just before light stimulation. The latency period is the interval between the light stimulation and the onset of the pupil contraction. The light reflex amplitude was determined as the difference between the initial and the minimum pupillary diameter after light stimulation.[31] The initial pupillary diameter reflects the sympathetic/parasympathetic balance, whereas latency and light reflex amplitude are parameters reflecting parasympathetic pupillary modulation.[31,32] Measurements were conducted at 0 (baseline) and 30 minutes and at 1, 2, 4, 6, 8, 10, and 24 hours after administration.

Respiration rate (in breaths per minute) was measured by means of a respiratory band placed around the participant's chest. The respiratory signal was digitized and recorded on a computer and later analyzed offline. The number of respiratory events in 1 minute was counted at each recording time point. Measurements were conducted at −15 (baseline 1), 0 (baseline 2), and 30 minutes and at 1, 1.5, 2, and 2.5 hours after administration.

Neuroendocrine Measures

Blood samples (3 mL, plain tubes without clot activator) were drawn at −40 (baseline 1), −10 (baseline 2), and 30 minutes and at 1, 1.5, 2, 4.5, and 6 hours after administration and were allowed to stand at room temperature. Serum was separated by centrifugation and aliquots stored for the analysis of GH, prolactin, and cortisol.

Serum GH and prolactin concentrations were determined by a chemiluminescence immunoassay system (Immulite 2000; Diagnostic Products Corp, EURO/Diagnostic Products Corporation, Llanberis, UK). The GH immunoassay, with a sensitivity of 0.06 mIU/L, uses the WHO first IRP 80/505 and shows intra-assay and interassay coefficients of variation (CVs) of 5.3% to 6.1% and 5.7% to 6.5%, respectively. The prolactin immunoassay uses the third IS 84/500, with an analytical sensitivity of 3.4 mIU/L and intra-assay and total CV between 2.2% to 2.3% and 6.9% to 7.9%, respectively. Serum cortisol concentrations were measured by electrochemiluminescent immunoassay (Elecsys Modular Analytics E170; Roche Diagnostics GmbH Manheim, Germany) with functional sensitivity of less than 8 nmol/L and intra-assay and total CVs of 1.7% and 2.8%, respectively, for mean human serum concentrations between 129 and 717 nmol/L.

Obtained values were transformed to nanograms per milliliter (prolactin and GH) and micrograms per deciliter (cortisol).

Lymphocyte Subpopulations

Blood samples (3 mL, heparin tubes) were drawn at baseline and at 1.5, 2, 4.5, and 24 hours after administration and were subjected to lymphocyte immunophenotyping. The following lymphocyte subpopulations were quantified: CD8 T, CD4 T, CD3 T, CD19 B, and NK cells.

For lymphocyte immunophenotyping, blood samples were stained with the Lymphogram (Cytognos, Salamanca, Spain) reagent kit; each tube contains 5 different murine MoAbs with 3 fluorochromes: CD8 and CD19 with fluorescein isothiocyanate, CD3 and CD56 with phycoerythrin. CD4 were labeled in tandem with PE and cyanate 5. The procedure has been detailed elsewhere.[33] Lymphocyte subpopulations were expressed as percentage of all blood cells.

DMT Plasma Levels

Blood samples (10 mL, EDTA tubes) were drawn at −10 (baseline) and 30 minutes and at 1, 1.5, 2, 2.5, 4.5, 6, and 10 hours after administration for analysis of DMT. Samples were centrifuged at 2000 revolutions per minute for 10 minutes at 4°C, and plasma was immediately frozen at −20°C. Frozen plasma samples were stored at −80°C until analysis. *N,N*-dimethyltryptamine was quantified by the method described by McIlhenny and coworkers,[34] which uses high-pressure liquid chromatography with electrospray ionization and tandem mass spectrometry. The method was adapted to quantify DMT in a plasma matrix using a protein precipitation/dilution protocol.

Protein precipitation 96-well plates (Thermo Scientific, Waltham, Mass) were used to prepare the samples. Analyses were conducted using a Thermo Open Autosampler and a Thermo Accela pumping system interfaced to a Thermo Velos linear ion trap-ion trap system with a heated electrospray ionization probe and operated in the positive ion mode as described.[35] The observed maximum concentration in plasma (C_{max}) and the time to reach this concentration (t_{max}) were determined for each individual.

Statistical Analyses

Subjective Effect measures

Before statistical analysis, ARCI scores were transformed to differences from preadministration values. The transformed ARCI scores and the raw HRS scores were analyzed using a 1-way repeated-measures analysis of variance (ANOVA) with drug (placebo, ayahuasca, amphetamine) as factor. When a significant effect was observed, pairwise comparisons were performed by means of Student *t* test.

EEG, Autonomic, Neuroendocrine, and Lymphocyte Measures

Preadministration values were subtracted from postadministration measures. Subsequently, we calculated peak variations (the maximum absolute change from baseline values). The obtained values were analyzed using a 1-way repeated-measures ANOVA with drug (placebo, ayahuasca, amphetamine) as factor. When a significant effect was observed, pairwise comparisons were performed by means of Student *t* test. In addition, a 2-way repeated-measures ANOVA was conducted, with drug (placebo, ayahuasca, amphetamine) and time point as factors to study the time course of effects. When this ANOVA yielded a significant drug or drug-by-time interaction, individual repeated-measures ANOVAs with drug as factor were performed at each postadministration time point followed by pairwise comparisons using Student *t* test.

DMT Plasma Levels

Descriptive statistics were used to report the characteristics of the time course of plasma DMT concentration. Maximum concentration (C_{max}) and time taken to reach the maximum concentration (t_{max}) were calculated and are reported as mean (SD) and as median and range, respectively.

In all tests performed, differences were considered statistically significant for $P < 0.05$. However, given the exploratory nature of the present study with regard to autonomic, neuroendocrine, and lymphocyte measures, pairwise comparisons between treatments are also reported in those cases where the main ANOVA yielded a $P < 0.1$.

RESULTS

Subjective Effects

Subjective effects results are shown in Table 1.

Compared with placebo, the administration of ayahuasca led to significant increases in all scales of the HRS and in the A, MBG, and LSD of the ARCI. On the other hand, differences from placebo were found for amphetamine in the affect, cognition, and intensity scales of the HRS and in the amphetamine scale of the ARCI. When the 2 active treatments were compared, ayahuasca led to significantly higher scores in the perception, cognition, volition, and intensity scales of the HRS. Regarding the ARCI, the comparison between active treatments found statistically significant differences in the BG scale, with amphetamine leading to increases and ayahuasca to decreases; in the PCAG scale where amphetamine showed decreases and ayahuasca increases; and finally in the LSD scale. Here both treatments led to increases that were much larger after ayahuasca.

EEG Effects

Treatment effects on relative β power are shown in Table 2 and Figure 1. As shown therein, ayahuasca induced significant increases in this variable, which was not modified by

TABLE 1. Subjective Effects Induced by Placebo, D-Amphetamine 20 mg, and Ayahuasca 1 mg DMT/kg

					Pairwise Comparisons		
	Placebo	D-Amphetamine	Ayahuasca	GLM	PLA:AMP	PLA:AYA	AYA:AMP
HRS							
Somaesthesia	0.07 (0.22)	0.5 (0.60)	1.23 (0.70)	<0.01	NS	*	NS
Affect	0.28 (0.09)	0.79 (0.52)	1.36 (0.69)	<0.01	†	*	NS
Perception	0.03 (0.09)	0.24 (0.45)	1.46 (0.97)	<0.001	NS	*	*
Cognition	0.01 (0.03)	0.49 (0.062)	1.58 (1.16)	<0.01	†	*	†
Volition	0.87 (0.74)	0.86 (0.43)	1.84 (0.75)	<0.01	NS	†	*
Intensity	0.00 (0.00)	1.10 (0.85)	2.23 (1.10)	<0.001	*	‡	†
ARCI							
A	0.30 (0.67)	2.20 (2.25)	3.30 (2.67)	<0.01	†	*	NS
BG	0.70 (0.82)	2.90 (2.85)	−0.70 (3.34)	<0.01	NS	NS	*
MBG	−0.10 (0.99)	2.20 (3.26)	3.10 (4.48)	0.069	NS	†	NS
PCAG	−1.60 (3.50)	−3.9 (3.38)	0.30 (5.38)	0.039	NS	NS	†
LSD scale	0.40 (1.65)	1.10 (1.52)	4.20 (2.25)	<0.01	NS	*	*

Values are mean (SD) of the scores obtained for the HRS and ARCI questionnaires subscales (n = 10) and results of the statistical analysis performed.

A indicates Amphetamine; AMP, D-amphetamine; AYA, ayahuasca; BG, benzedrine group; GLM, general linear model; MBG, morphine-benzedrine group; NS, not statistically significant; PCAG, pentobarbital-chlorpromazine-alcohol group; PLA, placebo.

*$P < 0.01$.

†$P < 0.05$.

‡$P < 0.001$.

amphetamine or placebo. The analysis of the time course of effects showed that ayahuasca-induced increases were significant, relative to placebo at 1.5 and 2 hours after dosing. In addition, ayahuasca was different from amphetamine at 1.5 hours.

Autonomic Measures

Autonomic effects results are shown in Table 2 and Figure 1.

Body Temperature

After placebo administration, body temperature showed a steady increase throughout the day. After amphetamine and ayahuasca administration, however, a biphasic pattern was observed; an initial decrease between 0 and 1 hour was followed by a gradual increase thereafter. This increase was larger for amphetamine. The overall analysis did not find any significant modification in peak values after either of the active treatments. However, the analysis of the time course of effects showed a significant decrease for ayahuasca as compared with placebo at 30 minutes after dosing. A significant increase relative to placebo was observed for amphetamine at 2 hours after administration.

Pupillometry

As shown in Figure 1, mean pupillary diameter values before light stimulation were larger for amphetamine and ayahuasca than for placebo. The overall statistical analysis showed a trend increase in peak values. Pairwise comparisons showed significant increases for amphetamine and no overall effect for ayahuasca. The analysis of the time course of effects showed significant elevations relative to placebo for amphetamine from 1 hour onward. Values remained significantly elevated at 24 hours at which time point they also differed significantly from those obtained after ayahuasca. This latter treatment significantly increased pupillary diameter relative to placebo between 0.5 and 2 hours after dosing.

Mean amplitude of the PLR was reduced to varying degrees by the 2 active treatments. The overall statistical analysis showed a trend effect in the general ANOVA for peak values. Pairwise comparisons showed a significant decrease after ayahuasca relative to placebo and no significant effect after amphetamine. The analysis of the time course of effects showed a significant decrease relative to placebo for ayahuasca at 30 minutes and a significant decrease relative to amphetamine at 2 hours.

Opposed patterns were seen between ayahuasca and amphetamine for pupillary reflex latency. Whereas placebo-like variations were observed for amphetamine, ayahuasca increased the mean values. Again, the overall ANOVA only showed a trend to significance. Pairwise comparisons showed trend increases relative to placebo for ayahuasca and amphetamine. Analysis of the time course of effects showed significant increases for ayahuasca relative to placebo and amphetamine at 2 hours.

TABLE 2. Effects Induced by Placebo, D-Amphetamine 20 mg, and Ayahuasca 1 mg DMT/kg on Peak Values for EEG and Autonomic Measures, Neuroendocrine Parameters, and Lymphocyte Subpopulations

					Pairwise Comparisons		
	Placebo	D-Amphetamine	Ayahuasca	GLM	PLA:AMP	PLA:AYA	AYA:AMP
EEG measures							
EEG relative β power	−1.41 (4.96)	−2.05 (4.46)	8.89 (10.56)	<0.01	NS	*	†
Autonomic measures							
Body temperature	0.33 (0.16)	0.49 (0.28)	0.43 (0.28)	>0.1	—	—	—
Pupillary diameter	0.58 (1.11)	1.66 (1.13)	1.50 (1.02)	0.053	†	NS	NS
PLR amplitude	0 (0.31)	−0.07 (0.26)	−0.33 (0.23)	0.065	NS	†	0.074
PLR latency	−3.5 (15.64)	−4.3 (13.67)	11.8 (17.63)	0.068	NS	0.093	0.051
Respiration rate	0.1 (2.86)	−0.45 (4.75)	0.05 (3.20)	>0.1	—	—	—
Hormones							
Prolactin	3.86 (5.07)	1.72 (5.06)	15.53 (12.03)	<0.01	NS	*	†
Cortisol	−5.28 (4.68)	5.31 (6.42)	11.64 (7.39)	<0.001	*	‡	0.070
GH	5.41 (4.74)	6.30 (9.34)	14.06 (15.25)	>0.1	—	—	—
Lymphocyte subpopulations							
Total lymphocytes	−2.40 (7.66)	−2.80 (7.10)	−4.40 (14.39)	>0.1	—	—	—
CD3	0.30 (6.22)	−7.30 (2.67)	−9.10 (6.61)	0.009	†	†	NS
CD4	−2.60 (6.87)	−8.90 (3.45)	−10.50 (6.52)	0.008	†	†	NS
CD8	−0.10 (3.84)	−1.00 (4.57)	−2.70 (4.08)	>0.1	—	—	—
CD19	−0.89 (4.01)	1.89 (6.51)	−1.44 (6.27)	>0.1	—	—	—
NK cells	1.70 (5.25)	7.70 (3.37)	11.60 (10.06)	0.014	†	†	NS

Mean (SD) of the scores obtained (n = 10), and results of the statistical analysis performed. Peak relative β power expressed as percentage; peak body temperature in °C; peak pupillary diameter in mm; peak PLR amplitude in mm; peak PLR latency in milliseconds; peak respiration rate in breaths/min; peak prolactin and peak GH in ng/mL; peak cortisol in μg/dL; peak lymphocyte subpopulations expressed as percentage.

*$P < 0.01$.

†$P < 0.05$.

‡$P < 0.001$.

AMP indicates D-amphetamine; AYA, ayahuasca; NS, not statistically significant; PLA, placebo.

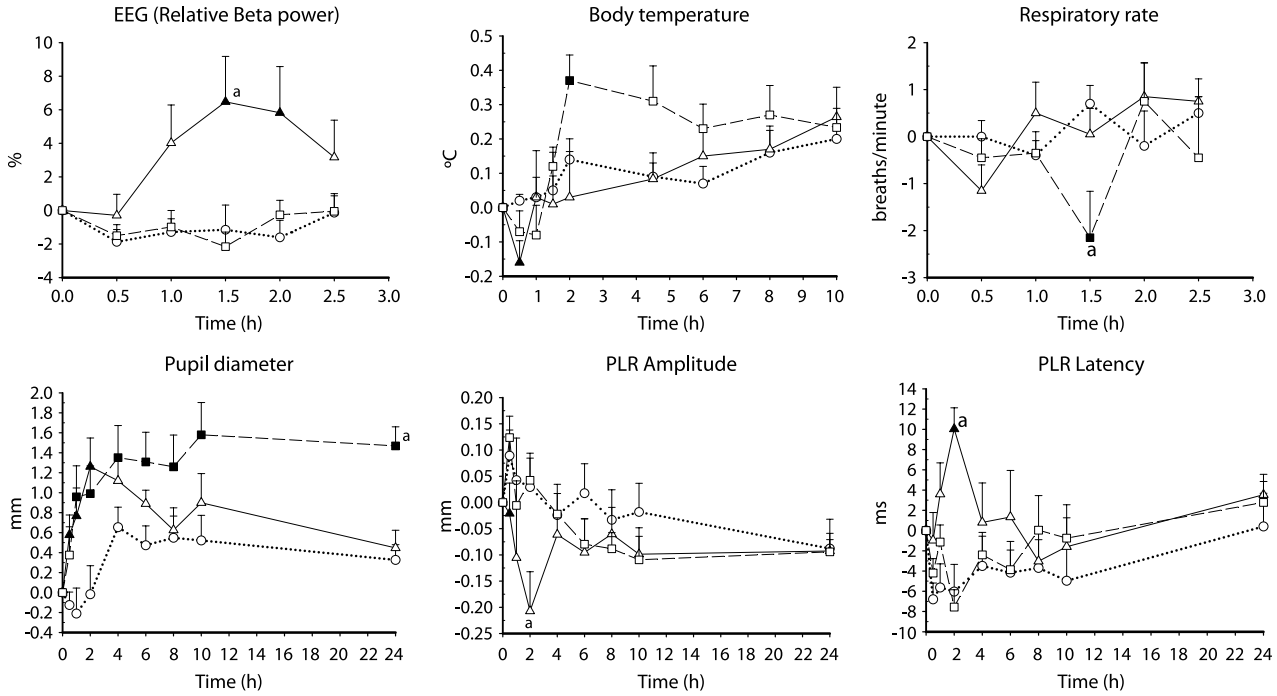

FIGURE 1. Time course of EEG and autonomic measures (means from 10 volunteers) after administration of placebo (circle, dotted line), 20 mg D-amphetamine (square, dashed line), and 1.0 mg DMT/kg body weight ayahuasca (triangle, solid line). Filled symbols indicate a significant difference from placebo. ''a'' indicates a significant difference between ayahuasca and amphetamine. Error bars denote 1 SEM.

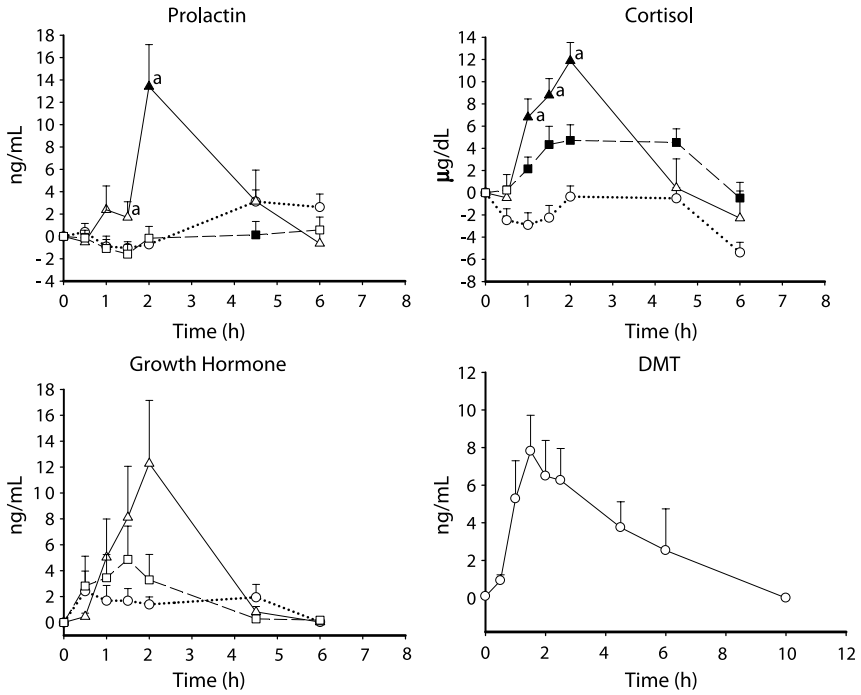

FIGURE 2. The upper panels and the lower left panel show the time course of neuroendocrine measures (means from 10 volunteers) after administration of placebo (circle, dotted line), 20 mg D-amphetamine (square, dashed line), and 1.0 mg DMT/kg body weight ayahuasca (triangle, solid line). Filled symbols indicate a significant difference from placebo. ''a'' indicates a significant difference between ayahuasca and amphetamine. The lower right panel shows the time course of DMT plasma concentrations after 1.0 mg DMT/kg body weight ayahuasca. Circles indicate means from 10 volunteers. Error bars denote 1 SEM.

Respiration Rate

No significant effect was observed for either active treatment in the overall ANOVA. The analysis of the time course of effects showed significant decreases for amphetamine relative to placebo and ayahuasca at 1.5 hours after dosing.

Neuroendocrine Measures

Neuroendocrine effects results are shown in Table 2 and Figure 2.

Prolactin peak levels after ayahuasca were significantly higher than those after placebo and amphetamine. The analysis of the time course of effects showed that ayahuasca significantly increased prolactin levels relative to placebo at 2 hours. Ayahuasca differed from amphetamine at 1.5 and 2 hours. Interestingly, although amphetamine did not modify prolactin in the peak value analysis, the time course analysis showed significantly reduced levels at 4.5 hours as compared with placebo.

Both active treatments significantly increased cortisol levels relative to placebo. Peak increases after ayahuasca showed a trend to be larger than those after amphetamine. Analyses at the different time points showed significant differences versus placebo in the time interval between 1 and 2 hours for ayahuasca and between 1 and 6 hours for amphetamine. Ayahuasca induced significantly higher cortisol levels than amphetamine at 1, 1.5, and 2 hours.

Regarding GH, although mean values after ayahuasca were higher than after placebo and amphetamine, no significant results were found in the ANOVA or at the individual time points.

Lymphocyte Subpopulations

Treatment effects on lymphocyte subpopulations are shown in Table 2 and Figure 3.

Total lymphocyte percentages in the 24-hour period did not show any significant changes after either of the 2 active treatments. However, the time course analysis showed an increase after ayahuasca relative to placebo at 1.5 hours and a decrease at 4.5 hours. The decrease was significant versus placebo and versus amphetamine. Interestingly, amphetamine nonsignificantly decreased total lymphocyte percentage at this time point. No differences were observed between treatments at 24 hours.

CD3 lymphocyte levels were found to be significantly decreased after ayahuasca and amphetamine. Time course analysis showed significant decreases at 1.5 and 2 hours after ayahuasca and at 1.5, 2, and 4.5 hours after amphetamine. No differences were found between treatments at 24 hours, although mean values were still lower than those after placebo.

Peak CD4 levels were significantly decreased after both active treatments. Time course analysis showed significant decreases at 1.5 and 2 hours for both ayahuasca and amphetamine. Again, no differences were found between treatments at 24 hours.

No significant changes were found for CD8 lymphocytes in the global and time course analyses. CD19 levels were also not found to be modified by any treatment in the ANOVA. However, the time course analysis found a significant decrease after ayahuasca at 2 hours.

Natural killer cells were significantly increased after ayahuasca and amphetamine. The time course analysis showed significant increases versus placebo at 1.5 and 2 hours after ayahuasca and at 1.5, 2, and 4.5 after amphetamine.

DMT Plasma Levels

The time course of DMT plasma concentrations is shown in Figure 2. The mean (SD) of the maximum concentration values

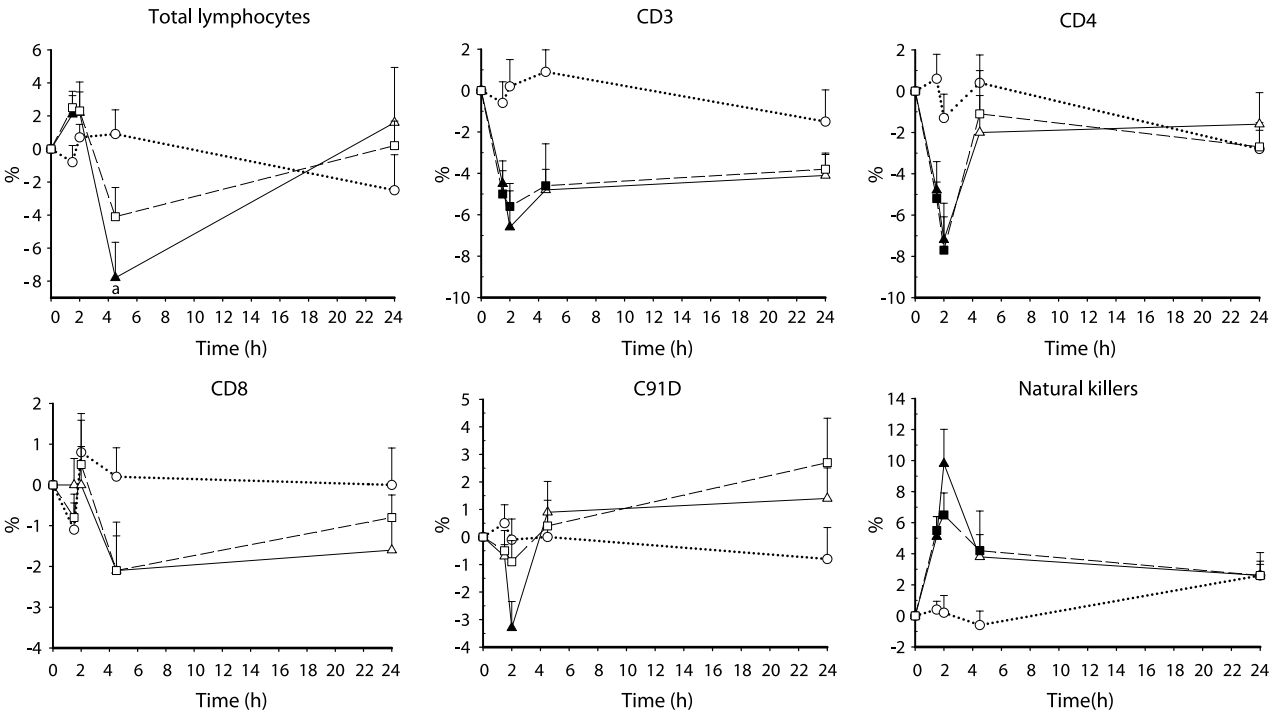

FIGURE 3. Time course of effects on lymphocyte subpopulations (means from 10 volunteers) after administration of placebo (circle, dotted line), 20 mg D-amphetamine (square, dashed line), and 1.0 mg DMT/kg body weight ayahuasca (triangle, solid line). Filled symbols indicate a significant difference from placebo. ''a'' indicates a significant difference between ayahuasca and amphetamine. Error bars denote 1 SEM.

(C_{max}) was 11.8 (SD, 6.4) ng/mL. The median (range) time at which the C_{max} was attained was 1.8 hours (range, 1–4.5 hours) after dosing.

DISCUSSION

The present investigation was undertaken to explore the autonomic, neuroendocrine, and immunomodulatory profile of ayahuasca. Considering the current expansion of ayahuasca use worldwide, we wished to further investigate the impact of this plant tea on human physiology. Results showed that ayahuasca induces relevant modifications in autonomic, neuroendocrine, and immune parameters as discussed below.

Systemic and CNS access of its main active principle was confirmed, respectively, by measurable DMT plasma levels and significant subjective effects. Maximum DMT concentrations were attained at 1.8 hours, in line with previously reported data.[10] Subjective effects included stimulant-like activation (ARCI-A), positive mood (ARCI-MBG), and somatic effects (ARCI-LSD, HRS-somaesthesia), in addition to perceptual modifications (HRS-perception), changes in thought processes and content (HRS-cognition), increased impairment (HRS-volition), and increased emotional lability (HRS-affect). These results replicate previous results after acute ayahuasca administration.[10,16]

The inclusion of amphetamine as an active control helped further characterize the psychotropic effect profile of ayahuasca. Thus, ayahuasca led to significantly higher scores than amphetamine in 4 of the 6 HRS scales (except HRS-somaesthesia and HRS-affect) and in the ARCI-LSD scale. On the other hand, as compared with ayahuasca, amphetamine significantly increased subjective feelings of intellectual energy and efficiency (ARCI-BG) and decreased scores on the sedative-sensitive ARCI-PCAG scale. Increases in the BG and decreases in the PCAG scale are known features of psychostimulants.[28] Although ayahuasca and amphetamine share some sympathomimetic properties, such as mydriasis and increases in blood pressure,[10] the divergences in subjective effects point to differential central mechanisms. Whereas amphetamine was perceived subjectively to increase intellectual energy, ayahuasca effects were rather felt as impairing. Differential effects on the CNS were further evidenced by the statistically significant increases observed in EEG relative β power. This effect was absent after amphetamine and replicates previous findings.[17] Although $5HT_{2A}$ receptor activation has repeatedly been found to cause physical signs of sympathetic activation,[36,37] the serotonergic mechanism leads to a pattern of CNS effects, which clearly differs from that by dopamine- and noradrenaline-enhancing drugs.

Ayahuasca effects on autonomic measures were not particularly robust. Body temperature showed a biphasic time course after ayahuasca and amphetamine. Mean temperature decreased below placebo levels between drug administration and the first hour and gradually rose thereafter. The initial decrease was significant for ayahuasca, but the subsequent increase was not. Previous studies involving the parenteral administration of DMT found inconsistent results for this measure, with 1 study reporting increases and 3 others reporting no change or ambiguous results.[36–39] Amphetamine, on the other hand, caused a nonsignificant initial decrease in body temperature followed by an increase that was larger than after ayahuasca, attaining statistical significance at 2 hours after dosing. Interestingly, a similar biphasic pattern had been previously described for body temperature after amphetamine and after the amphetamine derivative and serotonin releaser MDMA, although changes were statistically different from placebo only for the latter drug.[40,41] Weak changes were also found in the present study for respiration, which was reduced after amphetamine but only at 1 time point. Ayahuasca did not modify this variable. Early controlled studies with parenteral DMT did not find significant changes for this variable,[38] and it has not been measured in more recent studies.

Effects on pupillary diameter were more intense than on other autonomic variables, and a mydriatic effect was observed for both ayahuasca and amphetamine. However, whereas the effect of amphetamine was long lasting (still significant at 24 hours after administration), the effect after ayahuasca was significant only until 2 hours after dosing; mean values had fallen back to placebo levels at 8 hours. These findings are consistent with many previous studies. Mydriasis has been demonstrated in several controlled studies for parenteral DMT[36,38,39] and also for oral amphetamine and other psychostimulants.[28,40] As pupillary diameter is controlled by a balance between sympathetic and parasympathetic tone, based on our present findings ayahuasca seems to display sympathomimetic properties like those of amphetamine. However, ayahuasca was also found to decrease the amplitude and increase the latency of the PLR, effects typically ascribed to anticholinergic drugs. However, none of the components of ayahuasca seem to display affinity at muscarinic receptor sites.[3] A potential explanation is that the observed effects could be due to the noradrenergic inhibition of parasympathetic neurotransmission in the Edinger-Westphal nucleus, the CNS nucleus that controls constriction of the iris. The mixed serotonin/noradrenaline reuptake inhibitor venlafaxine has also been found to increase PLR latency and decrease PLR amplitude in the absence of any affinity of the compound for muscarinic receptors.[42,43] Results in those studies were also interpreted in terms of parasympatholytic effects mediated by noradrenergic inhibition of the Edinger-Westphal nucleus.

This is the first study in which the aforementioned autonomic variables have been measured after ayahuasca in a controlled clinical trial. In the only previous study known to us, the authors reported increases in respiration rate, pupillary diameter, and oral temperature.[20] However, as they did not include a nondrug (placebo) condition, the reported effects cannot be directly compared with ours. The autonomic effects of ayahuasca suggest sympathetic activation of lower intensity than that elicited by parenteral DMT in a dose range of 0.05 to 0.4 mg/kg.[36]

Ayahuasca and amphetamine produced significant time-dependent modifications in neuroendocrine variables and lymphocyte subpopulations. Ayahuasca, which contains the direct serotonergic agonist DMT, increased prolactin levels, whereas amphetamine, which increases noradrenergic and dopaminergic neurotransmission, did not. These results are in line with published data showing prolactin increases after DMT and serotonergic drugs such as MDMA, fenfluramine, and citalopram but not after amphetamine.[40,44] It is well known that dopamine is a potent inhibitor of prolactin secretion.[45] On the other hand, stimulation of the serotonin $5\text{-}HT_{1A}$, $5\text{-}HT_{2A}$, and $5\text{-}HT_{2C}$ receptors, which are all targets of DMT, increases prolactin release.[26] Neither of the active treatments produced significant changes in GH levels, but mean values were increased after ayahuasca. Growth hormone secretion is stimulated by selective $5\text{-}HT_{1A}$ agonists.[25] The lack of a significant result could be due to the lower affinity of DMT for the $5\text{-}HT_{1A}$ receptor.[7,8] Both ayahuasca and amphetamine produced significant increases in cortisol and variations in lymphocyte subpopulations. Activation of the sympathetic nervous system (SNS) and cortisol release have a well-known modulatory effect on lymphocytes.[21] Thus, besides a potentially direct receptor-mediated action, either

mechanism could be responsible for the changes observed in the present study, that is, reductions in CD3 and CD4 and enhanced NK cell levels. These changes appeared to be transient, with baseline values recovered after 24 hours.

A recent study found DOI reduces CD8 lymphocytes in mice via activation of the 5-HT_{2A} receptor.[24] Although no significant changes in CD8 percentages were found in the present study, data from the study in mice indicate that serotonergic psychedelics can modulate the immune function using a direct mechanism. Nevertheless, the remarkable similarity between the effects of ayahuasca and amphetamine on lymphocyte distribution in the present study suggests that changes may rather have been caused by an indirect mechanism common to both treatments rather than by specific drug-target interactions with immune cells. As mentioned above, this indirect mechanism could involve the hypothalamic-pituitary-adrenal axis and the SNS. Experimental evidence shows that despite their different molecular mechanisms of action; both DMT and amphetamine stimulate the hypothalamic-pituitary-adrenal axis, as reflected by increases in adrenocorticotropic hormone release.[36,46] Regarding the SNS, there is abundant literature on the procatecholaminergic effects of amphetamine,[28] and there is also evidence for increased urine excretion of noradrenaline metabolites after ayahuasca, suggesting an increased activity of the SNS after both drugs.[10] Further evidence on the nonspecific nature of ayahuasca effects is the similarity with the profile of changes induced by MDMA. Effects by this drug have been consistently replicated in many studies and basically involve decreases in CD3 and CD4 T lymphocytes and increases in NK cells.[47,48] Other psychoactive substances, such as cocaine, cannabis, alcohol, nicotine, and opiates, have also been found to modify the status of the immune system, both after acute intake and following chronic exposure.[23,48–50]

The health impact of ayahuasca ingestion in terms of susceptibility to disease is difficult to ascertain with the present data. Reductions in CD3 and CD4 are usually interpreted as detrimental. CD4 cells regulate cytotoxic T cells such as CD8 lymphocytes, which in turn destroy cells infected with intracellular microbes. CD4 cells also regulate B lymphocytes (CD19), which are responsible for antibody secretion.[51] On the other hand, increases in NK cells could be beneficial, these cells being involved in fighting virally infected and cancerous cells.[52,53] However, the overall time-dependent neuroendocrine and immunological profile observed in the present study mimics that observed in humans under stress.[54,55] Increased glucocorticoid levels and lymphocyte redistribution in acute stress have traditionally been regarded as immunosuppressant.[21] However, more recent views emphasize that, contrary to chronic stress, acute stress may have modulatory rather than inhibitory effects on immunity.[56] Considering the increasing popularity of ayahuasca and that ingestion of the tea on a regular basis is a central feature of the ayahuasca religions, the long-term impact of regular use on immunity warrants further investigation.

To conclude, the present findings indicate that acute ayahuasca has a moderate impact on the autonomous nervous system and a more robust activation of the hypothalamic-pituitary-adrenal axis. In addition, acute ayahuasca administration shows modulatory capacity on cell-mediated immunity, inducing a time-dependent redistribution of lymphocyte subtypes. A limitation of this study is the use of single doses only of the administered active drugs, thus precluding the assessment of dose-response relationships for the studied variables. Future studies should evaluate both the acute impact of different ayahuasca doses on immune function and the effects of chronic exposure in frequent users.

AUTHOR DISCLOSURE INFORMATION

The authors declare no conflicts of interest.

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ORIGINAL INVESTIGATION

Pharmacology of ayahuasca administered in two repeated doses

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Abstract
Rationale Ayahuasca is an Amazonian tea containing the natural psychedelic 5-$HT_{2A/2C/1A}$ agonist *N*,*N*-dimethyltryptamine (DMT). It is used in ceremonial contexts for its visionary properties. The human pharmacology of ayahuasca has been well characterized following its administration in single doses.
Objectives To evaluate the human pharmacology of ayahuasca in repeated doses and assess the potential occurrence of acute tolerance or sensitization.
Methods In a double-blind, crossover, placebo-controlled clinical trial, nine experienced psychedelic drug users received PO the two following treatment combinations at least 1 week apart: (a) a lactose placebo and then, 4 h later, an ayahuasca dose; and (b) two ayahuasca doses 4 h apart. All ayahuasca doses were freeze-dried Amazonian-sourced tea encapsulated to a standardized 0.75 mg DMT/kg bodyweight. Subjective, neurophysiological, cardiovascular, autonomic, neuroendocrine, and cell immunity measures were obtained before and at regular time intervals until 12 h after first dose administration.

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Results DMT plasma concentrations, scores in subjective and neurophysiological variables, and serum prolactin and cortisol were significantly higher after two consecutive doses. When effects were standardized by plasma DMT concentrations, no differences were observed for subjective, neurophysiological, autonomic, or immunological effects. However, we observed a trend to reduced systolic blood pressure and heart rate, and a significant decrease for growth hormone (GH) after the second ayahuasca dose.
Conclusions Whereas there was no clear-cut tolerance or sensitization in the psychological sphere or most physiological variables, a trend to lower cardiovascular activation was observed, together with significant tolerance to GH secretion.

Keywords Ayahuasca · Psychedelics · Repeated dose administration · Tolerance

Introduction

Ayahuasca is a psychoactive tea that was originally used for its visionary properties in shamanic, religious, and medicinal contexts in the Amazon but is now used worldwide in ceremonial and lay contexts (Tupper 2008). The tea is prepared from *Banisteriopsis caapi*, its key botanical ingredient, plus several other plants, typically *Psychotria viridis* (see, for a review, Riba 2003). Chemical analyses have shown that the main components of ayahuasca are alkaloids with β-carboline structure (harmine, harmaline, and tetrahydroharmine (THH)) from *B. caapi* plus *N,N*-dimethyltryptamine (DMT; Yritia et al. 2002; Riba 2003) from *P. viridis.* The monoamine-oxidase-inhibiting properties of the β-carbolines block the metabolic degradation of DMT, an orally labile psychedelic 5-$HT_{2A/2C/1A}$ receptor agonist (Smith et al. 1998; Riba 2003), and allow its access to systemic circulation after ayahuasca ingestion (McKenna et al. 1984; Riba 2003). In recent years, ayahuasca has been the object of various biomedical studies that have assessed its pharmacological profile in humans. Its effects when administered in single doses are well characterized. In a clinical research setting, it has been found to induce transient perceptual, cognitive, and affective modifications typical of the psychedelics, plus physiological effects that include elevations in diastolic blood pressure, cortisol and prolactin and lymphocyte redistribution, and electroencephalographic changes (Riba et al. 2002; 2003; Santos et al., in press). Ayahuasca is relatively well tolerated in healthy volunteers. The most commonly reported unpleasant effects are nausea and physical discomfort (Riba et al. 2001a).

In the context of the ceremonial use of ayahuasca, it is a common practice to drink several doses in each session, but the pharmacology of the drug in repeated doses has not yet been studied in a clinical trial. Laboratory information regarding possible increased toxicity after repeated administration is thus lacking. Furthermore, studying the pharmacology of ayahuasca administered in repeated doses is of interest from a basic science perspective. DMT appears to be different from other serotonergic psychedelics such as LSD, mescaline, and psilocybin, in terms of its tolerance-inducing capacity. While tolerance development to LSD was described over 50 years ago (Isbell et al. 1956), tolerance to DMT has not been conclusively demonstrated either in animals (Cole and Pieper 1973; Gillin et al. 1973; Kovacic and Domino 1976) or in humans (Gillin et al. 1976; Strassman et al. 1996).

In this present work, we studied the pharmacology of two consecutive doses of ayahuasca on subjective, physiological, and neurophysiological variables and assessed for potential acute tolerance or sensitization.

Materials and methods

Volunteers

A total of 17 volunteers (all male) with experience in psychedelic drug use were recruited. Eligibility criteria required prior use of psychedelics on at least ten occasions without sequelae derived thereof, i.e., psychedelic-related disorders as described in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (*DSM-IV*; American Psychiatric Association, 1994). A physician conducted a physical examination, ECG, and standard laboratory tests in all volunteers, confirming their good health. Prior to physical examination, volunteers were interviewed by a clinical psychologist (Spanish version of the Structured Interview for *DSM-IV* [*SCID*]; First et al. 1999). We excluded any volunteers who had a present or past history of Axis-I disorders (including alcohol or other substance dependence) and any who had parents or siblings with a present or past history of psychotic disorders. The study was conducted in accordance with the Declarations of Helsinki and Tokyo concerning experimentation on humans and was approved by the hospital ethics committee and the Spanish Ministry of Health. The volunteers received detailed information on the nature of ayahuasca and the general psychological effects of psychedelics and their possible adverse effects as reported in the psychiatric literature. All volunteers gave their written informed consent to participate.

Drug

Ayahuasca was not administered in liquid form but as a freeze-dried encapsulated formulation. Based on previous

studies by our group (Riba et al. 2001a; 2003), the dose of ayahuasca administered was equivalent to 0.75 mg DMT/kg body weight. Lactose capsules were used as a placebo.

Study design

Volunteers participated in three experimental sessions at least 1 week apart. Each experimental session involved two administrations separated by 4 h. In the first experimental session, all participants received the treatment pair placebo–placebo in an open-label fashion. This first session was intended to familiarize the volunteers with the study setting and to minimize the stress associated with the experimental interventions.

Volunteers were informed that, in sessions 2 and 3, they would randomly receive any of the following treatment pairs: placebo–placebo, placebo–ayahuasca, ayahuasca–placebo, or ayahuasca–ayahuasca. In fact, only two of the four combinations were administered: placebo–ayahuasca and ayahuasca–ayahuasca. Treatment pairs were administered in a double-blind fashion. The designation and order of administration for each of the four individual treatments was as follows: (a) for the placebo–ayahuasca pair: at zero hours, the treatment denominated *placebo* and at 4 h an ayahuasca treatment, designated here as *Aya0*; and (b) for the ayahuasca–ayahuasca pair: at zero hours, an ayahuasca treatment, designated here *Aya1,* and, at 4 h, an ayahuasca dose, designated here *Aya2*.

This approach was chosen to reduce the number of times volunteers were exposed to ayahuasca from four to three. Since *Aya0* and *Aya2* were administered in the afternoon and *Aya1* in the morning, *Aya1* is not comparable with the other two treatments due to circadian changes and to the influence of the light meal served to the participants before administration of *Aya0* and *Aya2*. Consequently, values for *Aya1* are shown for illustrative purposes in the figures in the results section, but they were excluded from the statistical analyses and are omitted from the tables. To test whether a single repeated dose administration of ayahuasca leads to higher absolute effects and acute tolerance or sensitization, *Aya0* was compared vs. *Aya2*. Comparisons vs. the placebo administered in the placebo–ayahuasca pair were merely conducted to confirm that *Aya0* and *Aya2* were active (see the statistical analyses explanation below).

Volunteers were requested to abstain from any medication or illicit drug use in the 2 weeks before the experimental sessions and until after the study was completed. Volunteers also were requested to abstain from alcohol, tobacco, and caffeinated drinks in the 24 h before each experimental day. Urinalysis for alcohol and illicit drug use was performed on each experimental day. Urine samples were tested for alcohol, benzodiazepines, cannabis, amphetamine, opiates, and cocaine using automated homogeneous enzyme immunoassays (Multigent, Architect C16000 System, Abbott Diagnostics, Abbott Laboratories, Abbott Park, IL, USA). Participants arrived at 7:00 AM under fasting conditions of at least 10 h and had a light breakfast before 10:00 AM. The first treatment was administered at approximately 11:00 AM and the second at 15:00 PM after a light meal. Throughout the experimental session, the volunteers remained seated in a comfortable reclining chair in a quiet, dimly lit room. Volunteers remained overnight in the laboratory and were discharged at 15:00 PM the following day.

Measurements

Subjective ratings

The subjective effects elicited by ayahuasca were measured by means of visual analog scales (VAS) and self-report questionnaires including the Hallucinogen Rating Scale (HRS) and the Addiction Research Center Inventory (ARCI).

VAS were 100-mm horizontal lines anchored with the words “Not at all” and “Extreme” with the following labels: “any effect,” indicating any physical or psychological modification that the volunteer attributed to the administered drug; “good effects,” indicating any effect, physical or psychological, the volunteer valued as good; “bad effects,” indicating any effect the volunteer valued as bad; “visual effects,” indicating modifications in visual perception, including any variations in object shape, brightness, or color and any illusion, abstract or elaborate, seen with eyes either closed or open; “auditory effects,” indicating modifications in auditory perception; “dizzy,” indicating near-syncope or lightheadedness; “liking,” reflecting that the volunteer liked the effects of the administered substance; “stimulated,” indicating any increases in thought speed and/or content, or any increases in associations and/or insights; and “high,” which reflected any positive psychological effect the volunteer attributed to the drug. The volunteers were requested to answer the VAS immediately before (baseline) and at 15, 30, 45 min, and 1, 1.5, 2, 2.5, 3, 4, 4.25, 4.5, 4.75, 5, 5.5, 6, 6.5, 7, 8, 10, and 12 h after administration of the first treatment.

The HRS includes six subscales: Somaesthesia, reflecting somatic effects; Affect, reflecting emotional and affective responses; Volition, indicating the volunteer’s capacity to willfully interact with his/her “self” and/or the environment; Cognition, describing modifications in thought processes or content; Perception, measuring visual, auditory, gustatory, and olfactory experiences; and Intensity, reflecting the strength of the overall experience (Strassman et al. 1994). A Spanish version of the questionnaire was used (Riba et al. 2001b). Scores for all subscales is 0 to 4.

The short version of the ARCI (Martin et al. 1971) consists of five scales or groups: MBG, the morphine–benzedrine group, measuring euphoria and positive mood; PCAG, the pentobarbital–chlorpromazine–alcohol group, measuring sedation; LSD, the lysergic acid diethylamide scale, measuring somatic–dysphoric effects; BG, the benzedrine group, measuring intellectual energy and efficiency; and the A scale, an empirically derived scale measuring amphetamine-like effects. Scores range from 0 to 16 for MBG, from −4 to 11 for PCAG, −4 to 10 for LSD, −4 to 9 for BG, and 0 to 11 for A. The questionnaire had been translated into Spanish and validated by Lamas et al. (1994). Volunteers were requested to answer the HRS and ARCI at 4 and 8 h after the first treatment.

Neurophysiological measures (EEG)

The EEG was recorded, preprocessed, and quantified following standard procedures as previously described (Riba et al. 2002). Recordings were obtained at 19 scalp locations according to the international 10/20 system by means of a Neuroscan SYNAMPS amplifier (Compumedics Neuroscan, Charlotte, NC, USA). A 3-min EEG with eyes closed was recorded at 0 (baseline) and 15, 30, 45 min, and 1, 1.5, 2, 2.5, 3, 4, 4.25, 4.5, 4.75, 5, 5.5, 6, 6.5, 7, and 8 h after administration of the first treatment. Following a fast Fourier transform, the target variables were calculated: relative power (expressed as percentage) in the beta (13–35 Hz) frequency band and in the beta-4 (25–30 Hz) and beta-5 (30–35 Hz) sub-bands. Modifications of these variables have been detected following acute ayahuasca administration (Riba et al. 2002; Santos et al., in press). Target variables were calculated at each electrode and time point. Averages for each variable in all 19 leads at each time point were used in the subsequent statistical analysis.

Cardiovascular measures

Systolic (SBP) and diastolic (DBP) blood pressures and heart rate (HR) were measured with the volunteer seated, before (baseline) and at 30 min, 1, 1.5, 2, 2.5, 3, 4, 4.5, 5, 5.5, 6, 6.5, 7, and 8 h after the first treatment using a Dinamap 8100 vital signs monitor (Critikon, Tampa, FL). The cuff was placed around the volunteer's left arm. Determination time was between 20 and 45 s.

Autonomic measures

Temperature and pupillary diameter were measured before administration (baseline) and at 30 min, 1, 1.5, 2, 2.5, 3, 4, 4.5, 5, 5.5, 6, 6.5, 7, and 8 h after the first treatment. Axillary temperature readings were obtained with a standard mercury-in-glass thermometer placed in the volunteer's armpit for at least 3 min. Pupillary diameter was measured with a portable pupillometer (NeurOptics Pupillometer; NeurOptics, Irvine, CA). The pupillometer was placed over the volunteer's eye immediately after turning the lights off and maintained in place until the pupillometer indicated that a valid reading had been obtained.

Neuroendocrine measures

Blood samples (3 mL, plain tubes without clot activator) were drawn before administration (baseline), and at 1, 2, 4, 5, 6, 8, and 28 h after administration of the first treatment and were allowed to stand at room temperature. Serum was separated by centrifugation and aliquots stored for the analysis of growth hormone (GH), prolactin, and cortisol.

Serum GH and prolactin concentrations were determined by a chemiluminescence immunoassay system (Immulite 2000®, Diagnostic Products Corp, EURO/Diagnostic Products Corporation, Llanberis, UK). The GH immunoassay, with a sensitivity of 0.06 mIU/L, uses the WHO 1st IRP 80/505, and shows intra- and interassay coefficients of variation (CV) of 5.3–6.1% and 5.7–6.5%, respectively. The prolactin immunoassay uses the 3rd IS 84/500, with an analytical sensitivity of 3.4 mIU/L, and intra-assay and total CV between 2.2–2.3% and 6.9–7.9%, respectively. Serum cortisol concentrations were measured by electrochemiluminescent immunoassay (Elecsys Modular Analytics E170®, Roche Diagnostics GmbH Manheim, Germany) with functional sensitivity <8 nmol/L, and intra-assay and total CVs of 1.7% and 2.8%, respectively, for mean human serum concentrations between 129 and 717 nmol/L.

Obtained values were transformed to nanograms per milliliter (prolactin and GH) and micrograms per deciliter (cortisol).

Lymphocyte subpopulations

Blood samples (3 mL, heparin tubes) were drawn before (baseline), and at 1, 2, 4, 5, 6, 8, and 28 h after administration and were subjected to lymphocyte immunophenotyping. The following lymphocyte subpopulations were quantified: CD8 T cells, CD4 T cells, CD3 T cells, CD19 B cells, and natural killer (NK) cells.

For lymphocyte immunophenotyping, blood samples were stained with the Lymphogram™ (Cytognos, Salamanca, Spain) reagent kit; each tube contains five different murine MoAbs with three fluorochromes: CD8 and CD19 with FITC (fluorescein isothiocyanate), CD3, and CD56 with PE (phycoerythrin). CD4 were labeled in tandem with PE and Cy5 (phycoerythrin−cyanate 5). The procedure has been detailed elsewhere (Bellido et

al. 1998). Lymphocyte subpopulations were expressed as percentage of all blood cells.

DMT plasma levels

Blood samples (10 ml, EDTA tubes) were drawn at 0 (baseline) and 30 min, and 1, 1.5, 2, 2.5, 3, 4, 4.25, 4.5, 5, 5.5, 6, 6.5, 7, 8, 10, and 12 h after administration for analysis of DMT. Samples were centrifuged at 2,000 rpm for 10 min at 4°C, and plasma was immediately frozen at −20°C. Frozen plasma samples were stored at −80°C until analysis. DMT plasma concentrations were determined as previously described (Yritia et al. 2002).

All measurements conducted at 4 h were performed prior to administration of the second treatment.

Statistical analysis

Data obtained in the first placebo−placebo acclimatization session were not included in the statistical analysis. The *placebo* used for the statistical comparisons was that administered in the placebo−ayahuasca session. The comparisons of interest were between *Aya0* and *Aya2*. These two ayahuasca treatments were fully equivalent in terms of time of the day and preceding meal. Nevertheless, *Aya0* and *Aya2* were compared with placebo to confirm that they were pharmacologically active.

For the HRS and ARCI questionnaires, scores on the different subscales were calculated and subjected to statistical analysis.

VAS, EEG, cardiovascular, neuroendocrine, immunological, temperature, and pupillary diameter measurements were transformed into differences from baseline (0 h). The following parameters were then calculated for each of the three treatments, i.e., placebo, *Aya0*, and *Aya2*: (1) the 0–4 h post-administration E_{max} ($E_{max(0–4h)}$) or peak effect (maximum absolute change from the 0 h baseline values); (2) the 0–4 h post-administration area under the curve ($AUC_{0–4h}$) of effect versus time; and (3) the 0–4 h post-administration area under the curve ($AUC_{0–4h}$) normalized by the respective $AUC_{0–4h}$ of the DMT plasma concentrations vs. time. These normalized AUCs were designated as AUC_{norm}. All AUCs were calculated using the trapezoidal rule. The comparison between AUC_{norm} after *Aya0* and AUC_{norm} after *Aya2* allowed making inferences regarding acute tolerance or sensitization development, taking the DMT plasma concentrations into account.

HRS and ARCI scores and the parameters described above for the pharmacodynamic variables were analyzed using paired Student's *t* tests.

For each ayahuasca treatment, the maximum DMT plasma concentration (C_{max}) was calculated and reported as mean±standard deviation (SD). The time to reach the maximum concentration (t_{max}) was reported as median and range. Areas under the concentration−time curves between treatment administration and 4 h ($AUC_{0–4h}$) were also calculated and reported as mean±SD. C_{max} and $AUC_{0–4h}$ were compared between ayahuasca treatments by means of Student's *t* test. T_{max} values were compared using non-parametric Wilcoxon's test.

In all tests performed, differences were considered statistically significant for *p* values lower than 0.05.

Results

Only nine of the 17 volunteers completed the study. One volunteer was excluded before the start of the acclimatization session due to a positive result for alcohol in the urinalysis. Two decided to voluntarily withdraw from the study, and five more were excluded due to vomiting. Vomiting was self-induced in one case; in another case, it occurred after the administration of the first ayahuasca dose in the ayahuasca−ayahuasca session, and in the remaining three cases, it occurred after the administration of the second ayahuasca dose in the ayahuasca–ayahuasca session. The data reported in the present paper refer only to the nine volunteers that completed all three experimental sessions. Participants in the final sample had a mean age of 32.8 (range, 24–41 years), a mean weight of 69.69 kg (range, 57–91), and a mean height of 177 cm (range, 170–186). Additionally, one subject showed no measurable DMT plasma levels after *Aya0*. Consequently, pharmacokinetic data are reported for eight volunteers only (see the "Pharmacokinetic analysis" section below). This also precluded the calculation of the normalized AUCs after *Aya0* for this participant. For this reason, the statistical comparison of normalized AUCs after *Aya0* vs *Aya2* was conducted for a sample of eight volunteers.

Subjective effects

Subjective effect results are shown in Fig. 1 and Table 1.

Both administered ayahuasca treatments proved psychoactive. Compared with placebo, the administration of both *Aya0* and *Aya2* led to significant increases in all subscales of the HRS and in the A and MBG subscales of the ARCI. Additionally, *Aya0* led to significant increases in the BG subscale and *Aya2* to significant increases in the LSD subscale. The effects of *Aya2* on the HRS subscales Somaesthesia and Volition were significantly higher than those of *Aya0*. The effects of *Aya2* on the HRS subscale Intensity showed a trend to significantly higher values than after *Aya0*. Higher increases were also observed in the Perception subscale, and these were marginally significant (p=0.050). No differences between doses were

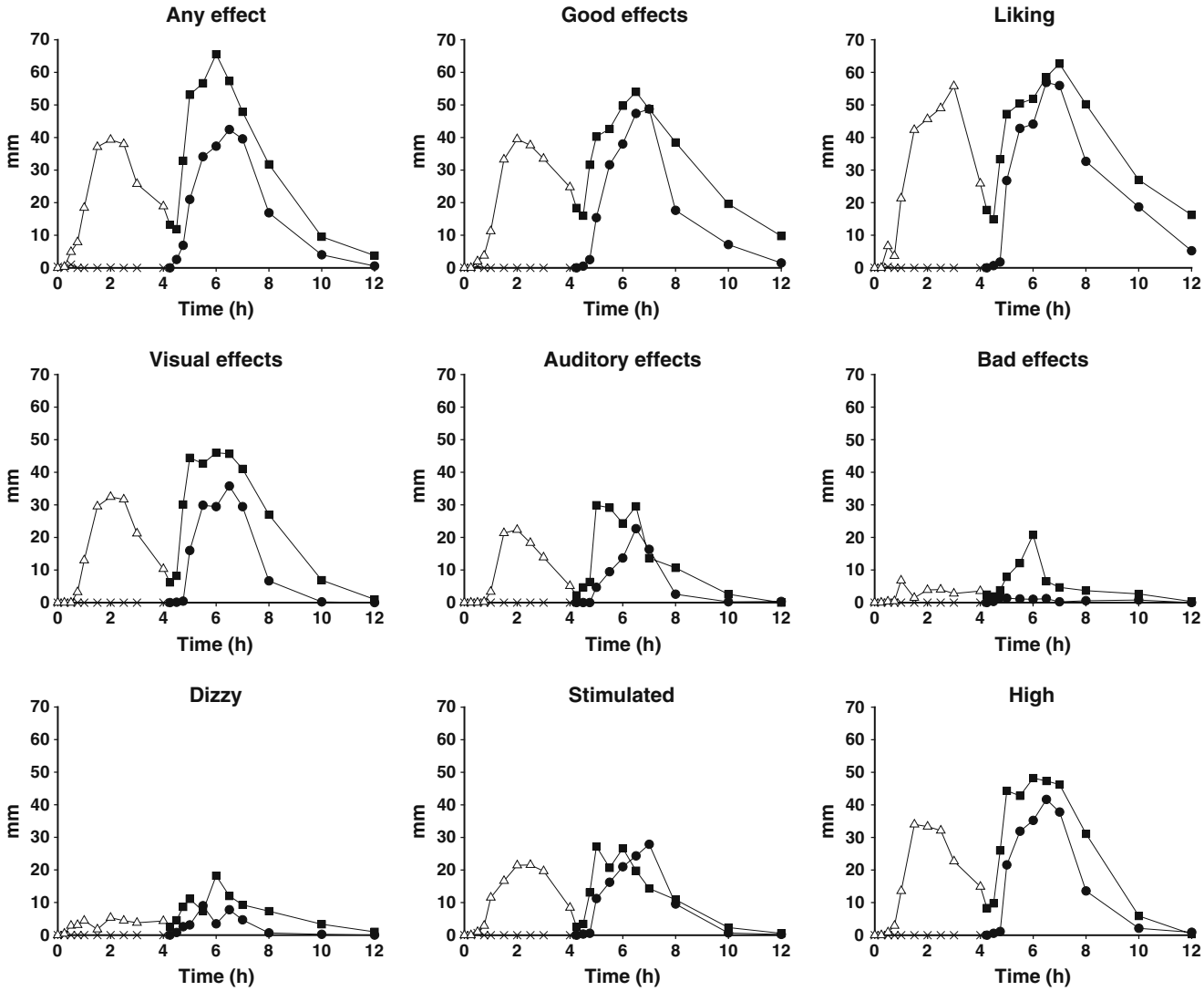

Fig. 1 Time course of scores on the nine VAS items (means from nine volunteers) after administration of placebo (*star*) and each of the three 0.75 mg DMT/kg body weight ayahuasca doses: *Aya1* (*open triangle*), *Aya0* (*filled circle*) and *Aya2* (*filled square*). *Aya0* was preceded 4 h by the placebo and *Aya2* was preceded 4 h by *Aya1*

found for Affect, Cognition, or ARCI subscales. Regarding the VAS items, both ayahuasca treatments produced significant increases relative to placebo in the peak values and $AUC_{0\text{-}4h}$ values of eight items (except for "bad effects", where only *Aya2* produced significant effects). For two VAS items ("any effect" and "bad effects"), values after *Aya2* were significantly higher than those after *Aya0*. The $AUC_{0\text{-}4h}$ for the "auditory effects" VAS item was higher for *Aya2* than for *Aya0*. The same parameter for the items "visual effects" and "dizzy" showed a trend to significantly higher values after *Aya2*. When DMT plasma levels were taken into account (AUC_{norm}), the VAS item "stimulated" showed a trend to lower effects after *Aya2* than after *Aya0*, while the item "bad effects" showed a trend for higher effects after *Aya2* than after *Aya0*.

EEG effects

Treatment effects on relative global beta power and relative beta-4 and beta-5 powers are presented in Fig. 2 and Table 2.

As shown therein, *Aya0* (AUC) induced significant increases in relative beta-4 and beta-5 powers and a marginally significant increase (p=0.050) in relative global beta power. However, no significant effects for *Aya0* peak values were observed in any of the EEG variables. On the other hand, *Aya2* (AUC and peak) induced significant increases in all three measures. There was only a trend in relative global beta power (peak) between ayahuasca treatments; and *Aya2* induced significantly larger increases in relative beta-4 power than *Aya0* (AUC and peak) and in

Table 1 Subjective effects induced by placebo, *Aya0* and *Aya2*

	Placebo	Ayahuasca0	Ayahuasca2	Pair-wise comparisons		
				PLA/AYA0	PLA/AYA2	AYA0/AYA2
HRS						
Somaesthesia	0.00 (0.00)	0.82 (0.53)	1.25 (0.55)	**	***	*
Affect	0.27 (0.66)	1.19 (0.55)	1.20 (0.53)	**	**	ns
Perception	0.00 (0.00)	1.41 (0.53)	1.81 (0.78)	***	***	0.050
Cognition	0.00 (0.00)	1.42 (0.71)	1.52 (0.68)	***	***	ns
Volition	0.19 (0.31)	1.05 (0.36)	1.51 (0.45)	***	***	**
Intensity	0.00 (0.00)	1.97 (0.65)	2.53 (0.78)	***	***	0.073
ARCI						
A	0.22 (0.67)	4.22 (2.86)	4.55 (3.68)	**	**	ns
BG	0.11 (0.78)	2.22 (2.39)	1.67 (3.12)	*	ns	ns
MBG	3.33 (0.71)	5.89 (4.48)	7.00 (5.57)	**	**	ns
PCAG	0.67 (1.41)	1.44 (3.28)	2.89 (4.25)	ns	ns	ns
LSD	−0.67 (1.12)	0.78 (2.49)	1.22 (2.17)	ns	*	ns
VAS						
Any effect_peak	1.33 (3.04)	51.67 (25.20)	76.33 (21.88)	***	***	*
Any effect_AUC	20.83 (54.57)	6,317.50 (4,326.05)	10,738.33 (5,313.29)	**	***	*
Any effect_AUC_norm	–	5.17 (3.63)	3.76 (3.05)	–	–	ns
Good effects_peak	0.44 (1.33)	61.33 (33.44)	68.55 (24.88)	**	***	ns
Good effects_AUC	6.67 (20.00)	7,503.33 (5,044.29)	10,231.67 (4,745.73)	**	***	ns
Good effects_AUC_norm	–	6.62 (5.96)	3.55 (2.63)	–	–	ns
Liking_peak	0.33 (1.00)	64.22 (31.76)	72.55 (28.86)	***	***	ns
Liking_AUC	5.00 (15.00)	8,479.17 (5,506.50)	10,995.00 (5,209.33)	**	***	ns
Liking_AUC_norm	–	7.52 (6.70)	3.67 (3.00)	–	–	ns
Visual effects_peak	0.00 (0.00)	46.78 (22.67)	65.89 (26.61)	***	***	ns
Visual effects_AUC	0.00 (0.00)	4,746.67 (2,841.53)	8,464.17 (6,172.66)	**	**	0.083
Visual effects_AUC_norm	–	3.91 (2.15)	2.67 (2.31)	–	–	ns
Auditory effects_peak	0.00 (0.00)	28.67 (24.50)	43.55 (29.81)	**	**	ns
Auditory effects_AUC	0.00 (0.00)	2,290.00 (2,419.08)	4,350.83 (3,952.53)	*	*	*
Auditory effects_AUC_norm	–	1.82 (1.88)	1.56 (2.22)	–	–	ns
Bad effects_peak	0.00 (0.00)	1.89 (3.51)	23.22 (22.24)	ns	*	*
Bad effects_AUC	0.00 (0.00)	183.33 (357.81)	1,829.17 (1,784.32)	ns	*	*
Bad effects_AUC_norm	–	0.17 (0.31)	0.48 (0.42)	–	–	0.057
Dizzy_peak	0.00 (0.00)	14.55 (12.03)	31.67 (25.11)	**	**	ns
Dizzy_AUC	0.00 (0.00)	956.67 (868.11)	2,295.83 (2,307.40)	*	*	0.064
Dizzy_AUC_norm	–	0.57 (0.38)	0.73 (0.43)	–	–	ns
Stimulated_peak	0.67 (2.00)	35.00 (21.30)	38.89 (25.22)	**	**	ns
Stimulated_AUC	10.00 (30.00)	3,654.17 (2,811.56)	3,955.83 (3,292.43)	**	**	ns
Stimulated_AUC_norm	–	2.64 (1.74)	1.41 (1.90)	–	–	0.064
High_peak	0.00 (0.00)	52.89 (23.76)	64.22 (28.76)	***	***	ns
High_AUC	0.00 (0.00)	5,880.00 (4,197.34)	8,940.83 (5,378.52)	**	**	ns
High_AUC_norm	–	4.79 (3.47)	2.63 (2.07)	–	–	ns

Mean (SD) of the scores obtained for the HRS and ARCI questionnaires subscales and for the VAS and results of the statistical analysis performed. $N=9$, except for normalized AUCs where $n=8$

PLA placebo, *AYA0* ayahuasca0, *AYA2* ayahuasca2, *A* amphetamine, *BG* benzedrine-group, *MBG* morphine–benzedrine-group, *PCAG* pentobarbital–chlorpromazine–alcohol-group, *LSD* lysergic acid diethylamide scale

*$p<0.05$, **$p<0.01$, ***$p<0.001$. Exact p values are given when $p<0.1$

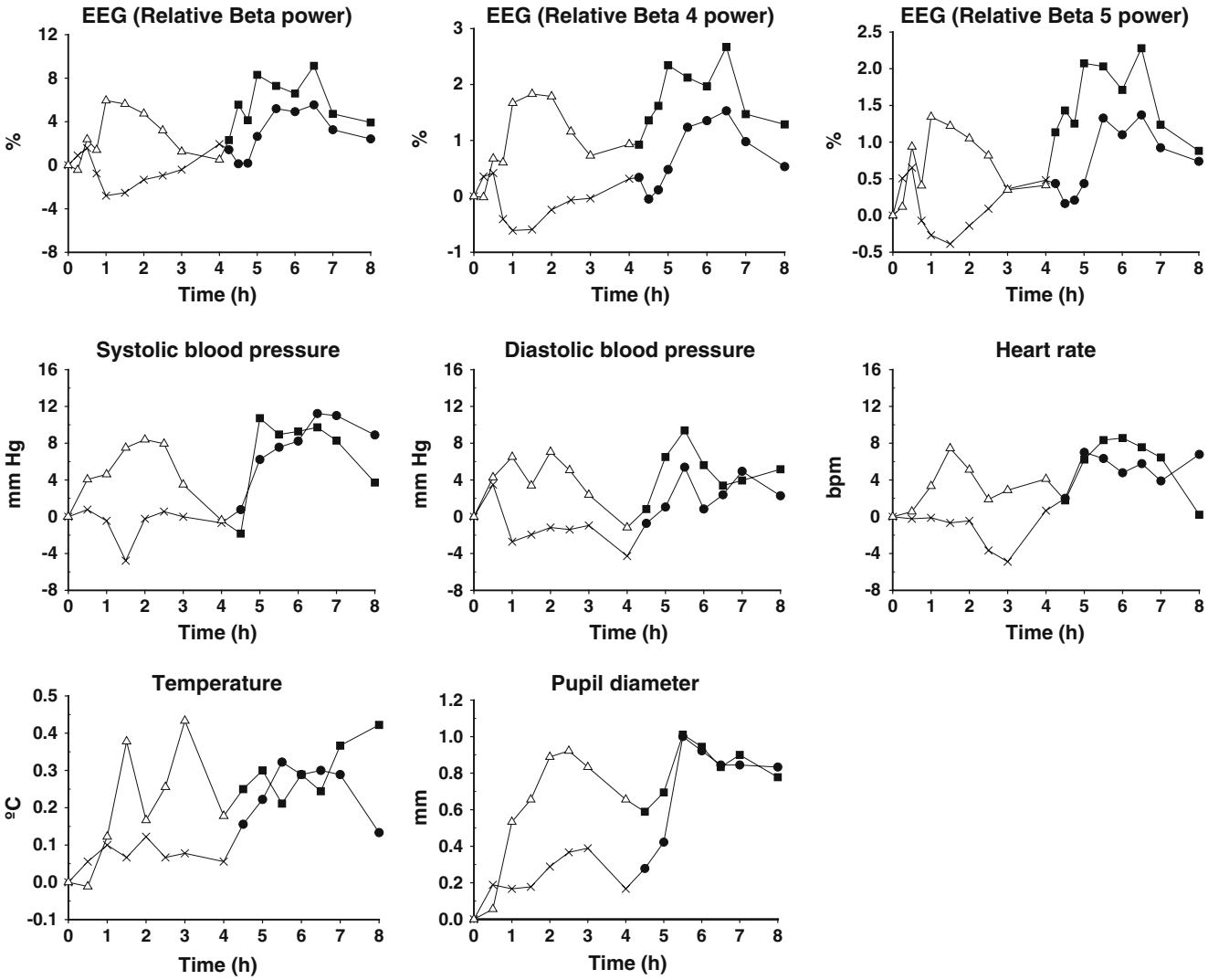

Fig. 2 Time course of electroencephalographic (EEG), cardiovascular and autonomic variables (means from nine volunteers) after administration of placebo (*star*) and each of the three 0.75 mg DMT/kg body weight ayahuasca doses: *Aya1* (*open triangle*), *Aya0* (*filled circle*), and *Aya2* (*filled square*). *Aya0* was preceded 4 h by the placebo and *Aya2* was preceded 4 h by *Aya1*

relative beta-5 power (peak). When DMT plasma levels were taken into account, no differences between active treatments appeared for any of the variables.

Cardiovascular effects

Cardiovascular effects are shown in Fig. 2 and Table 2. Peak and AUC values after both ayahuasca treatments were significantly higher than after placebo for SBP. No significant differences were found between active treatments. For peak DBP values, only *Aya2* produced increases significantly different from placebo. No significant differences were found between active treatments. In terms of AUC values, effects after both ayahuasca treatments were significantly larger than after placebo. No significant differences were found between ayahuasca treatments. For HR, *Aya0*, and *Aya2* produced significant increases compared with placebo, in terms of both peak and AUC values. No significant differences were found between active treatments. When DMT plasma levels were taken into account, there was a trend for lower values after *Aya2* for SBP and HR.

Occurrence of hypertension and/or tachycardia was examined for each participant. SBP rose above 140 mm Hg in three volunteers after *Aya0* (141 mm Hg; and 146 mm Hg, two volunteers) and in two volunteers after *Aya2* (147 mm Hg; and 142 mm Hg). Most of these events lasted 15–30 min. DBP values did not reach values above 90 mm Hg for any participant. HR rose above 100 beats/min (105 beats/min) in one volunteer after *Aya0*.

Table 2 Effects induced by placebo and *Aya0* and *Aya2* on EEG and cardiovascular and autonomic measures

	Placebo	Ayahuasca0	Ayahuasca2	Pair-wise comparisons		
				PLA/AYA0	PLA/AYA2	AYA0/AYA2
EEG measures						
Relative beta power_peak	−0.68 (8.37)	5.15 (12.85)	12.06 (18.07)	ns	*	0.074
Relative beta power_AUC	−140.86 (624.87)	790.33 (1,325.02)	1,391.50 (1,986.39)	0.050	*	ns
Relative beta power_AUC_norm	–	0.80 (1.23)	0.64 (0.87)	–	–	ns
Relative beta-4 power_peak	0.23 (2.08)	1.28 (3.44)	3.16 (3.52)	ns	**	**
Relative beta-4 power_AUC	−27.47 (191.92)	202.61 (327.20)	426.05 (334.23)	*	**	**
Relative beta-4 power_AUC_norm	–	0.20 (0.36)	0.16 (0.18)	–	–	ns
Relative beta-5 power_peak	0.41 (1.63)	1.21 (2.26)	4.17 (1.95)	ns	**	*
Relative beta-5 power_AUC	27.98 (113.85)	203.08 (188.09)	369.69 (374.27)	*	*	ns
Relative beta-5 power_AUC_norm	–	0.19 (0.18)	0.16 (0.20)	–	–	ns
Cardiovascular measures						
Systolic blood pressure_peak	−5.44 (14.49)	19.44 (7.99)	19.33 (10.96)	**	***	ns
Systolic blood pressure_AUC	−145.00 (868.58)	1,810.00 (1,088.56)	1,583.33 (904.40)	***	***	ns
Systolic blood pressure_AUC_norm	–	1.59 (1.56)	0.55 (0.63)	–	–	0.065
Diastolic blood pressure_peak	−1.33 (12.69)	4.67 (15.37)	12.22 (8.87)	ns	**	ns
Diastolic blood pressure_AUC	−301.33 (1,024.69)	501.67 (1,774.43)	1,113.33 (959.79)	*	**	ns
Diastolic blood pressure_AUC_norm	–	0.27 (2.00)	0.44 (0.33)	–	–	ns
Heart rate_peak	−0.78 (10.24)	12.55 (8.70)	14.67 (10.46)	*	*	ns
Heart rate_AUC	−365.00 (1,034.73)	1,186.67 (685.56)	1,371.67 (1,262.21)	**	*	ns
Heart rate_AUC_norm	–	0.88 (0.62)	0.45 (0.62)	–	–	0.096
Autonomic measures						
Temperature_peak	0.10 (0.61)	0.27 (0.59)	0.45 (0.69)	ns	ns	ns
Temperature_AUC	−12.50 (86.66)	27.67 (99.72)	81.54 (137.94)	*	0.055	ns
Temperature_AUC_norm	–	−0.01 (0.10)	0.02 (0.07)	–	–	ns
Pupillary diameter_peak	0.32 (0.70)	1.03 (0.65)	1.09 (0.40)	*	*	ns
Pupillary diameter_AUC	52.67 (77.98)	166.83 (100.40)	188.00 (86.68)	**	*	ns
Pupillary diameter_AUC_norm	–	0.14 (0.11)	0.05 (0.03)	–	–	ns

Means (SD) of the scores obtained and results of the statistical analysis performed. $N=9$, except for normalized AUCs where $n=8$

PLA placebo, *AYA0* ayahuasca0, *AYA2* ayahuasca2. Peak beta power expressed as percentage; Peak systolic blood pressure in mmHg; Peak diastolic blood pressure in mmHg; Peak heart rate in beats/minute; Peak body temperature in °C; Peak pupillary diameter in millimeters

*$p<0.05$, ** $p<0.01$, *** $p<0.001$. Exact p values are given when $p<0.1$

Autonomic effects

Autonomic effects are shown in Fig. 2 and Table 2. No significant differences between ayahuasca and placebo or between active treatments were found for temperature peak values. For pupillary diameter, *Aya0* and *Aya2* produced significant increases in peak values relative to placebo. No significant differences were found between active treatments. For AUC values, only *Aya0* produced statistically significant increases in temperature relative to placebo. *Aya2* only showed a trend for significantly higher AUC values than placebo. No significant differences were found between ayahuasca treatments. For pupillary diameter, *Aya0* and *Aya2* produced significant AUC increases relative to placebo. No significant differences were found between ayahuasca treatments. Finally, no differences were found between *Aya0* and *Aya2* in temperature and pupillary diameter when the normalized AUCs were compared.

Neuroendocrine effects

Neuroendocrine effects results are shown in Fig. 3 and Table 3.

Peak and AUC prolactin values after *Aya0* and *Aya2* were significantly increased relative to placebo. Increases after *Aya2* were significantly higher than after *Aya0*. For cortisol, only *Aya2* produced increases significantly different from placebo. A trend was seen for AUC values after *Aya0*. Increases after *Aya2* were significantly higher than after *Aya0* in terms of both peak and AUC values. For growth hormone, only *Aya0* produced increases in peak values significantly

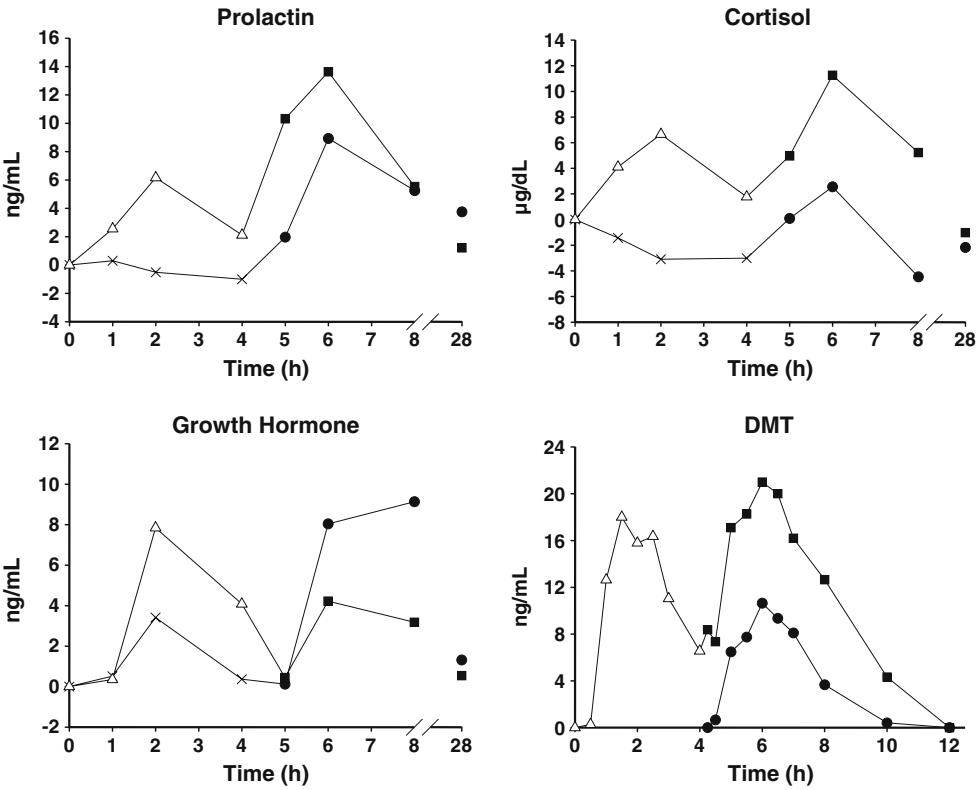

Fig. 3 Time course of neuroendocrine measures (means from nine volunteers) and DMT plasma concentrations (means from eight volunteers) after administration of placebo (*star*) and each of the three 0.75 mg DMT/kg body weight ayahuasca doses: *Aya1* (*open triangle*), *Aya0* (*filled circle*), and *Aya2* (*filled square*). *Aya0* was preceded 4 h by the placebo, and *Aya2* was preceded 4 h by *Aya1*

different from placebo. A trend to lower peak values was found for *Aya2* when compared with *Aya0*. In terms of AUC values, *Aya2* produced significant increases from placebo, whereas a marginally significant effect (p=0.052) was observed for *Aya0*. No significant differences were observed between active treatments. The comparison of the normalized AUCs between active treatments yielded non-significant results for prolactin and cortisol and significantly lower values for growth hormone.

Lymphocyte subpopulations

Treatment effects on lymphocyte subpopulations are shown in Fig. 4 and Table 3.

The total lymphocyte percentage did not show any significant changes after either of the two ayahuasca treatments in terms of AUC or peak values. However, *Aya2* (AUC) decreased total lymphocyte percentage more than *Aya0*. CD3 lymphocyte levels were found to be decreased after *Aya0*, but not after *Aya2*. No differences were found between ayahuasca treatments. Peak CD4 levels showed a trend for a significant decrease after both ayahuasca treatments. Furthermore, CD4 AUC value decreases reached statistical significance after both ayahuasca treatments. But again, no differences were found between active treatments. No significant changes were found for CD8 lymphocytes (peak and AUC), but there was a trend for a significant reduction after *Aya0*. No differences were found between *Aya0* and *Aya2*. The analysis of CD19 levels yielded mixed results. Whereas *Aya0* produced a marginally significant reduction (p=0.050) in AUC, *Aya2* significantly reduced peak values. No differences were found in AUC between ayahuasca treatments, but *Aya2* produced a significantly higher reduction than *Aya0* in peak values. NK cells were significantly increased after both ayahuasca administrations (AUC) and after *Aya0* (peak value). There was a trend for a significant increase after *Aya2* (peak). No differences were found between ayahuasca treatments. The comparison of the normalized AUCs between active treatments yielded non-significant results for all lymphocyte subpopulations.

Pharmacokinetic analysis

The time course of DMT plasma concentrations is shown in Fig. 3. One volunteer did not show measurable levels of DMT after *Aya0* and was excluded from the pharmacokinetic analyses. The mean±SD of the maximum concentration values (C_{max}) was 13.97±9.35 ng/ml for *Aya0* and 32.57±20.96 ng/ml for *Aya2*. These values were statistically different [$t(7)=-2.92$, $p=0.022$]. The median (range) time at which the C_{max} was attained (t_{max})was 2.0 h (1–3) for *Aya0* and 2.0 h (1–3) for *Aya2*. These values were not statistically different [$z=-0.32$, $p>0.1$]. The AUC values were 1,703 mg/ml·min^{-1} for *Aya0* and 4,078 mg/ml·min^{-1} for *Aya2*. These values were statistically different. [$t(7)=-2.78$, $p=0.027$]. To test whether the higher DMT AUCs obtained

Table 3 Effects induced by placebo, *Aya0*, and *Aya2* on neuroendocrine parameters and lymphocyte subpopulations

	Placebo	Ayahuasca0	Ayahuasca2	Pair-wise comparisons		
				PLA/AYA0	PLA/AYA2	AYA0/AYA2
Hormones						
Prolactin_peak	−0.78 (3.03)	12.89 (9.41)	16.78 (10.11)	**	**	*
Prolactin_AUC	−88.37 (442.52)	1,206.35 (1,295.05)	2,241.37 (1,280.70)	*	**	*
Prolactin_AUC_norm	–	1.36 (2.24)	0.81 (0.73)	–	–	ns
Cortisol_peak	−2.89 (7.75)	−0.55 (9.59)	11.33 (6.30)	ns	**	**
Cortisol_AUC	−544.50 (1,286.94)	−114.07 (1,286.70)	1,678.33 (1,186.12)	0.078	**	**
Cortisol_AUC_norm	–	−0.08 (1.58)	0.53 (0.44)	–	–	ns
GH_peak	3.44 (5.70)	15.00 (11.77)	6.22 (4.18)	*	ns	0.076
GH_AUC	359.78 (539.55)	1,290.16 (1,024.17)	719.85 (421.33)	0.052	*	ns
GH_AUC_norm	–	1.30 (1.20)	0.26 (0.22)	–	–	*
Lymphocyte subpopulations						
Total lymphocytes_peak	−0.13 (0.35)	−0.02 (0.60)	−0.33 (0.88)	ns	ns	ns
Total lymphocytes_AUC	−21.50 (38.22)	−7.32 (69.26)	−64.15 (123.23)	ns	ns	*
Total lymphocytes_AUC_norm	–	0.00 (0.07)	−0.01 (0.03)	–	–	ns
CD3_peak	−4.22 (5.02)	−10.00 (7.24)	−11.11 (15.98)	*	ns	ns
CD3_AUC	−516.67 (790.43)	−1,460.00 (1,337.61)	−1,343.30 (2,009.07)	*	ns	ns
CD3_AUC_norm	–	−1.40 (2.06)	−0.29 (0.94)	–	–	ns
CD4_peak	−3.89 (4.23)	−8.55 (6.52)	−10.44 (8.40)	0.058	0.097	ns
CD4_AUC	−396.67 (741.37)	−1,190.00 (1,104.92)	−1,576.67 (1,382.53)	*	**	ns
CD4_AUC_norm	–	−1.01 (1.36)	−0.56 (0.63)	–	–	ns
CD8_peak	0.11 (3.29)	−1.67 (4.21)	−0.89 (3.95)	0.082	ns	ns
CD8_AUC	−50.00 (457.19)	−270.00 (681.96)	−93.95 (621.16)	ns	ns	ns
CD8_AUC_norm	–	−0.39 (1.03)	−0.01 (0.20)	–	–	ns
CD19_peak	1.67 (4.24)	0.89 (4.75)	−3.22 (1.79)	ns	*	*
CD19_AUC	250.00 (713.41)	126.67 (817.02)	−203.33 (160.00)	0.050	ns	ns
CD19_AUC_norm	–	0.06 (0.43)	−0.07 (0.08)	–	–	ns
NK_peak	2.33 (5.72)	8.33 (6.10)	8.78 (9.46)	*	0.096	ns
NK_AUC	293.33 (817.85)	1,293.33 (1,074.30)	1,433.33 (1,387.46)	**	*	ns
NK_AUC_norm	–	1.15 (1.66)	0.42 (0.55)	–	–	ns

Means (SD) of the values obtained and results of the statistical analysis performed. $N=9$, except for normalized AUCs where $n=8$

PLA placebo, *AYA0* ayahuasca0, *AYA2* ayahuasca2

*$p<0.05$, **$p<0.01$. Exact p values are given when $p<0.1$

after *Aya2* were larger than the mere superposition over the remaining DMT levels of the preceding ayahuasca dose (*Aya1*), the $AUC_{4\text{-}8h}$ of *Aya1* was calculated for each volunteer and subtracted from the AUC obtained after *Aya2*. The corrected values were again compared vs. the AUC values obtained after *Aya0*. The corrected AUC value was 2,993 mg/ml·min^{-1}. The comparison vs. *Aya0* yielded non-significant results [$t(7)=-1.71$, $p>0.1$].

Discussion

The aim of the present investigation was to study the pharmacology of two consecutive doses of ayahuasca and to test whether acute tolerance or sensitization phenomena occurred. To our knowledge, this is the first study of this nature conducted to date. In our view, it is important to gather this information considering the increasing popularity of ayahuasca preparations worldwide (Tupper 2008) and the common practice of ingesting several doses in a single session.

The administered dose of 0.75 mg DMT/kg was above the threshold of psychoactivity and proved physiologically active on many levels. Results for the individual ayahuasca treatments replicate and extend previous findings. Statistically significant psychological and physiological effects were observed when compared with placebo. This dose had been found to be psychoactive in a previous study (Riba et al. 2001a). In the present work, the administration of two

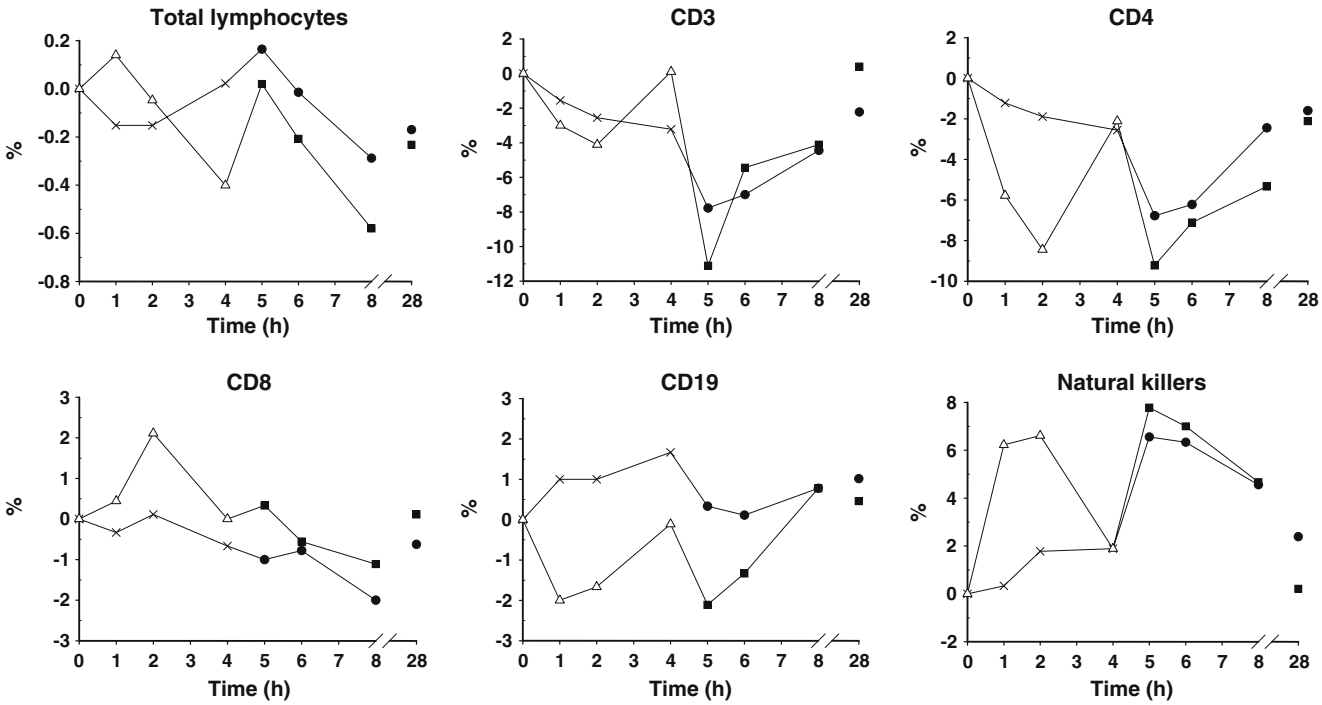

Fig. 4 Time course of effects on lymphocyte subpopulations (means from nine volunteers) after administration of placebo (*star*) and each of the three 0.75 mg DMT/kg body weight ayahuasca doses: *Aya1* (*open triangle*), *Aya0* (*filled circle*), and *Aya2* (*filled square*). *Aya0* was preceded 4 h by the placebo, and *Aya2* was preceded 4 h by *Aya1*

identical doses in succession with an interval of 4 h, led to virtually all subjective effects measures showing higher mean values after the second dose. Psychotropic effects were more intense, but unpleasant somatic effects and impairment also increased. In this respect, it is worth noting that several volunteers had to be excluded from the study due to vomiting after *Aya2*. Vomiting is commonly reported for liquid ayahuasca but rarely observed after single dose administration of the encapsulated freeze-dried formulation (Riba et al. 2001a; Riba et al. 2003).

The increase in psychotropic effects after the second dose can be explained by the significantly higher DMT levels attained. DMT was still present in blood at 4 h after *Aya1*, and DMT levels from the second dose were superimposed upon the first. The comparison of AUCs (see "Pharmacokinetic analysis" section) showed that the superimposition was linear, that is, no disproportionately higher DMT levels were attained after *Aya2*, when the DMT remaining from *Aya1* is taken into account.

At the subjective level, the results obtained are in line with those in the aforementioned study by Riba et al. (2001a) where researchers found that 0.75 mg DMT/kg produced significant increases in the same VAS items measuring overall psychotropic effects, perceptual modifications, and the VAS item "liking". The present time course of effects is also analogous to that previously reported, with effects peaking at 2 h after dosing. Furthermore, the pattern of responses in the HRS and ARCI are also equivalent. However, in the present study, ayahuasca significantly increased all HRS subscales including Volition, the only subscale that was not modified in the 2001 study. Here, both ayahuasca treatments consistently increased scores on the MBG and A scales of the ARCI. Identical findings were obtained by Riba et al. (2001a). In the present study, the comparison between ayahuasca treatments showed significantly higher somatic and unpleasant effects and impairment after *Aya2*. Auditory effects were also significantly enhanced. However, we did not obtain statistically robust evidence of sensitization. When VAS scores were normalized by DMT levels, we observed only a trend for increased unpleasant effects and for decreased stimulation. These results are in line with those by Strassman et al. (1996) who did not find differences in subjective scores (measured with the HRS) between the first and the fourth of four doses of intravenous DMT administered at 30-min intervals. However, contrary to the present study, the only significant effect observed was a reduction in Volition scores.

Similar to subjective measures, effects after *Aya2* on spontaneous brain electrical activity were larger than after *Aya0*. Ayahuasca increased relative power in the higher end of the beta EEG frequency band. This increase is an objective measure of the effects of ayahuasca on the CNS and has been reported in the past (Riba et al. 2002; Santos et al., in press). No tolerance or sensitization was observed when DMT levels were taken into account.

Unexpectedly, in contrast with subjective and EEG variables, increases in cardiovascular variables were not larger after *Aya2* than after *Aya0,* despite the fact that the 0.75 mg DMT/kg doses increased SBP, DBP, and HR significantly compared with placebo. The examination of the normalized AUCs showed that mean values after *Aya2* were lower than expected from the increased DMT levels present in plasma. The statistical comparison showed a trend towards a significantly lower response after *Aya2* in SBP and HR. This finding suggests that a certain acute tolerance might develop to the inotropic and chronotropic effects of ayahuasca. Strassman et al. (1996) had found non-significant reductions of mean arterial pressure after four closely spaced doses of DMT and a statistically significant reduction in heart rate. Decreases in these variables after repeated ayahuasca or DMT administration could be related to some level of desensitization (Roth et al. 1995; Romano et al. 2010), decreased signaling (Gresch et al. 2005), or downregulation of the 5-HT_{2A} receptor (Smith et al. 1999; Aloyo et al. 2001; Dougherty and Aloyo 2011).

Ayahuasca effects on autonomic variables appear to be more inconsistent than those of pure DMT. Temperature was affected by ayahuasca in the present study. However, despite larger mean values after *Aya2*, only *Aya0* produced statistically significant increases in temperature relative to placebo. A previous study in laboratory conditions did not find a clear-cut pattern of effects for this variable (Santos et al., in press). In contrast, Strassman and Qualls (1994) found increases in rectal temperature for intravenous DMT administered at doses of 0.2–0.4 mg/kg, an effect that was also observed previously for ayahuasca (Callaway et al. 1999) and for serotonergic compounds such as the mixed 5-HT agonist meta-chlorophenylpiperazine (*m*-CPP; Ghaziuddin et al. 2003), which suggests a non-specific hyperthermic effect of serotonergic stimulation. After repeated administration of DMT, Strassman and colleagues found non-significant reductions for this variable (Strassman et al. 1996).

In the present work, pupillary diameter was significantly increased after both ayahuasca doses. Mydriasis has been consistently described for DMT (Rosenberg et al. 1963; 1964; Strassman and Qualls 1994), whereas myosis has been observed for the 5-HT_2 antagonist ketanserin (Koudas et al. 2009). Increases in pupillary diameter were reported for ayahuasca by Callaway et al. (1999), who administered a lower dose (0.48 mg DMT/kg) and compared changes vs. baseline values, and by Santos et al. (in press) for a higher 1.0 mg DMT/kg dose. Increases in pupil diameter after *Aya2* were lower than expected when DMT levels were considered, but this effect did not reach statistical significance, and tolerance development cannot be concluded with certainty. A previous study in humans did not find any tolerance to the mydriatic effect of intramuscular DMT given twice daily for 5 days (Gillin et al. 1976).

At the neuroendocrine level, the observed increases in prolactin and cortisol also replicate previous findings (Santos et al., in press). Though increases after *Aya2* were significantly larger than after *Aya0* for prolactin and cortisol, when DMT levels were taken into account, no tolerance or sensitization was observed. However, the statistical comparison of the normalized AUCs suggests that tolerance develops to GH liberation after repeated exposure to ayahuasca. Strassman et al. (1996) had described acute tolerance development to the neuroendocrine effects of DMT.

Decreased GH after the second ayahuasca dose could be explained by changes at the 5-HT_{1A} receptor. Agonism at this site enhances GH release (Seletti et al. 1995; Pitchot et al. 2002), and consequently, higher GH levels would have been expected after the second ayahuasca dose. However, increased serotonergic tone and prolonged 5-HT_{1A} receptor stimulation have been found to decrease responsiveness at this level. Reduced GH secretion has been observed in humans given the 5-HT_{1A} agonists gepirone and buspirone after pretreatment with paroxetine and fluvoxamine, respectively (Sargent et al. 1997; Anderson et al. 1996). In another study, the same effect was observed for ipsapirone, also a 5-HT_{1A} agonist, after pretreatment with fluoxetine (Lerer et al. 1999). Furthermore, the repeated administration of selective 5-HT_{1A} agonists can lead to decreased receptor responsiveness in rats not only after several days (Assié et al. 2006), but also as early as 15 min after a single dose of 8-OH-DPAT (Riad et al. 2001). The rapid desensitization observed after 8-OH-DPAT was caused by the internalization of the 5-HT_{1A} receptor. Both prolonged activation of the 5-HT_{1A} receptor sites by DMT and increased serotonin caused by MAO inhibition (harmine and harmaline; McKenna et al. 1984) and serotonin reuptake inhibition (THH; Buckholtz and Boggan 1977) could have led to 5-HT_{1A} desensitization and decreased GH after the second dose.

The immunomodulatory effects of ayahuasca were analogous to those previously reported (Santos et al., in press), i.e., decreased CD4 and elevated NK subpopulations compared with placebo. Effects on CD19 and CD3 cells were less consistent and non-significant. No tolerance or sensitization was observed for any of the studied variables.

The absence of acute tolerance development for most of the variables assessed in the present investigation mimics results for DMT, the main active principle in ayahuasca. Clear tolerance has not been reported in animals (Cole and Pieper 1973; Gillin et al. 1973; Kovacic and Domino 1976) or in humans (Gillin et al. 1976) in the older literature. In the more recent study in humans by Strassman et al. (1996),

differential effects were observed depending on the studied variable. Subjective effects remained unchanged, but heart rate and ACTH, and prolactin levels, showed acute tolerance after repeated administration within a single experimental session. An analogous dissociation would be observed to a certain extent for ayahuasca.

The present study was limited by the small sample size. This was largely due to the adverse events associated with repeated ayahuasca intake. Five volunteers were excluded due to vomiting, which in three instances occurred after the administration of the second dose. Consequently, our results were obtained from those participants who tolerated ayahuasca better and may not be easily generalized.

In conclusion, the administration of two consecutive doses of ayahuasca led to higher DMT concentrations in plasma and increased psychotropic effects. The second dose was less well-tolerated leading to a higher incidence of unpleasant effects and vomiting. With regard to acute tolerance or sensitization development, a certain dissociation was observed. Whereas neither phenomenon was found for subjective, neurophysiological, autonomic, and immunological effects, tolerance was observed for GH and a trend for SBP and HR.

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Summary of results

Main results

Study 1

a) Statistically significant differences between ayahuasca and *d*-amphetamine in the HRS-Perception and HRS-Cognition scales highlighted the psychedelic effects of ayahuasca, absent after *d*-amphetamine. However, psychostimulant-like activation after ayahuasca was confirmed by significant increases relative to placebo in the ARCI-A scale. Ayahuasca did not statistically differ from *d*-amphetamine in this scale, nor in the euphoria-measuring ARCI-MBG scale.

b) Ayahuasca induced time-dependent increases were found for relative power of the beta EEG band. These effects were significantly different from those induced by the two control treatments, i.e., placebo and *d*-amphetamine.

c) Ayahuasca showed only mild effects on autonomic variables. Body temperature showed an initial decrease that was significant only at 30 min after dosing. No changes were observed thereafter, nor for respiration rate, throughout the study period. A mydriatic effect was observed both for ayahuasca and *d*-amphetamine. Increases in pupil diameter after ayahuasca were significant between 0.5 and 2 hours, while those after *d*-amphetamine remained significant even after 24 hours. Ayahuasca decreased the amplitude of the pupillary light reflex and increased its latency.

d) Prolactin levels were increased after ayahuasca but not after *d*-amphetamine. Neither drug elevated growth hormone levels significantly, although mean values appeared to be larger after ayahuasca than after placebo and *d*-amphetamine. Both active treatments significantly increased cortisol. Cortisol levels after ayahuasca were significantly higher than after *d*-amphetamine between 1 and 2 hours after dosing.

e) Ayahuasca and *d*-amphetamine induced similar modifications in the distribution of lymphocyte subpopulations. Percent CD4 and CD3 were decreased, while NK cells were increased. CD8 and CD19 were not consistently modified. These changes peaked at 2 hours after dosing and had returned to baseline levels at 24 hours.

Study 2

a) Ayahuasca was less well tolerated after administration of the second dose. Nausea and vomiting events were more frequently observed.

b) DMT plasma levels after the second of two consecutive ayahuasca doses were larger than after a single dose. However, this increase was not disproportionate and DMT levels after the second dose resulted from the superimposition over the remaining DMT levels of the preceding dose.

c) Some aspects of the subjective experience presented more intense effects after the second dose. The HRS-Somaesthesia, HRS-Volition, VAS-Any Effect, VAS-Auditory Effects and VAS-Bad Effects showed significantly higher scores than after the single dose. The HRS-Perception scale showed higher scores that were marginally significant ($p=0.050$). At the neurophysiological level, relative power in the beta-4 and beta-5 subbands was significantly enhanced compared to the single dose.

d) Cardiovascular (SBP, DBP and HR) and autonomic measures (temperature and pupillary diameter) were not statistically different between the single dose and the second of two consecutive doses. On the other hand, increases in prolactin and cortisol were significantly larger after the second of two consecutive doses than after a single dose. For growth hormone, a trend to lower secretion was observed after the second dose than after the single dose.

e) Analysis of lymphocyte subpopulations showed larger decreases in total lymphocytes and CD19 B cells after the second than after the single dose.

f) The analysis of VAS item scores normalized by DMT plasma concentrations showed neither tolerance nor sensitization for most items. However, a trend to non-linear relationships was found for two items. Scores on the VAS-Bad Effects were disproportionately larger after the second of two doses as compared with the single dose. This effect approached statistical significance ($p=0.057$). On the other hand,

scores on the DMT-normalized VAS-Stimulated item showed lower scores after the second dose. Again, this effect approached but did not reach statistical significance (p=0.064). No disproportionate increases or decreases were observed for neurophysiological variables between the second and the single ayahuasca doses taking DMT levels into account. Neither tolerance nor sensitization could be determined for these variables.

g) The analysis of the DMT-normalized data showed a trend decrease for SBP (p=0.065) and heart rate (0.096) after the second of two consecutive doses compared to a single dose. No statistical differences were found for autonomic variables, prolactin and cortisol. On the other hand, growth hormone levels were significantly lower after the second dose, suggesting tolerance development for this variable. No significant effects were observed for total lymphocytes, nor were for any of the lymphocyte subpopulations studied.

DISCUSSION

Previous clinical studies with ayahuasca involved the administration of single doses in the absence of active comparators. In Study 1, the simultaneous investigation of ayahuasca and *d*-amphetamine, a classic psychostimulant, in the same participant sample allowed a better characterization of the neurobiology of ayahuasca. Study 2 addressed the pharmacology of two repeated doses of ayahuasca, in terms of tolerability and the potential occurrence of tolerance or sensitization for a wide number of study variables. Repeated dose assessment is relevant given the common practice of ingesting multiple doses in a single session, especially in the context of the ayahuasca religions.

The assessment of subjective effects in Study 1 showed some overlapping between the effects of ayahuasca and *d*-amphetamine. Both drugs induced subjectively perceived arousal and euphoria. The general activation effects of psychedelics were already described in the early studies with LSD (see for instance the review by Brawley and Duffield, 1972) but the mechanism underlying it has not been clearly elucidated. Despite this shared arousing effect, *d*-amphetamine was differentially perceived by participants to increase intellectual energy and efficiency. The dopaminergic and noradrenergic mechanism of *d*-amphetamine could account for this. Differences at the neurochemical level could also account for the differential effects observed on the EEG. The energy shift observed after ayahuasca, which involved relative power increases in the faster beta subbands, was absent after *d*-amphetamine.

In contrast with subjective effects, pharmacological activity after both drugs was not so easily evidenced when autonomic variables were analyzed. Body temperature and respiration rate remained virtually unchanged. Previous studies with DMT found inconsistent results for body temperature (Rosenberg et al., 1963, 1964; Strassman and Qualls, 1994; Strassman et al., 1996) and increases after LSD and psilocybin, also 5-HT_{2A} agonists (Gouzoulis-Mayfrank et al., 1999; see for a review Passie et al., 2002, 2008; Hintzen and Passie, 2010). An early study with DMT did not find changes in respiration (Rosenberg et al., 1963) and no changes have been reported for LSD (Passie et al., 2008; Hintzen and Passie, 2010).

The most robust indicator of sympathetic activation after ayahuasca was pupil diameter. A mydriatic effect was observed for this drug and, as expected, also for *d*-amphetamine. Increases in pupil diameter after ayahuasca were significant between 0.5 and

2 h, mean values returning to placebo levels at 8 h. On the other hand, the mydriatic effect after *d*-amphetamine was much longer, remaining significant even after 24 h. Mydriasis, and hence sympathomimetic activation, was consistently demonstrated for DMT in the past (Rosenberg et al., 1963, 1964; Strassman and Qualls, 1994). In the study by Callaway et al. (1999) with ayahuasca, the authors also reported increased pupillary diameter. This effect could be explained by DMT agonism at the 5-HT_{2A} receptor and simultaneous increase in norepinephrine release after MAO inhibition by the β-carbolines (Riba et al., 2003). LSD and psilocybin also produce pupillary dilation (Passie et al., 2002, 2008; Hintzen and Passie, 2010).

Ayahuasca also decreased the amplitude and increase the latency of the pupillary light reflex, effects typically ascribed to anticholinergic drugs. Since none of the components of ayahuasca seem to display affinity at muscarinic receptor sites (see for a review Riba, 2003), a potential explanation is that the observed effects could be due to the noradrenergic inhibition of parasympathetic neurotransmission in the Edinger-Westphal nucleus (EWN), the CNS nucleus that controls constriction of the iris. The mixed serotonin/noradrenaline reuptake inhibitor venlafaxine has also been found to produce similar effects (Bitsios et al., 1999; Siepmann et al., 2007).

Regarding neuroendocrine variables, only ayahuasca produced significant increases in prolactin levels. Neither of the active treatments produced significant changes in GH levels, but mean values were increased after ayahuasca. Cortisol, a stress indicator, was increased by both drugs, with ayahuasca leading to higher increases. Cortisol levels after ayahuasca were significantly higher than after *d*-amphetamine between 1 and 2 h after dosing. This effect suggests an intense activation of the hypothalamic-pituitary-adrenal axis by ayahuasca. Previous studies with ayahuasca reported increases in prolactin, cortisol and GH levels (Callaway et al., 1999; Pomilio et al., 1999, 2003).

Modulation of prolactin, cortisol and GH may well be mediated by the serotonergic effects of ayahuasca. Agonism at the 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors has been found to increase plasma prolactin *in vivo* (Freeman et al., 2000). DMT is an agonist at the 5-HT_{1A} receptor (Pierce and Peroutka, 1989; McKenna et al., 1990; see for a review Riba, 2003; Nichols, 2004), and administration of 5-HT_{1A} agonists induces increases in GH, cortisol and prolactin secretion (Seletti et al., 1995; Pitchot et al., 2002; see also Nichols

and Nichols, 2008). Moreover, Strassman (1996) found reduced prolactin response in a DMT study after a pre-treatment with a 5-HT_{1A} antagonist, which supports a stimulatory role for the 5-HT_{1A} site in human prolactin secretion. Other serotonergic drugs also produce similar effects in neuroendocrine measures: e.g., citalopram (Seifritz et al., 1996; Attenburrow et al., 2001; Nadeem et al., 2004; Hawken et al., 2009), fenfluramine (Coccaro et al., 1996), and meta-chlorophenylpiperazine (*m*-CPP) (McCann et al., 1999; Ghaziuddin et al., 2003). Nevertheless, the lack of a significant result in GH secretion after ayahuasca could be due to the lower affinity of DMT for the 5-HT_{1A} receptor (Pierce and Peroutka, 1989; McKenna et al., 1990). Since the effects of *d*-amphetamine are dopaminergic and noradrenergic, this drug was not expected to produce increases in prolactin levels (Fitzgerald and Dinan, 2008).

The 5-HT_{2A} agonism of DMT can explain the overall neuroendocrine profile of ayahuasca. DMT increases serum levels of prolactin, GH and cortisol in humans (Meltzer et al., 1982; Strassman and Qualls, 1994; Strassman et al., 1996). Psychedelics similar to DMT produce similar neuroendocrine changes. LSD increases GH levels (Passie et al., 2008; Hintzen and Passie, 2010), while psilocybin increases prolactin and cortisol levels (Hasler et al., 2004).

Another aspect we wanted to address in the present work was the potential immunomodulating effects of ayahuasca. Both ayahuasca and *d*-amphetamine induced temporary redistributions of lymphocyte subpopulations. Decreases in CD3 and CD4 percentages were observed, together with increases in NK cells. These effects were time-limited, returning to baseline levels at 24 h. In the case of ayahuasca, these effects could possibly be produced by direct activation of serotonergic receptors, since 5-HT_{1A} and 5-HT_{2A} receptors can modulate the immune system (Idova et al., 2008; Davydova et al., 2010). Nevertheless, since *d*-amphetamine produced similar effects, they could also be explained by the activation of the sympathetic nervous system (SNS) and the increased cortisol release produced by both drugs. SNS activation and cortisol release have a modulatory effect on lymphocytes (Friedman and Irwin, 1997). The acute stress-like reaction and stimulation of the hypothalamic-pituitary-adrenal axis by both drugs could also explain these effects (Strassman and Qualls, 1994; Breznitz et al., 1998; Van de Kar and Blair, 1999; Armario, 2010).

It is not easy to speculate about the possible health impact of acute ayahuasca ingestion in the immune system. Reductions in CD3 and CD4 cells could potentially lead to deleterious effects by decreasing the body's ability to fully destroy infected cells and to produce antibodies (Chaplin, 2010). However, increases in NK cells could be potentially beneficial by increasing the body's defense against virally infected and cancerous cells (Caliguri, 2008; Lanier, 2008). Future studies should investigate the long-term effects of ayahuasca in the immune function after chronic consumption of the brew.

Ingesting several ayahuasca doses within a single session is a common practice amongst ritual ayahuasca users. One of the research questions we wished to address was the degree of tolerability of two consecutive doses as compared to a single ayahuasca dose. Results showed that ayahuasca was less well tolerated after administration of the second dose. This is reflected by the fact that only nine of the original 17 volunteers completed the study. In this regard, it is important to note that four volunteers were excluded due to ayahuasca-induced vomiting. Whereas only one of the four vomited after receiving the first ayahuasca dose, the other three did after receiving the second dose. Whereas vomiting has been reported in studies of *β*-carbolines in humans (Pennes and Hoch 1957; Naranjo, 1967, 1976, 1987) and in ayahuasca ceremonies, it has been rarely observed in studies involving single dose administration of encapsulated freeze-dried ayahuasca (Riba et al. 2001b; Riba et al. 2003).

Some researchers have proposed that nausea, vomiting and diarrhea may result from increasing levels of unmetabolized serotonin after MAO inhibition by the *β*-carbolines, with subsequent increased vagal stimulation and intestinal motility, produced by central and peripheral serotonin, respectively (Callaway et al., 1999; Callaway, 2005b). The second consecutive ayahuasca dose could have produced more nausea and vomiting by increasing the ingestion of MAO-inhibiting *β*-carbolines. On the other hand, 5-HT_{2A} receptor agonism might also be involved in the nausea and vomiting induced by ayahuasca, since in the case of LSD nausea may also occur, although emesis is exceptional (Passie et al., 2008; Hintzen and Passie, 2010). Psilocybin may also produce nausea (Passie et al., 2002).

DMT plasma levels after the second of two consecutive ayahuasca doses were larger than after a single dose. However, DMT was still present in blood 4 h after the first

ayahuasca dose, and DMT levels from the second dose were superimposed over the remaining DMT levels of the preceding dose. The statistical analysis showed that the superimposition was linear, that is, no disproportionately higher DMT levels were attained after the second of two consecutive ayahuasca doses, when the DMT remaining from the preceding ayahuasca dose was taken into account.

In line with the higher DMT levels attained, virtually all subjective effects measures showed higher mean values after the second consecutive ayahuasca dose. Psychotropic effects were more intense as were unpleasant somatic effects and impairment. This increase in unpleasant effects corroborated the reduced tolerability of ayahuasca after the second dose. Analogously, neurophysiological effects were also higher after the second dose.

After the single dose, ayahuasca produced cardiovascular effects compatible with sympathomimetic activation: moderate but significant increases in systolic and diastolic blood pressure and in heart rate. Although observed increases were not large, some participants met criteria for hypertension and tachycardia, again pointing out a worse tolerability of repeated dosing. Cardiovascular effects could be explained by agonism at the 5-HT_{2A} receptors and potentially by the indirect increase in norepinephrine release after MAO inhibition. Activation of 5-HT_{2A} receptors causes rises in blood pressure and generalized sympathetic activation (Ramage and Villalón, 2008). LSD produces increases in heart rate and blood pressure (Passie et al., 2008; Hintzen and Passie, 2010), as well as psilocybin (Gouzoulis-Mayfrank et al., 1999; Passie et al., 2002; Hasler et al., 2004; Griffiths et al., 2006; Moreno et al., 2006; Griffiths et al., 2011; Grob et al., 2011). Unexpectedly, despite the increased DMT plasma levels after the second dose, increases in cardiovascular variables were not larger than after the first dose.

Ayahuasca effects on body temperature were inconsistent. Only one of the two treatment combinations, i.e., the ayahuasca dose after placebo, showed significant modifications. On the other hand, increases in pupillary diameter replicated results in Study 1. A mydriatic effect was observed after both treatment combinations. However, no significant differences were found between the single dose and the second of two consecutive doses for either temperature or pupillary diameter.

Ayahuasca produced significant increases in prolactin, GH and cortisol levels, which have been already reported in previous studies (Callaway et al., 1999; Pomilio et al., 1999, 2003). Except for GH, these findings also replicate the results from Study 1. As discussed above, the specific effects of DMT on 5-HT_{1A} (Seletti et al., 1995; Strassman, 1996; Pitchot et al., 2002; see also Nichols and Nichols, 2008) and especially on 5-HT_{2A} (Meltzer et al., 1982; Strassman and Qualls, 1994; Strassman et al., 1996) receptor activation might explain these results. Increases in prolactin and cortisol levels were significantly larger after the second of two consecutive doses than after a single dose. For GH, a trend was observed to lower secretion after the second dose than after the single dose.

The immunomodulatory effects of ayahuasca were analogous to those reported in Study 1, i.e., decreased CD4 and elevated NK subpopulations compared with placebo. Larger decreases in total lymphocytes and CD19 B cells were reported after the second consecutive ayahuasca dose than after the single dose. As pointed above, these effects could be produced by direct activation of serotonergic 5-$HT_{1A/2A}$ receptors (Idova et al., 2008; Davydova et al., 2010), activation of the SNS and increased cortisol release (Friedman and Irwin, 1997), and production of a stress-like reaction and stimulation of the hypothalamic-pituitary-adrenal axis (Strassman and Qualls, 1994; Breznitz et al., 1998; Van de Kar and Blair, 1999; Armario, 2010).

In Study 2, the possible occurrence of tolerance or sensitization was evaluated. Tolerance is the occurrence of reduced responses to a given drug dose after repeated administration of the compound. Acute or rapid tolerance is the shortened duration and decreased intensity of drug effects that occurs within hours of administration (Barbanoj et al., 2007); whereas chronic tolerance is the occurrence of the same process in a larger period of time (Pazos, 2003). The mechanisms involved in the development of tolerance are only partially understood, and can be related to the induced synthesis of the hepatic enzymes involved in drug biotransformation, resulting in increased metabolism and reduced concentrations of the drug at the sites of action (*dispositional* or *pharmacokinetic tolerance*) or to some type of cellular adaptation within the affected systems, reducing the response in the presence of a given concentration of the drug (*pharmacodynamic tolerance*) (Jaffe, 1990; Nies, 1990). Pazos (2003) associates the definition of tolerance to the desensibilization of the receptors. Sensitization, sometimes also called “reverse

tolerance", on the other hand, would be characterized by an unexpected increase in the effects of a given substance after repeated administration, and is associated with a hipersensibilization of the receptors (Jaffe, 1990; Pazos, 2003).

Substances like alcohol, cocaine, nicotine, amphetamine, opioids, barbiturates, cannabis, benzodiazepines and the ring-substituted amphetamine MDMA can produce tolerance in animals and also in humans (Jaffe, 1990; Farré et al., 2001; Strakowski et al., 2001; Tomillero, 2001; Ayesta and Camí, 2003; Farré et al., 2004; Barbanoj et al., 2007; Gonzalez et al., 2007). On the other hand, sensitization has been described for alcohol, cocaine, nicotine, amphetamine, opioids, MDMA and Δ^9-tetrahydrocannabinol (Jaffe, 1990; Kalivas et al., 1998; Rubino et al., 2001; Strakowski et al., 2001; Fish et al., 2002; Todtenkopf and Carlezon, 2006; Liu et al., 2007; Biala and Staniak, 2010).

Regarding psychedelics, the exhaustive reviews of the pharmacology of these substances by Nichols (2004) and Fantegrossi et al. (2008) state that a very rapid tolerance known as tachyphylaxis is produced on repeated administration of these substances, both in animals and humans (Isbell et al., 1956; Rosenberg et al., 1964; see for a review Wyatt et al., 1976). The mechanism underlying such tolerance appears to be the down-regulation (Smith et al., 1999; Aloyo et al., 2001; Dougherty and Aloyo, 2011) and desensitization (Roth et al. 1995; Gresch et al., 2005; Romano et al., 2010) of 5-HT_{2A} receptors.

Nevertheless, DMT appears to be different from other serotonergic psychedelics such as LSD, mescaline, and psilocybin, in terms of its tolerance-inducing capacity. It is difficult to elicit tolerance to DMT in animals (Cole and Pieper, 1973; Gillin et al., 1973; Kovacic and Domino, 1976; Stoff et al., 1977, cited in Gillin et al., 1978; see for a review Gillin et al., 1978; Barker et al., 1981; Riba, 2003). In fact, some studies reported that the responses to DMT tended to increase in magnitude with repeated administration, suggesting the occurrence of sensitization (Cole and Pieper, 1973; Gillin et al., 1973; Kovacic and Domino, 1976).

In humans, a previous report demonstrated no tolerance development with twice daily sessions of DMT, separated by 5 h, for 5 consecutive days (Gillin et al., 1976). Strassman et al. (1996) conducted a study involving the administration to healthy volunteers of four consecutive doses of 0.3 mg/kg i.v. DMT at 30 min intervals. These

researchers did not find tolerance to the subjective effects or blood pressure increases, whereas neuroendocrine responses and heart rate decreased from the first to the fourth administered dose.

Cross-tolerance between LSD and mescaline and between LSD and psilocybin has been described in animals and humans (Wyatt et al., 1976; Jaffe, 1990; Spinella, 2001; Nichols, 2004). Nevertheless, little or no cross-tolerance occurs between DMT and LSD (Mahler and Humoller, 1959, cited in Hintzen and Passie, 2010; Kovacic and Domino, 1976; Jaffe, 1990), and LSD-tolerant individuals show undiminished responses to DMT (Rosenberg et al., 1964). These data would suggest that DMT shows some paradoxical characteristics that do not quite fit the typical profile of the serotonergic psychedelics. Smith et al. (1998) demonstrated that DMT displays agonist activity at the 5-HT_{2A} and 5-HT_{2C} receptor sites. This study also showed that DMT produces tolerance at the 5-HT_{2C} receptor, but not at the 5-HT_{2A}. This finding is important, since the mechanism underlying the development of tolerance to the other psychedelics appears to be the down-regulation and desensitization of 5-HT_{2A} receptors, as reported above.

Regarding ayahuasca, anecdotal reports appear to indicate that repeated use in a single session leads to more intense effects, rather than tolerance. This effect could be due to two main reasons. First, the aforementioned absence of tolerance observed for DMT and second, the MAO-inhibiting properties of a second ayahuasca dose, which could add up to those of the first dose.

Statistically robust evidence of tolerance or sensitization for the subjective effects was not obtained in Study 2. When VAS scores were normalized by DMT levels, only a trend to non-linear relationships for two items was observed. A trend ($p=0.057$) for increased unpleasant effects was reflected in disproportionately larger scores on the VAS-Bad Effects after the second consecutive ayahuasca dose as compared with the single dose. Again this result supported the reduced tolerability of the second consecutive ayahuasca dose. A trend ($p=0.064$) was also reported for decreased stimulation. Scores on the VAS-Stimulated item were reduced after the second consecutive dose. These results are in line with those by Strassman et al. (1996), i.e., no tolerance to the subjective effects of DMT. However, in contrast with the findings from Study 2, the only significant effect observed by Strassman et al. was a reduction in HRS-Volition scores.

Since no disproportionate increases or decreases were observed for neurophysiological variables (relative power of the beta EEG subbands) when taking DMT levels into account, neither tolerance nor sensitization could be determined for these variables, even considering the higher DMT levels attained after the second dose.

The examination of the DMT-normalized data for the cardiovascular variables showed that mean values after the second consecutive ayahuasca dose were lower than expected from the increased DMT levels present in plasma. The analysis showed a trend decrease for SBP ($p=0.065$) and heart rate ($p=0.096$) after the second of two consecutive doses compared to a single dose. These results suggest that a certain level of acute tolerance might develop for these measures. In the study by Strassman et al. (1996) on repeated DMT administration, non-significant reductions of mean arterial pressure and a significant reduction in heart rate were reported. Activation of 5-HT_{2A} receptors causes rises in blood pressure and generalized sympathetic activation (Ramage and Villalón, 2008). LSD and psilocybin produce increases in heart rate and blood pressure (Gouzoulis-Mayfrank et al., 1999; Hasler et al., 2004; Griffiths et al., 2006; Moreno et al., 2006; Griffiths et al., 2011; Grob et al., 2011; see for a review Passie et al., 2002, 2008; Hintzen and Passie, 2010). Decreases in SBP and heart rate after the second consecutive ayahuasca dose could be related to some level of down-regulation (Smith et al., 1999; Aloyo et al., 2001; Dougherty and Aloyo, 2011) and desensitization (Roth et al. 1995; Gresch et al., 2005; Romano et al., 2010) of 5-HT_{2A} receptors.

No robust indications of tolerance or sensitization were found for autonomic variables. Increases in pupil diameter after the second consecutive ayahuasca dose were lower than expected when DMT levels were considered, but this effect did not reach statistical significance. A previous study in humans did not find any tolerance to the mydriatic effect of DMT (Gillin et al. 1976). Strassman et al. (1996) found non-significant reductions for body temperature after repeated DMT administration.

Though increases after the second consecutive ayahuasca dose were significantly larger than after the single dose for prolactin and cortisol, when DMT levels were taken into account no tolerance or sensitization was observed. These results contrast with those reported by Strassman et al. (1996), who had described acute tolerance development to

prolactin after repeated DMT administration. The statistical comparison of the normalized GH data suggests that tolerance develops for this variable. Since DMT is an agonist at the 5-HT_{1A} receptor (Pierce and Peroutka, 1989; McKenna et al., 1990; see for a review Riba, 2003; Nichols, 2004), and administration of 5-HT_{1A} agonists induce increases in GH levels (Seletti et al., 1995; Pitchot et al., 2002; see also Nichols and Nichols, 2008), decreased GH after the second ayahuasca dose could be explained by changes at this receptor. Repeated administration of selective 5-HT_{1A} agonists can lead to decreased receptor responsiveness in rats after several days (Assié et al., 2006) and even as early as 15 min after a single dose (Riad et al., 2001). Moreover, increased serotonin release caused by ayahuasca could also contribute to 5-HT_{1A} receptor desensitization and decreased GH secretion. MAO inhibition by harmine and harmaline and serotonin reuptake inhibition by THH could potentially increase serotonin levels. Reduced GH secretion has been observed in humans given 5-HT_{1A} agonists after pretreatment with selective serotonin reuptake inhibitors (Anderson et al., 1996; Sargent et al., 1997; Lerer et al., 1999).

Finally, no tolerance or sensitization was observed for total lymphocytes or for any of the lymphocyte subpopulations studied, even taking into account the larger decreases in total lymphocytes and CD19 B cells reported after the second consecutive ayahuasca dose.

CONCLUSIONS

Main conclusions:

Study 1

a) Ayahuasca induced distinct psychedelic effects, which were absent after *d*-amphetamine. However, ayahuasca also induced general activation effects analogous to those of a prototypical psychostimulant.

b) Despite commonalities at the subjective level, the administration of ayahuasca produced a different pattern of neurophysiological effects. Ayahuasca-induced increases in the relative power of the EEG beta band were absent after *d*-amphetamine.

c) Increased pupillary diameter after both drugs suggest shared sympathomimetic effects. Noradrenergic inhibition of parasympathetic neurotransmission in the Edinger-Westphal nucleus could explain the observed mydriatic effect of ayahuasca.

d) Both ayahuasca and *d*-amphetamine activated the hypothalamic-pituitary-adrenal axis as reflected by elevations in plasma cortisol. However, mechanistic differences were highlighted by the pro-prolactinergic effects of ayahuasca, not observed for *d*-amphetamine. The neuroendocrine profile of ayahuasca further supports its interaction with serotonergic neurotransmission.

e) Ayahuasca and *d*-amphetamine induced similar modifications in the distribution of lymphocyte subpopulations, which possibly reflect an unspecific modulation of cell immunity caused by sympathomimetic activation and cortisol secretion after both drugs. No evidence was found of a specific effect of ayahuasca on cell immunity.

Study 2

a) The administration of ayahuasca in two consecutive doses led to poorer tolerability. This was evidenced by a higher incidence of undesirable effects, especially nausea and vomiting.

b) Higher DMT plasma levels were observed after the second of two consecutive doses. However, these increases were not disproportionate but linear.

c) Overall, somatic, auditory and incapacitating effects were more intense after the second of two consecutive ayahuasca doses. Neurophysiological effects, prolactin and cortisol levels were also increased, but not cardiovascular and autonomic effects.

d) At the cellular immunity level, the only variations observed were larger decreases in total lymphocytes and CD19 B cells after the second of two consecutive ayahuasca doses.

e) Neither tolerance nor sensitization were observed for subjective or neurophysiological effects. Only a trend was observed to more intense unpleasant effects.

f) Neither tolerance nor sensitization were observed for cardiovascular, autonomic, immunomodulatory effects, and prolactin and cortisol secretion. A trend was observed to tolerance for SBP and heart rate. Tolerance was observed for growth hormone secretion after the second dose.

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