TESI DOCTORAL

HUMAN PHARMACOLOGY OF *AYAHUASCA*

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A la Núria, el Marc i l'Emma.

No pasaremos en silencio una de las cosas que á nuestro modo de ver llamará la atención... toman un bejuco llamado *Ayahuasca* (bejuco de muerto ó almas) del cual hacen un lijero cocimiento...esta bebida es narcótica, como debe suponerse, i á pocos momentos empieza a producir los mas raros fenómenos...Yo, por mí, sé decir que cuando he tomado el *Ayahuasca* he sentido rodeos de cabeza, luego un viaje aéreo en el que recuerdo percibia las prespectivas mas deliciosas, grandes ciudades, elevadas torres, hermosos parques i otros objetos bellísimos; luego me figuraba abandonado en un bosque i acometido de algunas fieras, de las que me defendia; en seguida tenia sensación fuerte de sueño del cual recordaba con dolor i pesadez de cabeza, i algunas veces mal estar general.

Manuel Villavicencio
Geografía de la República del Ecuador (1858)

Das, was den Indianer den “Aya-huasca-Trank” lieben macht, sind, abgesehen von den Traumgesichten, die auf sein persönliches Glück Bezug habenden Bilder, die sein inneres Auge während des narkotischen Zustandes schaut.

Louis Lewin
Phantastica (1927)

Agraïments

La present tesi doctoral constitueix la fase final d'una idea nascuda ara fa gairebé nou anys. El fet que aquest treball sobre la farmacologia humana de l'*ayahuasca* hagi estat una realitat es deu fonamentalment al suport constant del seu director, el Manel Barbanoj. Voldria expressar-li la meva gratitud pel seu recolzament entusiàstic d'aquest projecte, molt allunyat, per la natura del fàrmac objecte d'estudi, dels que fins al moment s'havien dut a terme a l'Àrea d'Investigació Farmacològica de l'Hospital de Sant Pau. L'interès inexhaurible del Dr. Barbanoj per tots els aspectes de la psicofarmacologia humana i la llibertat que m'ha donat al llarg d'aquests anys per dur a terme aquest treball de recerca han estat uns dels principals atractius d'aquesta feina.

No puc oblidar en aquests agraïments als iniciadors de la idea i exploradors de terres incògnites Josep Maria Fericgla i Esther Martínez. A ells dec haver dirigit la meva atenció cap aquest fascinant preparat amazònic. Així mateix, sense la col·laboració inestimable de l'Esther Martínez, el Félix González i el Josep Maria Fàbregas l'*ayahuasca* no hauria arribat mai al nostre laboratori i aquest estudi mai no s'hauria portat a terme. El meu agraïment també als membres de CEFLURIS a Brasil per haver accedit a facilitar les mostres d'*ayahuasca* (*Daime*) que es van emprar a l'estudi clínic.

També estic en deute amb el Francesc Jané, pel seu suport com a cap del Servei de Farmacologia Clínica de l'Hospital de Sant Pau; amb el James C. Callaway, per dur a terme les anàlisi del contingut de DMT de l'*ayahuasca*; amb la Graciela Salazar, per ajudar-me en l'escriptura inicial del protocol de recerca i amb el Rick J. Strassman, per supervisar l'adaptació del qüestionari HRS i per facilitar-me les dades de farmacocinètica obtingudes al seu estudi d'administració intravenosa de DMT. El meu agraïment a la Gloria Urbano per la seva amistat incondicional en moments difícils i per les llargues hores que vam compartir durant la fase experimental de l'estudi. Gràcies al Toni Rodríguez, sobretot per fer-me de mestre en l'adquisició i processament dels registres neurofisiològics; al Peter Anderer, pel seu paper fonamental en la quantificació de les dades d'EEG i LORETA i a la Marta Valle, per la seva col·laboració en l'estudi i interpretació de les dades cinètiques.

El meu reconeixement també per la Mercedes Yritia, l'Araceli Castillo i la Yolanda Alfaro del laboratori d'anàlisi pel seu esforç en la determinació de les β-carbolines (tantes) i al Rafael de la Torre i al Jordi Ortuño del laboratori d'anàlisi de l'IMIM per la determinació de la DMT. Gràcies al Magí Farré, també de l'IMIM, per tota la informació facilitada directa o indirectament sobre les tècniques de mesura dels efectes subjectius dels fàrmacs i al Josep Maria Queraltó del Servei de Bioquímica de Sant Pau per la determinació del metabolits de les monoamines. Als companys del CIM, particularment a l'Adelaida Morte, la Susanna Clos, la Llúcia Benito, el David Martínez i la Sylvie Cotxet, per la seva col·laboració en la recollida de les dades experimentals. A l'Ángeles Funes i el Pablo Ayesta per la seva ajuda en l'edició dels manuscrits i a la Carolyn Newey per les seves pacients i exhaustives revisions de l'anglès.

Finalment estaré eternament agraït als meus avis, pares i germana per fer-me la vida molt més amable i a la Núria per haver estat sempre al meu costat tot aquest temps.

CONTENTS

INTRODUCTION

1. Early reports on *ayahuasca*, its botanical sources and uses

Ayahuasca is the Quechua name used to designate a traditional psychotropic plant beverage widely used by the indigenous peoples of northwestern South America. The area of use has been estimated to extend from Panama to Amazonian Peru and Bolivia and from the coastal areas of Colombia and Ecuador to the Río Negro in Brazil (Ott, 1993). While the term *ayahuasca*, which also designates the plant the beverage is made from, is used in Peru and some areas of Ecuador and Colombia, this psychotropic tea is also known by many other vernacular names. The term *caapi* is employed in the river Vaupés, *yajé* or *yagé* in southern Colombia, *Daime* or *Hoasca* in Brazil, *natema* in Ecuador and *pinde* along the Pacific coast of Colombia. More than 70 indigenous groups are known to employ *ayahuasca*, for which 42 different vernacular names have been reported (Luna, 1986). The use of this pan-Amazonian psychotropic beverage appears to be very old, since according to Plutarco Naranjo, several pottery artifacts which could have been used for *ayahuasca* preparation/ingestion date back to 2000–1000 B.C. (Naranjo, 1986). Oddly enough, *ayahuasca* use had apparently remained unknown by the outside world until very recent times. Despite the fact that European exploration of the Amazon had taken place as early as 1541, the first written reference to its use was made well into the 18th century by Jesuit priests travelling through the region (Naranjo, 1986). This is in sharp contrast with the ritual use of other visionary plants and preparations such as peyote and the *cohoba* snuff which had been known to the Spaniards since the early days of their arrival in the New World (Sahagún, 2001; Wassen, 1967).

The plant source of *ayahuasca* was first described in the 19th century. In 1852, the English botanist Richard Spruce observed the use of a "climbing plant" called *caapi* as an intoxicant by Tukanoan tribes in the Vaupés river in northwestern Brazil, and characterized the plant as an undescribed *Banisteria* of the Malpighiaceae family. Spruce named it *Banisteria caapi* (see Figure 1) and collected specimens for posterior chemical analysis, which would not be conducted until more than a hundred years later. He also described a communal celebration in which *caapi* was served and commented on the effects exerted by the brew (Spruce, 1908):

> In two minutes or less after drinking it, its effects begin to be apparent. The Indian turns deadly pale, trembles in every limb, and horror is in his aspect. Suddenly contrary symptoms succeed: he bursts into a perspiration, and seems possessed with reckless fury, seizes whatever arms are at hand, his murucú, bow and arrows,

> or cutlass, and rushes to the doorway, where he inflicts violent blows on the ground or the doorposts, calling out all the while, 'thus would I do to mine enemy (naming him by his name) were this he!' In about ten minutes the excitement has passed off, and the Indian grows calm, but appears exhausted.

Spruce partook of *ayahuasca* but all he experienced was a "strong inclination to vomit". However, he further commented that travelers he had talked to had experienced rather remarkable effects:

> Alternations of cold and heat, fear and boldness. The sight is disturbed, and visions pass rapidly before the eyes, wherein everything gorgeous and magnificent they have heard or read of seems combined; and presently the scene changes to things uncouth and horrible…intelligent traders on the Upper Rio Negro, Uaupés and Orinoco have all told me the same tale, merely with slight personal variations. A Brazilian friend that when he took a full dose of *caapi* he saw all the marvels he had read of in the Arabian Nights pass rapidly his eyes as in a panorama; but the final sensations and sights were horrible, as they always are.

Two years later, Spruce observed the use of *caapi* in the Orinoco by the Guahibo Indians who drank the infusion and also chewed the dried stem. Again, in 1857 in the foothills of the Andes in the area of the river Pastaza in Ecuador he saw *caapi* was cultivated by the Záparo Indians, although it was known under the name *ayahuasca*, meaning "Dead man's vine".

Spruce's contemporary, the Ecuadorian geographer Manuel Villavicencio, commented on the use of a vine by the Záparo, Angatero, Mazán and other tribes of the Río Napo region and wrote an account of his own experience with *ayahuasca*. According to Villavicencio (1858), the vine was used:

> To foresee and to answer accurately in difficult cases, be it to reply opportunely to ambassadors from other tribes in a question of war; to decipher plans of the enemy through the medium of this magic drink and take proper steps for attack and defense; to ascertain, when a relative is sick, what sorcerer has put on the hex; to carry out a friendly visit to other tribes; to welcome foreign travelers; or, at least, to make sure of the love of their womenfolk.

Villavicencio goes on:

> When I have partaken of aya-huasca, my head has immediately begun to swim, then I have seemed to enter on an aerial voyage, wherein I thought I saw the most charming landscapes, great cities, lofty towers, beautiful parks, and other delightful things. Then all at once I found myself deserted in a forest and attacked by beasts of prey, against which I tried to defend myself. Lastly, I began to come around, but with a feeling of excessive drowsiness, headache and sometimes general malaise.

The description of the botanical species identified by Spruce was first published by Grisebach, and years later Morton revised the plant's classification and found it to belong to the genus *Banisteriopsis* rather than *Banisteria*. The plant's current botanical name is *Banisteriopsis caapi* (Spruce ex. Griseb.) Morton. Two species of *Banisteriopsis, B. inebrians* Morton and *B. quitensis* (Nied.) Morton, formerly considered independent species also used in the preparation of the Amazonian psychotropic tea, are now considered to be synonyms of *B. caapi*. Other *Banisteriopsis* species reportedly used in the elaboration of *ayahuasca* are *B. martiniana*, *B. muricata*, *B. longialata* and *B. lutea* (Ott, 1993). Descriptions of the actual procedure used in the obtention of *B. caapi* extracts indicate variations from one geographical location to the other. Large pieces or cuttings of intact or pounded vine are used in the preparation of the tea, which in the Colombian Amazon is essentially a cold-water extract (Schultes and Raffauf, 1992), while in other geographical areas various degrees of cooking and concentration of the resulting brew have been described. Thus, while in the Purús river area in Peru brief boiling for 1 hour is used with no further processing (Rivier and Lindgren, 1972), in Pucallpa, also in Peru, a 10-15 h cooking period is followed by concentration of the tea. The latter process leads to much higher levels of active compounds being extracted (McKenna et al., 1984).

A relevant aspect of *ayahuasca*'s botany and pharmacology is the widespread practice of using a large number of plants as additives to the tea. This has caused some confusion regarding the botanical identity of *ayahuasca*, since the basic ingredient, *B. caapi*, and the tea derived from it are usually designated with the same name, irrespective of the admixture plants used. Furthermore, some plants added to the brew display potent psychotropic activity on their own, which has led several authors to confuse, for instance, species of *Brugmansia*

with *ayahuasca*. Another common error has been the identification of the apocynaceous *Prestonia amazonica* as the source of *yajé* (Schultes and Hoffman, 1980). Usual admixtures to *ayahuasca* are tobacco (*Nicotiana* spp.), coca (*Erythroxylum coca*), *Ilex guayusa*, several species from the solanaceae family such as *Brugmansia* spp. and *Brunfelsia* spp. and many others, totaling 90 different species belonging to 38 families (Ott, 1993). Among the most commonly used additives are the leaves of *chacruna*, the rubiaceous *Psychotria viridis* Ruiz & Pavón (see Figure 2) and the leaves of *oco-yajé* or *chagropanga*, the malpighiaceous *Diplopterys cabrerana* (Cuatrec.) B. gates, formerly known by the basionyms *Banisteriopsis rusbyana* (Nied.) Morton and *Banisteriopsis cabrerana* Cuatrec. (Schultes and Raffauf, 1992). *Psychotria viridis* is a shrub from the coffee family and is commonly used in Brazil, Peru and Ecuador, whereas *Diplopterys cabrerana* is a liana belonging to the same family as *B. caapi*, used mainly in Ecuador and Colombia (Schultes and Hofmann, 1980; McKenna et al., 1984). As will be discussed below, *ayahuasca* brews incorporating *chacruna* or *chagropanga* are thought to induce their visionary effects through the action of indole alkaloids present in the leaves of these admixture plants.

Figure 1: *Banisteriopsis caapi*. Photo courtesy of Josep Maria Fericgla.

Figure 2: *Psychotria viridis*. Photo courtesy of James C. Callaway

The traditional patterns of use of *ayahuasca* have been extensively researched by anthropologists. Despite varying degrees of acculturation, *ayahuasca* use still survives among the indigenous peoples who inhabit the Amazon Basin. Like many other psychotropic plants of the New World, *ayahuasca* brews are considered sacred and are usually employed by medicine men or shamans for the visionary experiences they elicit. Additionally, other members of the group may ingest the tea in specific ritual ceremonies, such as rites of passage, funerals or communal celebrations (Reichel-Dolmatoff, 1990). Dobkin de Rios (1984) has reviewed the anthropological literature and has summarized the following roles for *ayahuasca* in traditional indigenous societies: 1) as a means to contact the supernatural world, practise divination or witchcraft; 2) as a means to determine the causes of disease and cure the ill; and 3) as a means to obtain pleasure, facilitate sexual activity or social interaction. The following examples can be mentioned: the Jívaro or Shuar of Ecuador have been reported to use *natema* to contact the spirit world to obtain guidance (Fericgla, 1994; Karsten, 1935, cited in Dobkin de Rios, 1984); it has been used both to bring harm to others and for protection against the ill will of others (Fericgla, 1994; Harner, 1972); the Cubeo of Colombia have been known to use *ayahuasca* to achieve pleasurable ecstatic states (Goldman, 1963, cited in Dobkin de Rios, 1984); the Záparo of Ecuador reportedly use it to obtain insight into the future and for healing purposes (Reinburg, 1921, cited in Dobkin de Rios, 1984); and the Peruvian Cashinahua drink *nixi pae* "to learn of things, persons and events removed from them by time and/or space", and their shamans use it to consult the spirits concerning the

causes of someone's illness (Der Marderosian et al., 1970). Rivier and Lindgren mention *ayahuasca* use among the Sharanahua and Culina in Peru both for medical and social purposes (Rivier and Lindgren, 1972). Besides, they comment on the prohibition of *ayahuasca* use for women and children, a restriction also found in the Colombian and Ecuadorian Amazon (Reichel-Dolmatoff, 1990; Villavicencio, 1858).

Today, the use of *ayahuasca* is expanding beyond its original home in the South American rainforest to reach the urban areas of the continent. Around cities like Iquitos in Peru, mestizo folk healers known as *ayahuasqueros* or *vegetalistas* treat the emotional and psychological illnesses of their patients by means of *ayahuasca* sessions (Luna, 1984a; Dobkin de Rios, 1996). These healers use *ayahuasca* visions to diagnose the magic causes of disease or neutralize the evil magic responsible for certain types of illness. *Ayahuasqueros* themselves undergo an apprenticeship during which they observe strict diets and ingest *ayahuasca* and other psychotropic plants such as tobacco (Dobkin de Rios, 1984). Some researchers have emphasized the role of plants as "teachers" in the learning process of the future shaman. The initiate learns certain healing procedures directly from the plant. These can typically include certain magical melodies called "ícaros", that will later be used to combat the evil spirits responsible for illness (Luna, 1984a; Luna, 1984b). It is worth commenting that in the context of Indian and mestizo shamanism, *ayahuasca* does not seem to be used as a curative agent in itself but rather a means used by healers to deal with the supernatural causes of their patient's afflictions.

Another important cultural transformation of *ayahuasca* use is that which has taken place within the so-called *ayahuasca* churches in Brazil. These groups have blended Christian and/or Afro-Brazilian religious beliefs with the indigenous use of *ayahuasca*, which is consumed as a sacrament in the rituals (for a review see the book *O Uso Ritual da Ayahuasca* edited by Labate and Araújo). The oldest of these cults, the *Santo Daime* was founded by a former *seringueiro* or rubber tapper called Raimundo Irineu Serra. Mestre Irineu, as he is known by his followers, was initiated into *ayahuasca* in the 1920s in the jungle regions of Acre state close to the border with Bolivia and Peru (Fróes, 1986; McRae, 1998). During his experiences with *ayahuasca* he had revelations from a female entity, Nossa Senhora da Conceição or Rainha da Floresta, and around 1945 he founded the Centro de Iluminação Cristã Luz Universal (CICLU), also known as Alto Santo in Rio Branco, Acre. The name given to *ayahuasca*, *Daime*, was revealed to Irineu by the Rainha da Floresta and derives

from invocations such as "Dai-me amor, luz força" used in the rituals. The *Santo Daime* doctrine is recopilated in a series of hymns revealed to Mestre Irineu and other members of the church under the effects of *Daime* and sung during the ceremonies. It is essentially dualistic, with the ingredients of *ayahuasca*, i.e., *B. caapi,* known as *cipó*, *mariri* or *jagube,* representing masculinity and *P. viridis* or *folha*, *rainha* or *chachrona* representing femininity. Several groups originated from the original Alto Santo such as the Centro Ecléctico de Correntes da Luz Universal (CECLU) in Porto Velho, Rondônia, or the group led by Sebastião Mota de Melo, Padrinho Sebastião, a follower of Mestre Irineu, who split with the original Alto Santo and founded the Colônia 5000. The group at the Colônia 5000 originally incorporated the use of *Cannabis* in its rituals, leading to a police raid in 1981. Two years later, Padrinho Sebastião's group moved to a more remote location on the river Igarapé do Mapiá, a tributary of the Purús river, and founded the Céu do Mapiá. In 1982, the Céu do Mar was founded outside the jungle, in Rio de Janeiro. In 1989, the *Santo Daime* church adopted the denomination *Centro Ecléctico da Fluente Luz Universal Raimundo Irineu Serra* or CEFLURIS, led by Padrinho Sebãstiao's son, Padrinho Alfredo. In 1998 the church adopted its present name *Igreja do Culto Eclético da Fluente Luz Universal*.

Independently, Daniel Pereira de Matos founded the *Barquinha* in Acre in 1945, and in 1961 José Gabriel da Costa, also a *seringueiro*, established what is today the largest of the *ayahuasca* churches, the *Centro Espírita Beneficente União do Vegetal* (UDV) in Porto Velho, Rondônia. During the 1970s and 80s, both the UDV and the *Santo Daime* spread to the urban centers of southeast Brazil, while the *Barquinha* remained in Acre. The use of *ayahuasca* was temporarily banned in 1985 and after an official governmental investigation of the UDV and the *Santo Daime* the ban was lifted in 1987, an action which effectively legalized the use of *ayahuasca* within a ritual context in Brazil.

In recent years, the *ayahuasca* churches have exported their activities to other countries, contributing to the spread of *ayahuasca* use in the highly industrialized countries of Europe and North America. This phenomenon has been facilitated by the growing interest of many individuals interested in shamanic practices, and also by the fame of *ayahuasca* as a means to facilitate self-knowledge and introspection. Groups of *Santo Daime* and UDV followers have arisen in several European countries, including Germany, Great Britain, Holland, France and Spain as well as in the United States (Anonymous, 2000). Thus, individuals with a very different cultural background to that of the people from Amazonia have come into contact

with the ancient psychotropic beverage in rituals virtually open to all. Furthermore, in this ritual context *ayahuasca* sessions are typically held every 15 days, an unprecedented high frequency of use, and although the number of users is still relatively small outside of Brazil, *ayahuasca* use has raised concerns for public health (Callaway and Grob, 1998). The gradual increase of *ayahuasca* imports into Europe and North America recently attracted the attention of health and police authorities, which led to confiscation of tea shipments, arrests and trials of members in the Netherlands, France, Germany and Spain. In the course of these judicial processes it became evident that both prosecutors and the defense lacked accurate information on the nature of *ayahuasca* brews and on its effects in humans.

2. Chemical constituents of *ayahuasca*

The chief component of *B. caapi's* alkaloidal fraction and the first of its alkaloids to be identified is harmine, a β-carboline which was isolated from *Banisteriopsis* specimens by several researchers working independently. In 1905, the Colombian explorer Zerda Bayon wrote a report on the use of yagé on the Caquetá river and attempted to isolate the active principle from the brew. Although he could not obtain a crystalline compound, a crude precipitate appeared after addition of alkaline salts to the brew. This suggested the presence of an alkaloid he tentatively named telepathine (Perrot and Raymond-Hamet, 1927a). In 1923 Fisher Cardenas apparently isolated an alkaloid in crystalline form for which he conserved the name of telepathine (Perrot and Raymond-Hamet, 1927a), and in 1925 Barriga Villalba published the isolation of two crystalline alkaloids from the stems of yajé, which he erroneously thought to be the species *Haemodictyon amazonicum*. He named the alkaloids yajeine and yajenine (Barriga Villalba, 1925). Perrot and Raymond-Hamet (1927b) isolated telepathine from authentic *Banisteriopsis caapi* and Louis Lewin obtained an alkaloid he named banisterine from a specimen of *Banisteriopsis caapi* and described its pharmacological effects in animals and in human subjects (Lewin, 1928). In the same year, at the pharmaceutical company Hoffman-La Roche, Elger published a paper in which he described the obtention of harmine from a sample of the yagé liana provided by Raymond-Hamet (Elger, 1928). Elger found that the alkaloid in his yagé sample was identical to harmine from *Peganum harmala* and to the telepathine obtained by Perrot and Raymond-Hamet. Wolfes and Rumpf at Merck (1928) reported to have unexpectedly obtained harmine from a Colombian malpighiaceous liana, which was supposed to contain Villalba's yajeine. In 1939, Chen and Chen were able to obtain harmine from the stems, leaves and roots of *B. caapi* and

concluded that telepathine, yajeine and banisterine were the same compound, i.e., harmine. They consequently proposed that the other names given to harmine should be dropped (Chen and Chen, 1939). Years later, Hochstein and Paradies (1957) corroborated and extended these findings with the isolation of harmine, harmaline and *d*-tetrahydroharmine (*d*-THH) from *B. caapi*.

More recent analyses have verified the presence in *B. caapi* of *β*-carboline alkaloids, mainly harmine and *d*-THH, and to a lesser extent harmaline and traces of harmol and harmalol (McKenna et al., 1984; Rivier and Lindgren, 1972). Minor amounts of harmine-*N*-oxide, harmic acid methyl ester and harmalinic acid have been isolated (Hashimoto and Kawanishi, 1975) and in a later study, harmic amide, acetyl norharmine and ketotetrahydronorharmine have been identified (Hashimoto and Kawanishi, 1976). These same authors have also isolated other alkaloids which do not posses the *β*-carboline but the pyrrolidine skeleton: shihunine and dihydroshihunine (Kawanishi et al., 1982).

As mentioned beforehand, other psychoactive plants are frequently added to *ayahuasca* brews. Common admixtures are the nicotine-containing *Nicotiana tabacum* and *Nicotiana rustica*, *Erythroxylum coca* containing ecgonine alkaloids such as cocaine, *Ilex guayusa* and *Paullinia yoco*, both rich in caffeine, the solanaceous *Brugmansia sauveolens* and *Brugmansia insignis* which contain tropane alkaloids like atropine and scopolamine, and many others (Ott, 1993). Among the most commonly used are, however, the tryptamine-containing plants *Psychotria viridis* and *Diplopterys cabrerana* (formerly *Banisteriopsis rusbyana*). These plants are known to be rich in methylated tryptamines. Der Marderosian et al. (1970) found the psychedelic indole *N*,*N*-dimethyltryptamine (DMT) in the leaves of an unidentified species of *Psychotria* used as an *ayahuasca* admixture. Rivier and Lindgren (1972) found DMT in the leaves of *P. viridis* plus trace amounts of *N*-methyltryptamine (NMT) and 2-methyl-1,2,3,4-tetrahydro-*β*-carboline (MTHβC), and McKenna et al. (1984) also found DMT to be the major alkaloid in the leaves of *P. viridis*. The leaves of *D. cabrerana* are also rich in DMT (Agurell et al., 1968; Der Marderosian et al., 1968; Poisson, 1965); in this plant, trace amounts of *N*-methyltryptamine (NMT), 5-Methoxy-*N*,*N*-dimethyltryptamine, 5-hydroxy-*N*,*N*-dimethyltryptamine and *N*-methyltetrahydro-*β*-carboline have also been found (Agurell et al., 1968). Figure 3 shows the chemical structure of the major alkaloids found in *B. caapi, P. viridis* and *D. cabrerana.*

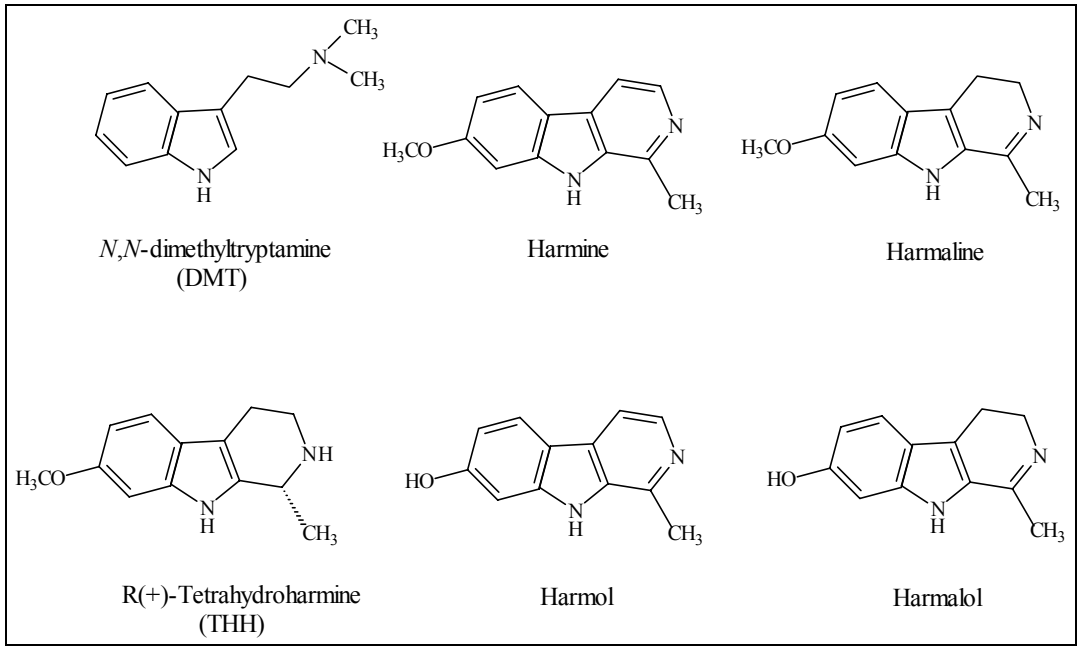

Figure 3: Chemical structures of the main alkaloids found in *Psychotria viridis* and *Diplopterys cabrerana* (*N*,*N*-dimethyltryptamine), and *Banisteriopsis caapi* (harmine, harmaline, THH, harmol and harmalol).

Quantitative Analyses of *Banisteriopsis* have shown the stems to contain an average of 0.4% dry weight of alkaloids, ranging from 0.05% to 1.36%, of which around two thirds are harmine (see Table 1). Other parts of the plant also contain alkaloids, in even higher concentrations. Rivier and Lindgren report 1.95% in the roots of one specimen of *B. caapi* and 1.9% in the leaves of another. However, the roots and leaves of *B. caapi* are rarely used in the preparation of the tea. The alkaloid content in the leaves of *Psychotria* is on average 0.3% (see Table 2), most of it DMT, although in some cases no alkaloids have been found at all, as for instance in a specimen of *Psychotria carthaginensis* Jacq. analyzed by McKenna et al. (1984). The leaves of *Diplopterys cabrerana* contain an average of 0.7% of alkaloids (see Table 2).

Table 1: Alkaloid contents of *Banisteriopsis caapi* dry stems. Figures indicate mean percentage (range).

	Harmine	Harmaline	*d*-THH	Harmol	Harmalol	Total
Hochstein & Paradies, 1957[a]	0.30	trace	trace	n.d.	n.d.	0.30
Poisson, 1965[b]	0.21	trace	n.d.	n.d.	n.d.	0.21
Rivier & Lindgren, 1972[c]	0.25 (0.04-0.51)	0.02 (0.00-0.06)	0.08 (0.00-0.31)	trace	n.d.	0.36 (0.05-0.83)
McKenna et al., 1984[d]	0.39 (0.06-0.64)	0.19 (0.05-0.38)	0.15 (0.03-0.33)	0.04 (0.001-0.12)	0.006 (0.00-0.35)	0.78 (0.17-1.36)

[a] 1 sample collected on the Napo river near Iquitos, Peru; [b] 1 sample collected on the Marañón river, Peru; [c] 14 samples from the Purús river, Peru and other origins; [d] 6 samples collected on different locations in Peru. n.d. = not determined

Table 2: DMT contents of the leaves of *Psychotria* spp. and *Diplopterys cabrerana*, both used as admixtures to *ayahuasca*.

	Species	DMT (%)	
		Mean	Range
Poisson, 1965[a]	*Diplopterys cabrerana*	0.64	---
Der Marderosian et al., 1968[b]	*Diplopterys cabrerana*	1.46	1.33-1.75
Agurell et al., 1968[c]	*Diplopterys cabrerana*	0.47	---
McKenna et al., 1984[d]	*Diplopterys cabrerana*	0.17	---
Der Marderosian et al., 1970[e]	*Psychotria spp.*	0.19	0.17 - 0.22
Rivier & Lindgren, 1972[f]	*Psychotria viridis*	0.17	0.00 - 0.34
Rivier & Lindgren, 1972[g]	*Psychotria carthaginensis*	0.66	---
McKenna et al., 1984[h]	*Psychotria viridis*	0.13	0.10 - 0.16
McKenna et al., 1984[i]	*Psychotria carthaginensis*	0.00	---

[a] 1 sample collected on the Marañón river, Peru; [b] 1 sample collected in eastern Ecuador; [c] 1 sample provided by H. Pinkley. Place of collection not specified; [d] 1 sample collected in Tarapoto, Peru; [e] 4 samples collected at Balta, upper Purús river, southeastern Peru; [f] 2 samples collected on the Purús river, Peru; [g] 1 sample collected on the Purús river, Peru; [h] 3 samples collected in Iquitos, Tarapoto and Pucallpa in Peru. [i] 1 sample collected in Tarapoto, Peru.

The most complete quantitative analyses of *ayahuasca* brews and their plant constituents are those undertaken by Rivier and Lindgren (1972) and McKenna and coworkers (1984). Rivier and Lindgren (1972) studied *ayahuasca* use among the Sharanahua and Culina Indians living on the river Purús in Peru and found that the brew was prepared from *B. caapi* plus *P. viridis* and that on some occasions *P. carthaginensis* was used instead of *P. viridis*. The chemical analyses of 14 samples of stems of authentic *B. caapi* and undetermined *Banisteriopsis* spp. from river Purús and other origins detected a mean alkaloid concentration of 0.36% (range: 0.05-0.83), constituted by the *β*-carbolines: harmine, harmaline, THH and harmol, and also by a tryptamine, 6-methoxytryptamine. Of the total alkaloids, harmine represented on average 74% (range: 42-96), THH represented 16% (range: 1-47) and harmaline 4% (range: 0-9). Harmol and 6-methoxytryptamine were trace constituents found in the stems of four and three samples, respectively. The analyses of *P. viridis* leaves showed the presence of DMT, NMT and traces of MTHβC. Alkaloids represented 0.23% (range: 0.11-0.34) of the dry weight of *P. viridis* and 0.66% of the dry weight of *P. carthaginensis*. Of the total alkaloids, DMT represented 99% in a sample of *P. viridis* and also 99% in a sample of *P. carthaginensis*. One of the two samples of *P. viridis* analyzed was found to be devoid of DMT. Instead, NMT acounted for 85% of the total alkaloids and MTHβC for another 12%. Four other *Psychotria* species: *P. bacteriophylla*, *P. emetica*, *P. undulata* and another not identified were found to be devoid of alkaloids. Analyses of 9 brew samples prepared by the Sharanahua, Culina and Piro tribes combining *Banisteriopsis* sp. and *Psychotria* sp. found a mean alkaloid content of 0.04% (range: 0.01-0.06) consisting of harmine 39% (range: 21-62%), THH 15% (range: 6-40), harmaline 2% (range: 0-4) and DMT 20% (range: 0-41).

McKenna et al. (1984) performed chemical analyses of 6 samples of authentic *B. caapi* collected in Tarapoto, Iquitos and other locations in Peru and found a mean alkaloid concentration in the stems of 0.78% (range: 0.17-1.36), made up of the following *β*-carbolines: harmine, harmaline, THH, harmol and also harmalol in one of the specimens. Of the total alkaloids, harmine represented on average 48% (range: 34-72), THH was 22% (range: 13-47) and harmaline 26% (range: 15-44), while harmol represented only 4% (range: 0.4-9%). The analyses of three specimens of *Psychotria viridis* used as admixtures showed the presence in the leaves of only DMT, except in one sample where traces of MTHβC were detected. Average alkaloid content was 0.13% (range: 0.10-0.16) of the dry weight. A sample of *P. carthaginensis* was found to be devoid of alkaloids. One sample of *Diplopterys*

cabrerana collected previously (in 1976) found 0.17% DMT plus traces of 5-OH-DMT. McKenna and coworkers (1984) performed qualitative analyses of eight *ayahuasca* brew *s*amples collected in the areas around the Peruvian cities of Iquitos, Pucallpa and Tarapoto. The samples studied had been prepared by local *ayahuasqueros* from *B. caapi* plus *P. viridis* and in one case *P. carthaginensis* was used instead of *P. viridis*. The qualitative analyses showed harmine, harmol, harmaline and THH in all samples. Harmalol was detected in one sample. All samples showed DMT except the *ayahuasca* prepared from *P. carthaginensis* which was devoid of this compound. Quantitative analyses of 5 brew samples prepared by Pucallpa *ayahuasqueros* combining *B. caapi* and *P. viridis* showed a mean alkaloid content of 0.73% (range: 0.59-0.82) consisting of harmine 65% (range: 53-67), THH 22% (range: 18-30), harmaline 6% (range: 5-6) and DMT 8% (range: 6-11). Interestingly, some of the *ayahuasca* samples collected by these researchers were freeze-dried and the alkaloid content was quantified in terms of mg/g freeze-dried material. The obtained concentrations are shown in Table 3 together with those found in the *Daime* batch used in the present work.

Table 3: Alkaloid concentrations in freeze-dried *ayahuasca* in mg/g expressed as mean (range).

	Harmine	**Harmaline**	***d*-THH**	**DMT**	**Total Alk.**
McKenna et al., 1984 [a]	23.8 (8.6-57.6)	5.1 (4.2-6.3)	11.1 (8.0-25.5)	6.4 (0.0-7.2)	46.9 (29.1-75.6)
Riba et al., 2001[b]	14.1	1.0	11.4	8.3	34.8

[a] 5 samples of Peruvian *ayahuasca* from Pucallpa, Iquitos and Tarapoto; [b] 1 sample Brazilian *Daime* used in this work

Other authors have also quantified the alkaloid contents in *ayahuasca* brews. The average values reported are shown in Table 4. Average values for DMT range from 0.14 to 1.18 mg/ml, with a mean of 0.48 mg/ml (0.05%). Average values for total β-carbolines range from 0.18 to 6.68 mg/ml with a mean of 2.68 mg/ml (0.27%). Based on the average values reported, the β-carbolines in *ayahuasca* brews represent roughly 76% of the total alkaloids (range: 55-92) and DMT the remaining 24% (range: 8-45). Among the β-carbolines, harmine represents around 44% of the total alkaloids (range: 23-64), THH around 25% (range: 22-41) and harmaline around 7% (range: 0-32).

Table 4: Alkaloid concentrations in *ayahuasca* brews expressed in mg/ml.

	Harmine	Harmaline	*d*-THH	*β*-carbolines*	DMT
Der Marderosian, 1970[a]	0.07	0.11	n.d.	0.18	0.14
Rivier & Lindgren, 1972[b]	0.15	trace	0.05	0.20	0.13
McKenna et al., 1984[c]	4.67	0.41	1.60	6.68	0.60
Liwszyc et al., 1992[d]	1.49	trace	1.39	2.88	0.53
Callaway et al., 1999[e]	1.70	0.20	1.07	2.97	0.24
Callaway, 1999[f]	2.25	0.13	1.82	4.20	1.18
Riba et al., 2001[g]	0.90	0.06	0.72	1.68	0.53

*β-carbolines = harmine + harmaline + *d*-THH; [a] 2 samples of Peruvian *nixi pae*; [b] 9 samples of Peruvian *ayahuasca*; [c] 5 samples of Peruvian *ayahuasca* from Pucallpa; [d] 1 sample of Brazilian *Daime*; [e] 1 sample of Brailian *Hoasca*; [f] 20 samples of Brazilian *Hoasca*; [g] 1 sample of Brazilian *Daime* used in this work. n.d. = not determined

Table 5 lists alkaloid amounts in typical doses. Those studies not clearly reporting their estimate of a typical dose have not been included. Thus, the mean volume of an *ayahuasca* dose is 166 ml (range: 60-237) and involves the ingestion of 193 mg of alkaloids (range: 65-437). Of these, roughly 161 (range: 40-401) correspond to the *β*-carbolines: 109 mg (range: 17-280) harmine, 35 mg (range: 0-96) THH, and 17 mg (range: 0-26) harmaline. Additionally, each dose contains an average of 31 mg (range: 25-36) DMT. However, the total alkaloid intake may be considerably higher in practice, considering that in the course of an *ayahuasca* session several doses are commonly ingested.

Table 5: Alkaloid amounts in mg ingested in reported typical *ayahuasca* doses.

	Harmine	Harmaline	*d*-THH	*β*-carbolines*	DMT
Der Marderosian, 1970[a]	17	26	n.s.	43	33
Rivier & Lindgren, 1972[b]	30	trace	10	40	25
McKenna et al., 1984[c]	280	25	96	401	36

*β-carbolines = harmine + harmaline + *d*-THH; [a] Estimated in 237 ml (8 ounces); [b] Estimated in 200 ml; [c] Estimated in 60 ml;.

Ayahuasca brews thus appear to be composed of two main alkaloid groups, tryptamines, whose main representative in the tea is DMT, and *β*-carbolines, mainly harmine. As was soon

realized by early researchers, *ayahuasca* combines a powerful visionary compound, DMT, with potent enzymatic inhibitors, the *β*-carbolines. As will be discussed in section 8, *ayahuasca* is thought to owe its psychotropic properties to the pharmacological interaction between these two alkaloid classes.

3. Pharmacology of DMT in humans

DMT had already been obtained as a synthetic product by Manske (1931) when it was isolated as the *N*-oxide from the seeds of *Anadenanthera peregrina* (referred to as *Piptadenia peregrina*, Fish et al., 1955a), the putative source of a Piaroa psychotropic snuff. Today, DMT is known to occur in over fifty plant species (Ott, 1994) and to be a major active component of *ayahuasca* and other psychotropic plant preparations. The presence of DMT in the seeds of *Anadenanthera* spp., used in the preparation of a psychotropic snuff, caught the attention of the Hungarian biochemist Stephen Szára who conducted a series of experiments with the drug in humans. Many other studies with the pure compound followed and its powerful visionary effects were highlighted. As will become evident from the data presented in this introduction, DMT appears to fit the characteristics of the so-called psychedelic or hallucinogenic drugs, which according to Hollister (1968) share the following characteristics regarding their human pharmacology: 1) modifications in thought processes, perception and mood predominate over other alterations; 2) intellectual capacity and memory is minimally affected; 3) stupor, narcosis or excessive stimulation are not the predominant effects; 4) autonomic side effects are moderate; 5) addictive craving is minimal. The classification of DMT into this pharmacological group is further supported by its chemical structure, and its receptor affinity profile, since according to Glennon, the "classical hallucinogens" are "agents that meet Hollister's original definition, but also: a) bind at 5-HT_2 serotonin receptors, and b) are recognized by animals trained to discriminate 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) from vehicle" (Glennon, 1999), two additional criteria which DMT has been demonstrated to meet.

3.1. Subjective effects

The first study published in the scientific literature on the subjective effects induced by DMT was reported by Szára, who conducted a series of experiments to investigate whether this tryptamine had a "psychotic effect" in humans (Szára, 1956). After observing that the drug was not orally active in doses as high as 150 mg (2 mg/kg), he and other members of his team self-administered DMT intramuscularly and found it to elicit psychotropic effects from 30 mg (0.2 mg/kg) onwards, the highest tested dose being 150 mg (2 mg/kg; Szára, 1957). The effects described at what the author considered the optimal dose (0.7-1.1 mg/kg) involved visual illusions, disturbances of thought and euphoria, accompanied by tingling sensations, tremors, mydriasis and elevations of blood pressure and pulse rate. The author regarded these effects as qualitatively similar to those elicited by mescaline and lysergic acid diethylamide (LSD), but with a characteristic time course. Indeed, the most striking aspect of DMT was the onset and duration of the "model psychosis": effects were first felt around 3-5 min following i.m. injection and had disappeared after 1 h. Szára (1957) gave the following account of the effects he had experienced when he first self-administered 75 mg i.m. of DMT:

> In the third or fourth minute after the injection vegetative symptoms appeared such as tingling sensation, trembling, slight nausea, mydriasis, elevation of the blood pressure and increase of the pulse rate. At the same time eidetic phenomena, optical illusions, pseudo-hallucinations, and later real hallucinations, appeared. The hallucinations consisted of moving, brilliantly colored oriental motifs, and later I saw wonderful scenes altering very rapidly. The faces of the people seemed to be masks. My emotional state was elevated sometimes to euphoria. At the highest point I had compulsive athetoid movement in my left hand. My consciousness was completely filled by hallucinations, and my attention was firmly bound to them; therefore I could not give an account of the events happening around me. After ¾ - 1 hour the symptoms disappeared and I was able to describe what had happened.

In a subsequent paper, Sai-Halász et al. (1958) summarized the main subjective effects elicited by i.m. DMT (0.7-1.0 mg/kg) in a group of 30 healthy volunteers, mainly medical doctors, as follows: 1) perceptual modifications which were mainly visual, rapidly changing colorful illusions and hallucinations; 2) modifications of spatial perception, with changes in

room dimensions; 3) modifications of bodily image, with subjects reporting that parts of their body no longer belonged to them; 4) modifications of time perception, with volunteers usually overestimating the duration of effects; 5) thought modifications with loosening of associations, incoherence of speech and difficulties to control their trains of thoughts. In some cases suspiciousness and paranoid ideation were observed; 6) affective modifications consisting mainly of euphoria or uncontrollable laughter. Fear was also common, mainly during the first minutes of intoxication; 7) in some cases, clouding of consciousness was observed, with volunteers being unable to recall events for several minutes.

The above observations were confirmed in general terms by Arnold and Hofmann (1957) in Germany employing i.m. doses of 1.0-1.2 mg/kg and replicated in other papers by the Hungarian group (Sai-Halász, 1962) who tested doses in the 0.35-0.83 mg/kg range, also by the i.m. route. It is interesting to note that while the subjective effects of this new "psychoticum", as the Hungarian research team had a priori labeled DMT (Sai-Halász et al., 1958), were quite reproducible in their group of normal volunteers, the researchers noted that the DMT experience did not definitively resemble schizophrenia or other endogenous psychoses. This led them to conduct drug-administration experiments with chronic schizophrenics. In these patients, 1-1.5 mg/kg i.m. doses of DMT induced feelings of strangeness, mood changes and autonomic effects but the visual images which characterized the experiences of healthy subjects were virtually absent (Böszörmenyi and Szára, 1958).

In the United States, initial research on the human pharmacology of DMT was conducted by Turner and Merlis (1959) in schizophrenic patients, first testing the drug intranasally (5-20 mg, i.e., 0.07-0.27 mg/kg) and orally (doses up to 350 mg, i.e., 4.7 mg/kg) and finding it to be inactive by these routes. These investigators only observed "unilateral flushing of the face and mydriasis" in one patient who had received 10 mg intranasally. In contrast, intravenous (5-25 mg, i.e., 0.07-0.33 mg/kg) and intramuscular (5-50 mg, i.e., 0.07-0.67 mg/kg) doses of the drug induced states of anxiety, restlessness, and a very intense dysphoric reaction in one patient (Turner and Merlis, 1959). At the Addiction Research Center in Lexington, Kentucky, results obtained by Rosenberg and coworkers (1963) in convicted former opiate addicts who "volunteered" for a DMT study, highlighted visual distortions and hallucinations, among other drug effects on the psychological sphere, after the administration of 0.75-1.0 mg/kg i.m. In a later cross-tolerance experiment in which LSD and DMT (0.5-1.0 mg/kg i.m.) were administered, these researchers found these two drugs to differ only in the time course of the

intoxication and concluded that they elicited similar autonomic and subjective effects. The latter included "euphoria, anxiety, visual hallucinations and perceptual distortions" (Rosenberg et al., 1964).

Subsequent studies conducted with non-psychotic subjects also stressed the predominance of visual phenomena during the intoxication (Gillin et al., 1976). However, these appear to be only part of the overall psychological experience. Bickel and coworkers (1976) found that DMT could be differentiated from placebo at low 0.25 mg/kg i.m. doses by means of self-report scales measuring (from highest to lowest scores): derealization, visual phenomena and altered body image. The lowest scores, although statistically significant, were obtained for scales measuring dysphoria, euphoria and delusion. The DMT syndrome was also characterized by somatic effects which included altered equilibrium, numbness in hands and feet, heaviness of the legs and dizziness. In a comparative study with 0.25 mg oral THC and placebo (Dittrich et al., 1976), 0.25 mg/kg i.m. DMT induced increases in scales measuring thought modifications, visual illusions and body image modification scales. Interestingly, at the administered doses, DMT could not be differentiated from THC. But again, the only aspect which predominated in the DMT-induced state relative to THC effects were the visual illusions which showed higher scores but with only a tendency towards statistical significance.

Strassman and coworkers administered doses from 0.05 to 0.4 mg/kg intravenously to healthy experienced psychedelic drug users (Strassman and Qualls, 1994; Strassman et al., 1994; Strassman et al., 1996). At the higher dose, drug effects were characterized by an intense "rush" which was followed by colorful hallucinations, very intense emotional effects and perceptual modifications conferring an oneiric quality to the experience. This modified state of awareness was described as being very compelling, totally replacing the previously ongoing mental activity. At 20-30 min following administration, when plasma levels decreased to levels slightly above the limit of detection, the subjective effects reported had completely disappeared. In one of these studies (Strassman et al., 1994), the authors obtained interesting dose-response data regarding subjective effects. At the lower 0.05 and 0.1 mg/kg doses, emotional and somatic effects appeared to predominate over perceptual modifications, whereas at the higher 0.2 and 0.4 mg/kg doses drug-induced visual effects and detachment from external reality were described by the volunteers as being overwhelming. In line with previous studies (see for example Sai-Halász et al., 1958), during the initial rush most

volunteers experienced anxiety and opposed emotions like fear and euphoria coexisted throughout the intoxication in some cases.

In summary, in the clinical studies reviewed, DMT was found to be psychologically inactive by the oral and intranasal routes in doses up to 4.7 mg/kg and 0.27 mg/kg, respectively. Distinct psychotropic effects were observed, however, when the drug was administered by the i.m. route, commonly in doses around 0.7-1 mg/kg, with the maximal reported dose being 2 mg/kg. The i.v. route has also proved to be effective, with a maximal reported dose of 0.4 mg/kg and the threshold dose for psychedelic effects around 0.2 mg/kg.

3.2. Pharmacokinetics

In the course of the first trials in which pure DMT was administered to humans, it soon became apparent that DMT was devoid of psychoactive effects after oral administration (Szára, 1957). This peculiarity was later corroborated by Turner and Merlis (1959), who extended the observation to intranasally administered DMT. The drug thus appears to be the only psychedelic known to be psychologically inactive per os, although this has also been postulated for 5-methoxy-DMT and contested in a recent paper (Ott, 2001). The lack of oral activity for DMT led early researchers to administer the drug parenterally, and the scarce human data available on the pharmacokinetics of DMT have been obtained after its i.m and i.v. administration. It is also interesting to note that despite the several papers indicating that DMT does not exert psychoactive effects in oral doses of several hundred milligrams (Szára, 1957; Turner and Merlis, 1959), no study has been conducted to date to assess the metabolic fate of DMT following its oral administration. Thus, the metabolic pathways involved in what appears to be an extensive first pass effect have not been established in humans and the data available on the in vivo degradation of DMT have been obtained after its parenteral administration, mainly in animals.

In his first paper on DMT, Szára (1956) identified 3-indoleacetic acid (IAA) in urine as a metabolite of the drug following its i.m. administration in doses of 0.7–1.1 mg/kg. The amounts recovered ranged from 8 to 25% of the administered dose and no unmetabolized DMT was found in the samples. The authors postulated that the rapid metabolism of DMT could explain the short duration of the drug-induced “psychosis”. In this paper, no data were given regarding plasma levels of the metabolite or the parent compound.

Kaplan and coworkers (1974) found mean peak concentrations of 100 ng/ml at 10-15 min following an i.m. injection of 0.7 mg/kg to eleven male subjects. The drug also disappeared from plasma very rapidly. By 1h, DMT had almost disappeared and the concentration vs. time figure showed that virtually no DMT could be quantified at 2 h. Subjective effects appeared to closely parallel DMT plasma levels. Both the subjective "high" and the maximum drug concentration in plasma were found at 10 min and both returned to baseline levels around 1 h. These researchers pointed out the large individual variability in peak plasma concentration, which oscillated approximately between 20 and 150 ng/ml, and the apparent similar time course between individuals. Drug levels in 24 h urine showed only an average of 0.069% of the administered dose was recovered in urine, most of which was excreted within the first 2 h. They consequently argued that the drug was rapidly metabolized, but no study was conducted in order to identify the putative metabolites.

Strassman and Qualls (1994) measured DMT plasma levels between 2 and 60 min following an i.v. bolus of 0.05, 0.1, 0.2 and 0.4 mg/kg doses. Drug effects were found to initiate almost instantaneously. DMT plasma levels could be measured up to 30 min after injection and had virtually disappeared at 60 min for all doses. Mean peak value at 2 min after the 0.4 mg/kg dose was approximately 90 ng/ml and showed marked interindividual variability, with a range of 32-204 ng/ml. As in the studies discussed above, pharmacokinetic parameters were not reported and the authors only stated that "generally, a doubling of dose resulted in a doubling of Δmax values". Neither were DMT metabolites assessed.

3.3. Cardiovascular effects

Early studies on DMT repeatedly described increases in blood pressure following drug administration to humans, both in uncontrolled (Szára, 1956; Böszörmenyi and Szára, 1958; Sai-Halász et al., 1958) and also in placebo-controlled studies in which 1 mg/kg i.m. doses were administered (Rosenberg et al., 1963; Rosenberg et al., 1964). Heart rate has been found to increase in relation to pre-drug values, although less markedly than blood pressure, in non-placebo-controlled studies enrolling healthy volunteers (Sai-Halász et al., 1958) and psychiatric patients (Böszörmenyi and Szára, 1958). However, other authors have failed to observe statistically significant increases for this variable compared with placebo (Rosenberg et al., 1963; Rosenberg et al., 1964). In a more recent study, Strassman and Qualls (1994)

reported dose-dependent statistically significant increases in mean arterial blood pressure and heart rate after 0.2 and 0.4 mg/kg doses of i.v. DMT.

3.4. Autonomic effects

Increases in pupil diameter were observed in early non-placebo-controlled studies with DMT, after its i.m. administration to both healthy volunteers in doses of 0.7-1.0 mg/kg (Sai-Halász et al., 1958) and to chronic schizophrenics in doses of 1.0-1.5 mg/kg i.m. (Böszörmenyi and Szára, 1958). Gillin and coworkers (1976) also described mydriasis after the i.m administration of a 0.7 mg/kg dose in an uncontrolled study. DMT-induced mydriasis after a 1 mg/kg i.m. dose was confirmed in placebo-controlled studies by Rosenberg and coworkers (1963), who replicated these findings in a subsequent study (Rosenberg et al., 1964) with the same dose. More recently, the same effect has been observed by Strassman and Qualls (1994), who found statistically significant increases in pupil diameter after 0.4 mg/kg i.v. DMT in double-blind placebo-controlled conditions.

Strassman and Qualls (1994) also measured the effects of DMT on rectal temperature and found increases after the higher 0.2 and 0.4 mg/kg doses. However, these results were not replicated in a subsequent study by these authors in which repeated 0.3 mg i.v. DMT doses were administered at 30 min intervals. The authors argued that the slow increase in this variable and the short interval between doses may have precluded significant elevations after the first DMT injection (Strassman et al., 1996).

3.5. Neuroendocrine effects

DMT increases serum levels of prolactin, growth hormone and cortisol in humans (Meltzer et al., 1982). These authors found that the administration of a 0.7 mg/kg i.m. dose produced increases in prolactin and cortisol beginning as early as 10 min after injection and peaking at 30 min. This was in contrast with growth hormone, which began to rise at 60 min. No effect of DMT on follicle stimulating hormone, thyroid stimulating hormone or luteinizing hormone secretion was observed. Pretreatment with the serotonin antagonist cyproheptadine only inconsistently inhibited the rise in cortisol and prolactin but effectively blocked the increase in growth hormone in all three subjects participating in the study. In a subsequent single-

subject experiment, pretreatment with haloperidol increased both the subjective effects of DMT and the rise in growth hormone.

Strassman and Qualls (1994) found DMT to dose-dependently increase blood levels of corticotropin, β-endorphin and prolactin after i.v. administration of doses between 0.05 and 0.4 mg/kg. Statistically significant increases were obtained after the higher 0.2 and 0.4 mg/kg doses. Peak levels for these hormones were found between 5 and 10 min after injection. Cortisol levels were also significantly raised but increases peaked later, at 15-30 min. No drug effects were seen for growth hormone or melatonin levels. In a subsequent study, Strassman et al. (1996) replicated and expanded their previous results, reporting significant increases for prolactin, cortisol and adrenocorticotropic hormone after an i.v. dose of 0.3 mg/kg.

3.6. Adverse effects

Under the Substance-Related Disorders, the Diagnostic and Statistical Manual of Mental Disorders in its 4th edition (American Psychiatric Association, 1994) lists the following hallucinogen-related disorders:

A) Hallucinogen use disorders, which include hallucinogen abuse and dependence.
B) Hallucinogen-induced disorders:
    1. Hallucinogen-Induced Anxiety Disorder
    2. Hallucinogen-Induced Mood Disorder
    3. Hallucinogen Persisting Perception Disorder (Flashbacks)
    4. Hallucinogen-Induced Psychotic Disorder, with Delusions
    5. Hallucinogen-Induced Psychotic Disorder, with Hallucinations
    6. Hallucinogen Intoxication Delirium
    7. Hallucinogen-Related Disorder Not Otherwise Specified

While repeated use of certain psychedelics, such as LSD, has been described to lead to tolerance to the psychological effects, abstinence symptomatology has not been described. The most frequently described acute adverse event in the course of psychedelic drug intoxication is the occurrence of an intense panic state commonly known as a “bad trip”. These events have been described to respond to verbal reassurance and to treatment with benzodiazepines (Strassman, 1984). At the subacute level, the occurrence of the intriguing

and controversial Hallucinogen Persisting Perception Disorder has been recently reviewed by Halpern and Pope (2003). In this work the authors conclude that the disorder "appears to be a genuine but uncommon disorder, sometimes persisting for months or years after hallucinogen use and causing substantial morbidity". Another aspect of psychedelic drug use that has been the object of controversy is whether the repeated exposure to these compounds leads to neuropsychological toxicity. This has also been reviewed by Halpern and Pope (1999) with inconclusive results. However, the authors indicate that "there are few, if any, long-term neuropsychological deficits attributable to hallucinogen use."

In the specific case of DMT, virtually all early DMT studies described episodes of anxiety and dysphoria after acute administration of the drug (see the Subjective Effects section above). More recently, Strassman also reported the occurrence of anxiety after i.v. DMT administration (Strassman et al., 1994) and commented on the potentially traumatic nature of high-dose hallucinogen sessions (Strassman, 1995). This author reported an incidence of flashbacks in 5-10% of his study participants who had received at least one high 0.4 mg/kg DMT dose (Strassman, 1995), and the withdrawal of one volunteer due to the development of a depression in the course of the study (Strassman, 1994).

4. Mechanism of action of DMT and related compounds

4.1. Receptor level interactions

Psychedelic phenylalkylamines and indolealkylamines show remarkable structural similarities with serotonin, norepinephrine and dopamine, the main endogenous neurotransmitter amines, and through the years, interaction with each of these neurotransmitter systems has been proposed as the mechanism of action of these drugs. However, while few data support a direct action of psychedelics on noradrenergic and dopaminergic neurotransmission, biochemical and behavioral data suggest the targeting of serotonergic receptors as a common feature of the classical psychedelics. Among serotonergic receptors, the candidate most likely to mediate psychedelic drug effects is the 5-HT_{2A}, as will be discussed below. Other receptor subtypes believed to be targeted by these drugs are the 5-HT_{2C}, which shows a high degree of homology with the 5-HT_{2A} site, and the 5-HT_{1A}, the latter showing high affinity for indolealkylamines, but not for phenylalkylamines. All three subtypes are G-protein-coupled receptors consisting of seven transmembrane helices connected by intracellular and

extracellular loops. While the 5-HT_{2A} and 5-HT_{2C} are positively coupled to phosphoinositide hydrolysis, the 5-HT_{1A} subtype is negatively coupled to adenylate cyclase. 5-HT_{2A} receptors show the highest densities in the neocortex, while 5-HT_{2C} receptors predominate in the choroid plexus and 5-HT_{1A} are mainly found in the hyppocampus, amygdala, limbic cortex and notoriously in the raphe nuclei where they act as somatodendritic autoreceptors (for a review see Glennon and Dukat, 1995).

Initial studies on LSD–serotonin interactions in peripheral tissue found that LSD antagonized smooth muscle contraction induced by serotonin (Gaddum, 1953) and serotonin antagonism was consequently proposed as the mechanism underlying psychedelic effects (Woolley and Shaw, 1954). In subsequent experiments, LSD was also found to display agonist activity in other systems and this was put forward as an alternative hypothesis explaining its psychotropic properties (Shaw and Woolley, 1956). Substantial information on the mechanism of action of these compounds was obtained later from research in behaving animals by means of various models, most prominently the drug discrimination paradigm. In these studies, rodents are trained to discriminate between a known psychedelic such as LSD, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) or DOM and saline, and subsequently, the percentage of substitution elicited by the drug under investigation is assessed. Early work employing this paradigm evidenced that the stimulus effects of the phenylalkylamine compounds such as DOM generalized to other structurally related phenylalkylamines, but also to indolealkylamines such as LSD and a large number of methylated tryptamines, including DMT (Glennon et al., 1983b). Alternatively, when the indolealkylamines LSD or 5-MeO-DMT were used as the training stimulus, adequate responding generalized to DOM, mescaline and many other phenylalkylamines (Glennon et al., 1993a). These results suggested a common mechanism of action underlying the interoceptive effects of both structural groups. It was also found that the serotonergic 5-HT_2 antagonists such as ketanserin and pirenperone blocked the discriminative stimulus effects of DOM and its generalization to LSD, 5-MeO-DMT and mescaline (Glennon et al., 1983c). Based on these results, it was proposed that the psychedelics act as agonists at 5-HT_2 receptors (Glennon et al., 1983c). In the specific case of DMT, complete stimulus generalization (greater than 80% appropriate responding) has been shown to occur in rats trained with LSD, 5-MeO-DMT and DOM (Glennon et al., 1983a), suggesting a common mechanism of action with these drugs. A more recent study has found a somewhat lower level

of substitution (77.9%) in rats trained with LSD as a discriminative stimulus (Helsley et al., 1998a).

Subsequent radioligand binding studies in animals examined the binding of various psychedelics at different populations of serotonin receptors and common sites could be identified for the two main structural families. Thus, in vitro autoradiography revealed high affinity binding sites for ^{125}I-DOI (McKenna et al., 1987) in regions previously shown to contain high densities of 5-HT_2 receptors as measured by means of ^{125}I-LSD autoradiography (Nakada et al., 1984). In a subsequent study, McKenna and Saavedra (1987) showed that LSD and 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane (DOB) cross-displaced ^{125}I-DOI and ^{125}I-LSD in specific rat brain regions, and the authors speculated that psychedelic drug effects might be mediated by receptors common to the ergolines and the phenylalkylamines. More detailed in vitro receptor binding studies showed differential affinity for the 5-HT_2 and the 5-HT_{1A} sites between phenylalkylamine and indolalkylamine compounds. While LSD and DMT displayed nanomolar affinity for both 5-HT_{1A} and 5-HT_2 receptors, the phenylalkylamines DOB, DOM, DOI and many others were selective for the 5-HT_2 (McKenna et al., 1990; Pierce and Peroutka, 1989; Titeler et al., 1988). In the study by Pierce and Peroutka (1989), the affinity of LSD, DMT, DOB and DOI for non-serotonin receptors was also assessed. While all four displayed virtually no affinity for benzodiazepine binding sites tested, the phenylalkylamines showed micromolar affinity for the muscarinic, α_1-, α_2- and β-adrenergic receptors. LSD also bound to the adrenergic receptors, displaying high (nanomolar) affinity for the α_2-adrenergic receptor. Finally, DMT displayed micromolar affinity for the α_1- and α_2-adrenergic receptor, and no interaction with the β-adrenergic, muscarinic and benzodiazepine receptor sites. Thus, despite particular interactions with other receptors which may modulate the overall effects, affinity for the 5-HT_2 site appears to be a common feature of compounds with a disparity of chemical structures but able to induce psychedelic effects in humans. Glennon and coworkers (1984) found a high correlation between psychedelic potency in humans and 5-HT_2 binding affinity, and also between ED_{50} values from drug discrimination studies and 5-HT_2 binding affinity. Thus, the 5-HT_2 receptor was singled out as a likely candidate for the site of action of psychedelic drugs.

Other serotonin receptor subpopulations such as the 5-HT_{1A} have been implicated in the mechanism of action of these drugs. As mentioned above, contrary to phenylalkylamines,

indolealkylamines display high affinity for the 5-HT_{1A} receptor (Pierce and Peroutka 1989; Titeler et al., 1988). This led researchers to investigate to which extent this receptor participates in their pharmacology. Two different studies have respectively either failed to find (Glennon et al., 1984) or observed (Titeler et al., 1988) a significant correlation between potency in humans or ED_{50} in animals and binding affinity for the 5-HT_{1A} receptor. However, the fact that DOM-stimulus generalization does not occur for 5-HT_{1A} agonists (Glennon et al., 1986), led Titeler and coworkers (1988) to conclude that 5-HT_{1A} agonism "may not play a primary role in the mechanism of action of hallucinogenic agents". More recently, Strassman (1996) reported that pretreatment with the 5-HT_{1A} antagonist pindolol enhanced, rather than decreased, the subjective effects of i.v. DMT in humans.

To make things more complicated, psychedelic phenylalkylamines and indolealkylamines have also been found to bind to the 5-HT_{2C} (formerly labeled 5-HT_{1C}) receptor, identified by Pazos and coworkers (Pazos et al., 1984). Psychedelic compounds such as DOI and DOM, used as training drugs in the drug discrimination paradigm, and which had been previously considered to bind selectively to the 5-HT_{2A} receptors, were also found to interact with the 5-HT_{2C} receptor (Glennon et al., 1992; Sanders-Bush, 1994). It was thus postulated that many actions initially attributed to the 5-HT_{2A} might have in fact been mediated by the 5-HT_{2C} receptor or a combination of 5-HT_{2A} and 5-HT_{2C} mechanisms (Glennon et al., 1992). However, more recent work has found that the 5-HT_{2A} receptor rather than the 5-HT_{2C} receptor mediates the stimulus effects of LSD (Fiorella et al., 1995). Similarly, Schreiber et al. (1994) and Smith et al. (1999) found that the discriminative stimulus effects of DOI are blocked by the selective 5-HT_{2A} antagonist MDL 100,907, but not by the selective 5-HT_{2C} antagonist SB 200,646. Furthermore, Smith et al. (1999) found an association between the behavioral tolerance to the effects of DOI and the down-regulation of the 5-HT_{2A} but not of the 5-HT_{2C} receptors. Table 6 shows DMT affinity values at various receptors.

Table 6: DMT radioligand binding data at serotonergic, benzodiazepine, muscarinic cholinergic, opioid and adrenergic receptors.

	K_i (nM)									
	5-HT$_{2A}$	**5-HT$_{2C}$**	**5-HT$_{1A}$**	**5-HT$_{2B}$**	**BZD**	**Musc.**	**Opioid**	**α_1**	**α_2**	**β**
Glennon et al., 2000[a]	323/ 660	1450	200	-	-	-	-	-	-	
Deliganis et al., 1991[b]	440/ 455	-	130/ 464	-	-	-	-	-	-	-
Sadzot et al., 1989[c]	462/ 1200	-	-	-	-	-	-	-	-	-
Lyon et al., 1988[d]	1200/ 64	-	-	-	-	-	-	-	-	-
	IC_{50} (nM)									
McKenna et al., 1990[e]	75	-	170	450	-	-	-	-	-	-
Pierce and Peroutka, 1989[f]	12	-	170	-	>100,000	88,000	-	4,400	1,200	>100,000

BZD=benzodiazepine, Musc.=muscarinic cholinergic, α_1=α_1-adrenergic, α_2=α_2-adrenergic, β=β-adrenergic. (-) = not determined.

[a] Radioligands: [^{3}H]DOB/[^{3}H]Ketanserin (5-HT$_{2A}$), [^{3}H]8-OH DPAT (5-HT$_{1A}$), [^{3}H]Mesulergine (5-HT$_{2C}$), [^{3}H]N-methylspiperone (D_2), [^{3}H]RO15,1788 (BZD).

[b] Radioligand: [^{3}H]Ketanserin (5-HT$_2$), [^{3}H]8-OH DPAT (5-HT$_{1A}$). Two values reported at each site corresponding to absence / presence of 10^{-4} M GTP.

[c] Radioligand: [^{3}H]Ketanserin (5-HT$_2$). Two values reported corresponding to human cortex / rat cortex, respectively.

[d] Radioligands: [^{3}H]Ketanserin / [^{3}H]DOB. Two values reported corresponding to: [^{3}H]Ketanserin / [^{3}H]DOB, respectively.

[e] Radioligands: [^{3}H]8-OH DPAT (5-HT$_{1A}$) [^{125}I]-R-(-)DOI (5-HT$_{2A}$), [^{3}H]Ketanserin (5-HT$_{2B}$).

[f] Radioligands: [^{77}Br]-R(-)DOB (5-HT$_{2A}$), [^{3}H]8-OH DPAT (5-HT$_{1A}$), [^{3}H]Flunitrazepam (BZD), [^{3}H]Quinuclidinylbenzylate (QNB, Muscarine Cholinergic), [^{3}H] WB-4101 (α_1-adrenoceptor), [^{3}H] Rauwolscine (α_2-adrenoceptor), [^{3}H] DHA (β-adrenoceptor).

Despite the evidence derived from animal studies, the nature of the interaction of psychedelics at the 5-HT$_{2A}$ sites has been the object of controversy regarding whether these drugs act as agonists or antagonists. As mentioned above, results from discriminative stimulus antagonism tests indicated that drug interoceptive effects are primarily mediated via an agonistic action at 5-HT$_{2A}$ receptors. Pierce and Peroutka (1988), however, challenged this concept based on results from second messenger studies. The 5-HT$_{2A}$ receptor is coupled to stimulation of phospholipase C through activation of a G-protein. Receptor stimulation results in the hydrolysis of phosphatidylinositol 4,5-biphosphate by phospholipase C and the generation of inositol 1,4,5-triphosphate and diacylglycerol. In turn, inositol triphosphate and diacylglycerol lead to a series of intracellular events, most prominently the activation of protein kinases (Sanders-Bush and Canton, 1995). Pierce and Peroutka (1988) found LSD to antagonize the

maximum effects of serotonin on phosphoinositide hydrolisis and proposed that LSD acts as a 5-HT_2 antagonist. In the case of DMT, a series of studies on drug-induced phosphoinositide hydrolysis led to seemingly contradictory profiles of activity, the drug being characterized either as a full agonist (Smith et al., 1998), a partial agonist (Cory et al., 1987; Rabin et al., 2002) or an antagonist (Deliganis et al., 1991). In this model, DMT has consistently been shown to elicit a maximum effect (intrinsic efficacy) below that of serotonin, the full agonist. Nevertheless, in all four studies, DMT increased phosphoinositide hydrolysis above baseline levels, which would argue for agonistic activity. Cory et al. (1987), Rabin et al. (2002) and Deliganis et al. (1991) all found for DMT an intrinsic efficacy which was 20% that of serotonin, i.e., the drug displayed partial agonist activity. Nevertheless, while the former two authors characterized the drug as a partial agonist, in the study by Deliganis et al. (1991), the fact that increasing doses of DMT decreased the maximum effect exerted by a fixed serotonin concentration -as would be expected from a partial agonist- was erroneously interpreted as an antagonistic effect of DMT. At the other end of the spectrum, Smith et al. (1998) found for DMT an intrinsic efficacy of 90% that of serotonin, which led the authors to characterize the drug as a full agonist. Thus, the largest discrepancy between these studies is the intrinsic efficacy of the drug, but all four indicate that DMT mimics the stimulatory effect of serotonin in phosphoinositide hydrolysis, suggesting partial agonist activity. Partial agonist activity has also been demonstrated for LSD, DOI, 5-MeO-DMT and mescaline in human neuroblastoma cells transfected with the 5-HT_2 receptor (Newton et al., 1996); for LSD DOI and DOM in rat cerebral cortex (Edwards et al., 1992; Sanders-Bush et al., 1988); for 5-MeO-DMT and bufotenine in rat tumoral cells (Cory et al., 1987); and for LSD, bufotenine, DOM, 5-MeO-DMT, DOI, and DOB in recombinant cells expressing 5-HT_2 receptors (Egan et al., 2000).

Further support for the 5-HT_{2A} (partial) agonism hypothesis arises from the fact that repeated exposure to indolealkylamine (LSD) and phenylalkylamine (DOI, DOB, DOM) psychedelics provokes a selective down-regulation of 5-HT_{2A} receptors (Aloyo et al., 2001; Anji et al., 2000; Buckoltz et al., 1985; Buckholtz et al., 1988; Buckholtz et al., 1990; Leysen et al., 1989; McKenna et al., 1989; Smith et al., 1999), a phenomenon that can be related to the development of tolerance to the behavioral effects of these drugs (Leysen et al., 1989; Smith et al., 1999). In contrast, it has been observed that repeated administration of LSD and DOM does not affect binding to 5-HT_{1A}, 5-HT_{1B}, β-adrenergic, α_1- or α_2-adrenergic or D_2-dopaminergic or to the serotonin transporter, suggesting that tolerance is not mediated by drug actions at these receptors (Buckholtz et al., 1990; Leysen et al., 1989).

Glennon (1990) reexamined the agonism-antagonism controversy and concluded that hallucinogens are not 5-HT_2 antagonists, but agonists, or at least partial agonists, at 5-HT_2 receptors. This author argued that some of these compounds display a low intrinsic activity; and may thus appear to antagonize the maximum effect of the full agonist (serotonin) when tested in combination.

4.2. DMT as a monoamine oxidase inhibitor (MAOI)

Monoamine oxidases A and B are flavoproteins located in the outer mitochondrial membrane. They catalyze the oxidation of amines to aldehydes. These enzymes oxidize endogenous neurotransmitters and also xenobiotics. MAO-A preferentially oxidizes norepinephrine and serotonin and is selectively inhibited by clorgyline. MAO-B preferentially degrades phenylethylamine and is selectively inhibited by *l*-deprenyl. The neurotransmitter dopamine is oxidized by isoforms A and B. Both forms are present in the human brain and peripheral organs, with high levels found in the liver (Saura et al., 1996). DMT is a substrate of MAO (Suzuki et al., 1981) and there is evidence that the drug displays MAO-inhibitory activity to some extent. Ho et al. (1970) examined a series of substituted dimethylaminoethylindoles and dimethylaminomethylindoles (gramines) and found for DMT an IC_{50} value in the millimolar range. This is, however, significantly less active than the β-carbolines, which have IC_{50} values between the micromolar and the 100 nanomolar range (see section 7.2. below). In the study by Ho et al. (1970), DMT was the most active in the dimethylaminoethylindole series followed in order of decreasing potency by 5-methyl-DMT > 5-MeO-DMT > 5-hydroxyl-DMT. Barlow (1961) found that millimolar concentrations of DMT inhibited tyramine oxidation by 80% and tryptamine oxidation by 44% in suspensions of guinea pig liver. Furthermore, DMT appeared to inhibit serotonin oxidation more potently, with 100 μM concentrations reducing MAO activity between 50 and 90%. These results indicate that DMT acts as a weak MAO inhibitor and suggest that it has a higher affinity for MAO-A than for MAO-B. A more recent study has found that, at low concentrations, DMT has affinity for both types of MAO, while at high concentrations the drug binds preferentially to isoform B (Suzuki et al., 1981).

4.3. Electrophysiological effects

The ability of the classical psychedelics to temporarily modify perception, cognition and mood has been interpreted as indicating a direct drug action on the neocortex or an indirect action on subcortical structures projecting to the neocortex (Marek and Aghajanian, 1998a). Early studies on the effects of psychedelics on neuronal electrical activity found LSD to potently and reversibly inhibit the tonic firing of serotonergic neurons in the dorsal raphe nucleus (Aghajanian et al., 1968), a subcortical structure located in the brainstem. This notion was later extended to other indolealkylamines such as DMT, 5-MeO-DMT and psilocin (Aghajanian and Haigler, 1975; Aghajanian et al., 1970; Rogawsky and Aghajanian, 1981), but was found to be inconsistent for the phenylalkylamines (Aghajanian et al., 1970; Haigler and Aghajanian, 1973). This ability to "antagonize" serotonergic neurotransmission was later found to be dependent on the interaction of these drugs at the 5-HT_{1A} somatodendritic autoreceptor (Aghajanian, 1995), of which serotonergic raphe neurons show a high density. At this level, indolealkylamines mimicked, rather than antagonized, serotonin. However, raphe neurons decreased their firing rate due to the autoreceptor-mediated inhibition. As discussed in the previous section, indolalkylamines demonstrate similar affinity for the 5-HT_{1A} and 5-HT_{2A} sites, whereas phenylalkylamines are fairly selective for the latter. It thus became evident that activity at the 5-HT_{1A} receptor level is not a common feature of the two main structural groups of psychedelic agents. Furthermore, the fact that not all drugs displaying agonist of partial agonist activity, e.g., buspirone, show psychedelic properties, together with the lack of a consistent correlation between affinity at this site and human psychedelic potency led researchers to the conclusion that inhibition of serotonergic neurotransmission must not be the underlying mechanism of psychedelic drug activity (Aghajanian, 1994).

What all classical psychedelics share is their ability to bind to the 5-HT_2 receptor family, and, as mentioned above, affinity at this level shows a good correlation with drug potency in humans and in drug discrimination animal models. Within the 5-HT_2 class, the 5-HT_{2B} receptor is mainly expressed in peripheral tissue and the 5-HT_{2A} predominates over the 5-HT_{2C} receptor throughout the neocortex (Pompeiano et al., 1994), making the former the most likely candidate for mediating psychedelic drug action. Thus, electrophysiological studies have mainly explored the effects of serotonin and psychedelics on 5-HT_{2A} receptors located in cortical regions.

The main effect of serotonin on cortical 5-HT_{2A} receptors is the induction of postsynaptic potentials, that can be inhibitory or excitatory depending on the brain region studied (Aghajanian and Marek, 1999a). In the rat piriform cortex, a paleocortical brain structure, serotonin evokes inhibitory postsynaptic potentials (IPSPs) in layer II pyramidal cells, through excitation of a subpopulation of GABAergic interneurons located in the border between layers II and III (Sheldon and Aghajanian, 1990; Sheldon and Aghajanian, 1991). This effect is mimicked by DOM, LSD and DOI but with a lower intrinsic efficacy, i.e., these compounds again appear to display partial agonist activity (Marek and Aghajanian, 1996; Marek and Aghajanian, 1998a; Sheldon and Aghajanian, 1990). In addition, the effect is blocked by selective 5-HT_{2A} antagonists (Marek and Aghajanian, 1994). Conversely, in the neocortex, serotonin and DOB interaction with the 5-HT_{2A} receptors lead to increases in membrane excitability of layer V pyramidal neurons (Araneda and Andrade, 1991). In a later study, bath application of serotonin was found to induce, via activation of the 5-HT_{2A} receptor, increases in the amplitude and frequency of spontaneous excitatory postsynaptic potentials (EPSPs) and currents (EPSCs). This effect was observed in layer V neurons of brain slices from transitional and neocortical areas, i.e., cingulate, medial prefrontal and fronto-parietal cortex (Aghajanian and Marek, 1997). Serotonin-induced spontaneous EPSPs/EPSCs were not blocked by GABA antagonists, but by selective 5-HT_2 receptors antagonists, and also by AMPA/kainate antagonists, suggesting the involvement of glutamate release in this effect. Despite the fact that glutamate was being released by presynaptic terminals, EPSCs did not appear to depend on the firing of neighboring neurons. In fact, they were also induced by direct application of serotonin at certain specific locations within the apical dendritic field of layer V pyramidal cells. However, selective group II metabotropic agonists acting on presynaptic inhibitory autoreceptors appeared to suppress the serotonin-induced increase in the frequency of EPSCs (Aghajanian and Marek, 1997). Similarly, μ-opiate receptor agonists also blocked the 5-HT induced EPSCs by means of a presynaptic mechanism (Marek and Aghajanian, 1998b). These findings would indicate that serotonin induces the excitatory effect by acting presynaptically, directly or indirectly, through a 5-HT_{2A}-mediated mechanism inducing focal glutamate release on apical dendrites of layer V pyramidal cells (Marek and Aghajanian, 1998c). In addition, the measured amplitude increases could indicate the participation of a postsynaptic amplification mechanism (Aghajanian and Marek, 1997) that could be mediated by 5-HT_2 receptors located in the apical dendrites of pyramidal cells (Jakab and Goldman-Rakic, 1998). The psychedelic phenylalkylamine DOI has been found to increase the amplitude of electrically evoked EPSPs

and also the frequency of spontaneous EPSCs, but to a lesser extent than 5-HT, suggesting partial agonist activity (Aghajanian and Marek, 1997). However, while DOI and LSD have a low efficacy in inducing spontaneous EPSCs, they have been found to enhance late EPSCs evoked by electrical stimulation of afferent fibers (Aghajanian and Marek, 1999a; Aghajanian and Marek, 1999b; Aghajanian and Marek, 2000). This late component involves a specific mechanism of neurotransmitter release termed asynchronous release, which is not promoted by serotonin. This difference between the endogenous neurotransmitter and the classical psychedelics has been proposed to explain why treatments that elevate endogenous serotonin levels do not elicit psychedelic effects (Aghajanian and Marek, 2000). A very recent study has observed DOI to increase the frequency of spontaneous EPSPs in the medial frontal cortex of mice, and also to induce a characteristic behavioral response in whole animals. Both effects could be effectively suppressed by the selective Group II mGlu presynaptic receptor agonist LY379268 (Klodzinska et al., 2002). In conclusion, the latest research suggests that psychedelic 5-HT_{2A} agonists interact with glutamatergic neurotransmission increasing glutamate release, presumably from thalamic afferents (Marek et al., 2001), without enhancing impulse flow. Also, activation of postsynaptic 5-HT_{2A} receptors probably counteracts dendritic inhibitory mechanisms, increasing the range of stimuli causing neurons to fire (Jakab and Goldman-Rakic, 1998).

4.4. Effects on immediate early gene expression

Immediate early genes are activated by external signals and do not require *de novo* synthesis of proteins. Their induction is fast and transient and they are thought to encode transcription factors which will, in turn, modify the expression of other genes known as target genes. The *c-fos* gene is regarded to link external cellular signals with phenotypic changes in brain cells and there exists evidence for the involvement of the Fos protein in the control of behavior. Neuronal activation leads to expression of *c-fos* and the study of this process is believed to provide an in vivo map of cellular responses to a given stimulus (for a review see Herrera and Robertson, 1996). Recently, LSD and DOI administration to rats has been found to stimulate *c-fos* expression in the rat cortex (Frankel and Cunningham, 2002; Gresch et al., 2002; Scruggs et al., 2000; Zhai et al., 2003). LSD increased the levels of the Fos protein in the medial prefrontal cortex and in the anterior cingulate cortex (Frankel and Cunningham, 2002; Gresch et al., 2002); whereas DOI-induced increases in *c-fos* mRNA and Fos protein were observed throughout the cortex (Zhai et al., 2003) and in the somatosensory cortex (Scruggs

et al., 2000), respectively. The involvement of glutamate release in this effect has been demonstrated by the fact that *c-fos* expression by DOI can be reduced by pretreatment with postsynaptic glutamate AMPA antagonists (Scruggs et al., 2000). Furthermore, pretreatment with the presynaptic receptor mGlu2/3 agonist LY379268 was found to block the DOI-induced increase in *c-fos* mRNA in the prefrontal cortex (Zhai et al., 2003). Scruggs and coworkers (2000) observed that lesions of the thalamic afferents to somatosensory cortex reduced the DOI-induced *c-fos* expression. Additionally, Gresch et al. (2002) showed that the majority of cells expressing Fos did not present 5-HT_{2A} receptors. These results suggest an indirect action of 5-HT_{2A} receptors on cortical pyramidal cells, presumably involving glutamate release from thalamo-cortical afferents.

4.5. Tolerance and cross-tolerance

In a review of a large number of studies conducted to determine whether tolerance developed to psychedelic drug administration (Wyatt et al., 1976), this effect was found to occur for LSD, mescaline, DOM and psilocybin in all human trials conducted. Tolerance was also found to develop for these drugs in the vast majority of animal studies examined. This is in sharp contrast with data from DMT. Gillin and coworkers (1976) reported that preliminary data on 4 normal volunteers who received two daily injections of 0.7 mg/kg DMT i.m. for five days were inconclusive in this respect. No consistent changes were found in autonomic variables, subjective ratings or behavior, although a certain decrease in ratings of subjective "high" was recorded. A more recent study (Strassman et al., 1996) found differential tolerance to develop after a dosing regime involving four doses of 0.3 mg/kg DMT i.v. administered at 30 min intervals. Thus, scoring on subjective rating scales or blood pressure increases did not attenuate, whereas neuroendocrine responses (adrenocorticotropic hormone, prolactin and cortisol) and heart rate decreased from the first to the fourth administered dose. Animal studies similarly indicate that tolerance does not develop easily. Thus, failure to develop tolerance has been observed for behavioral and EEG measures in cats (Gillin et al., 1973), and for DMT-induced disruption of operant behavior in primates (Cole and Pieper, 1973). Unlike these authors, Cooper et al. (1981) observed tolerance to the effects of DMT on unconditioned behavior in mice, and Kovacic and Domino (1976) were able to produce a certain degree of tolerance to operant behavior disruption in rats but only by administering very frequent DMT doses for a prolonged period of time, i.e., every 2 h for 21 days.

Another unsettled aspect of DMT pharmacology is whether it displays cross-tolerance with other serotonergic psychedelics. Cross-tolerance has consistently been found between LSD and mescaline and between LSD and psilocybin, both in animal and human studies (Wyatt et al., 1976), as would be expected from drugs with a common mechanism of action despite their different chemical structures. Although statistically significant decreases in subjective ratings and clinical evaluation scores were found after DMT administration to subjects tolerant to LSD in the only human cross-tolerance study performed to date with DMT, these decreases were regarded as moderate by the researchers. What is more, cross-tolerance to the mydriatic effect of DMT could not be demonstrated in this group of subjects who had developed a robust tolerance to the mydriatic effects of LSD (Rosenberg et al., 1964). In animals, Kovacic and Domino (1976) demonstrated that rats tolerant to LSD displayed cross-tolerance to a 3.2 mg/kg DMT dose, but not after a 10 mg/kg dose. Additionally, rats tolerant to 3.2 mg/kg of DMT displayed cross-tolerance to LSD, but strangely, rats tolerant to 10 mg/kg of DMT only displayed minimal tolerance to LSD and for a brief period of time.

4.6. Effects on sensory and sensorimotor gating

Current research with psychedelics has explored the possibility that drugs displaying agonist activity at the 5-HT_{2A} sites temporally disrupt inhibitory neural mechanisms thought to intervene in the normal filtering of information. This hypothesis is based on the assumption of the existence of brain mechanisms directed at filtering out, under normal conditions, the flow of sensory information reaching consciousness. According to this model, serotonergic psychedelics would interact with brain structures involved in the gating mechanisms, temporarily decreasing their functionality and giving rise to the characteristic perceptual and cognitive effects elicited by these agents (Vollenweider, 1994).

Two neurophysiological measures have been developed to evaluate the functionality of neural gating mechanisms: suppression of the P50 auditory evoked potential (AEP) and prepulse inhibition of the startle reflex (PPI). The P50 AEP is a midlatency potential appearing about 50 ms after the presentation of an auditory stimulus (Picton et al., 1974). The consecutive administration of two identical stimuli, a conditioning and testing stimulus, at a certain interstimulus interval, typically 500 ms, leads to a decrease in the amplitude of the second P50 wave (Adler et al., 1982). The amplitude decrement seen for the testing stimulus is thought to obey active inhibitory mechanisms triggered by the conditioning stimulus

(Freedman et al., 1983). P50 suppression is regarded as a measure of sensory gating, and its neural substrates have been located in the hippocampus, in the mesial temporal lobe (Adler et al., 1998).

The second operational measure, PPI, is based on the inhibitory effect of a weak sensory stimulus (the prepulse) on the motor response caused by a stronger startle reflex-eliciting stimulus. The startle reflex is a brainstem reflex occurring after the presentation of intense and sudden sensory stimuli. Prepulse inhibition is obtained when the startling stimulus is preceded 15-400 ms by the prepulse, and it manifests as a decrease in the intensity of the reflex (Blumenthal, 1999). Typically, in PPI studies the habituation of the startle reflex across the experimental session is also measured. In contrast to P50, PPI is considered a measure of sensorimotor gating, given that the response measured is the motor output to the presented stimulus. While the neural circuit mediating the startle reflex is located in the brainstem, prepulse inhibition is regulated by descending projections from areas in the forebrain (Swerdlow et al., 2001).

Studies in animals have evaluated suppression of the N40 potential in rodents in a paired stimuli paradigm, homologous to that of the human P50. In the only study reported to date on the effects of 5-HT_2 modulation of N40 suppression, an unexpected disruptive effect was found for the 5-$HT_{2A/2C}$ antagonist ketanserin. Conversely, the 5-$HT_{2A/2C}$ agonist DOI increased filtering and was also capable to revert the reductions in filtering caused by ketanserin and amphetamine (Johnson et al., 1998). To my knowledge, no study has been carried out to date on the influence of serotonergic psychedelics/entactogens on the human P50 suppression paradigm.

Braff and Geyer (1980) demonstrated an impairment in habituation of tactile startle in rats after administration of the mixed serotonergic agonist LSD. PPI has also been found to be impaired in rats after the 5-$HT_{2A/2C}$ agonist DOI, an effect which can be prevented by mixed 5-$HT_{2A/2C}$ (Sipes and Geyer, 1994) and selective 5-HT_{2A} antagonists (Padich et al., 1996; Sipes and Geyer, 1995). In a recent article, LSD was found to disrupt PPI in rats, and this effect was prevented only by selective 5-HT_{2A} antagonists. Other antagonists with affinity for the 5-HT_{2C}, 5-$HT_{2B/2C}$, 5-HT_{1A}, and 5-HT_6 did not counteract LSD-induced disruptions (Ouagazzal et al., 2001). In the only human study performed to date involving serotonergic psychedelics, the administration of psilocybin provoked a mild though significant increase of

PPI at a prepulse-to-pulse interval of 100 ms, with no significant effects on habituation (Gouzoulis et al., 1998).

4.7. Effects on regional cerebral blood flow and glucose metabolism

In recent years, nuclear medicine techniques have been incorporated to the in vivo study of psychedelic drugs in humans. Although DMT has not been specifically studied, other drugs with similar receptor affinity profiles have been investigated. A PET investigation utilizing ^{18}F-glucose revealed that the most important metabolic changes after psilocybin administration to humans occur predominantly in the temporomedial, frontomedial, frontolateral and anterior cingulate cortices, where an increase in glucose metabolism is observed (Vollenweider et al., 1997). Metabolic increases in frontomedial regions, and more specifically in the anterior cingulate cortex, have also been observed by another research group after psilocybin in a ^{18}F-glucose PET study (Gouzoulis-Mayfrank et al., 1999a). A previous investigation on regional cerebral blood flow after acute mescaline administration also found an increase in perfusion in the frontal lobes (Hermle et al., 1992). In view of these results, a metabolic "hyperfrontality" has been proposed as the whole-brain mechanism involved in the effects of psychedelics (Gouzoulis-Mayfrank et al., 1999a; Hermle et al., 1992; Vollenweider et al., 1997).

5. DMT metabolism and interactions with other drugs

5.1. DMT metabolism

Data from clinical trials have highlighted the short-lived nature of the subjective effects elicited by DMT, the drug's rapid disappearance from plasma, and the low percentage of the administered dose which can be recovered unmetabolized in urine (Kaplan et al., 1974). Also, in vivo studies in animals have attested a very rapid clearance of DMT from various tissues such as plasma, brain, and liver (Cohen and Vogel, 1972; Mandel et al., 1977; Sitaram et al., 1987b), which considered together with human data is suggestive of an extensive and efficacious metabolism. Unfortunately, as already mentioned in the pharmacokinetics section, only one study has assessed DMT metabolism in humans. Following the i.m. administration of DMT, Szára (1956) identified in urine IAA as the drug's degradation product, with no DMT being detected. This finding was in agreement with a previous study by Ersparmer

(1955), who had found this metabolite in rodent urine, and pointed at oxidative deamination, a reaction catalyzed by MAO, as the process involved in the metabolic breakdown of DMT.

Besides monoamine oxidase-catalized oxidative deamination, in vivo and in vitro studies have also identified *N*-oxidation as an important degradative pathway of DMT, and to a lesser extent *N*-demethylation (Sitaram and McLeod, 1990; Sitaram et al., 1997c). Oxidative deamination was found to be a major route in brain liver and kidney, whereas NADPH-dependent microsomal *N*-oxidation predominated in peripheral tissue and was a minor route in the brain (Sitaram et al., 1997c). According to these authors, *N*-demethylation is a minor degradation route for this compound in all tissues examined (Sitaram and McLeod, 1990). Earlier in vitro studies by Fish et al. (1955b) had already identified DMT-*N*-oxide (DMT-NO) and IAA as the main metabolic products of DMT. They also found NMT to be converted to IAA, but not DMT-NO. These investigators concluded that *N*-oxidation was the main metabolic route in the absence of mitochondrial MAO and that the *N*-oxide compound was not an intermediate to IAA formation by MAO. In order to explore routes other than oxidative deamination, Szára and Axelrod (1959) found that incubation of DMT in rabbit liver microsomal fraction (in vitro experiment) pretreated with the MAO inhibitor iproniazid yielded NMT, DMT-NO, 6-hydroxy-DMT and 6-hydroxy-DMT-NO. In the same paper, the authors reported that the administration of DMT in vivo to rats pretreated with iproniazid yielded NMT, tryptamine, 6-hydroxy-DMT, IAA, 6-hydroxy-IAA, but failed to detect DMT-NO and 6-hydroxy-DMT-NO, which had been found in the in vitro experiment. The presence of hydroxylated metabolites in these experiments and the finding that 6-hydroxy-diethyltryptamine appeared to be more active than diethyltryptamine in animal studies (Szára and Hearst, 1962) led to the speculation that some of the hydroxylated metabolites, specifically 6-hydroxy-DMT, might be responsible for the psychedelic effects of DMT (Szára, 1961; Szára and Hearst, 1962). Nevertheless, this was refuted in a later clinical trial in which DMT and 6-hydroxy-DMT were administered to humans and the latter was found to be inactive (Rosenberg et al., 1963). The relevance of hydroxylation as a metabolic pathway of DMT has received no further attention in the most recent studies and 6-hydroxyderivatives have not been assessed in several experiments on the in vivo and in vitro metabolism of DMT and 5-methoxy-DMT (Sitaram and McLeod, 1990).

Besides the metabolites mentioned above, studies by Barker and coworkers (1980) have detected cyclization derivatives of DMT. These investigators reported IAA, NMT, DMT-NO and 2-methyl-tetrahydro-*β*-carboline (MTHβC) as the main DMT metabolites in rat whole brain homogenates, together with traces of tryptamine and 1,2,3,4-tetrahydro-*β*-carboline. No hydroxylated metabolites were detected and the authors justified this arguing that this reaction takes place in peripheral tissue but not in the brain. They also reported that when DMT was incubated in brain homogenates of rats pretreated with iproniazid, IAA formation was reduced by 83%, but unexpectedly, NMT and DMT-NO formation were also reduced by 90%, and no tetrahydro-*β*-carboline could be measured. Based on these results, the authors pointed out that the enhancement of behavioral effects and increments in DMT half-life in tissue observed after pretreatment with iproniazid in other studies (Kovacic and Domino, 1973, cited in Wang Lu and Domino, 1976; Shah and Hedden, 1978; Wang Lu and Domino, 1976) might also be due to the inhibition of *N*-demethylation and *N*-oxidation rather than to a unique and selective inhibition of MAO. However, these results were not replicated by the group of Sitaram, who showed iproniazid to inhibit the formation of indoleacetaldehyde and IAA from DMT in liver homogenates (Sitaram et al., 1987c) but not the formation of DMT-NO. These authors also found iproniazid to increase the levels of DMT in vivo in rat brain, liver, kidney and blood and those of DMT-NO in rat liver (Sitaram et al., 1987b), and to increase rat urinary excretion of unmetabolized DMT, DMT-NO and NMT (Sitaram et al., 1987a). These results would indicate that after MAO inhibition, DMT metabolism is shifted to other functioning routes. The pharmacological modulation of metabolic pathways independent of MAO has been studied in a series of drug-interaction studies. Wang Lu et al. (1978) observed increases in brain and liver DMT levels after pretreatment with SKF-525A, an inhibitor of the microsomal CYP system, whereas Shah and Hedden (1978) did not. In the study by Wang Lu et al. (1978), chronic phenobarbital administration, a drug which stimulates microsomal CYP activity, reduced brain and liver DMT levels. Also, neuroleptics such as haloperidol and chlorpromazine, respectively, decreased and increased, brain DMT levels. In conclusion, all these studies point out that although oxidative deamination of the side chain by monoamine oxidase appears to be the main metabolic pathway of DMT, the drug can also be degraded by other routes, mainly *N*-oxidation, but possibly also by *N*-demethylation, 6-hydroxylation and cyclization. The extent to which these pathways may be active or even predominate when the drug is administered orally concomitantly with selective MAO inhibitors, as is the case in *ayahuasca* potions, remains to be assessed.

5.2. DMT interactions with MAOIs and other drugs in animals

In addition to the effects of drugs on DMT tissue levels and metabolism discussed in the previous section, a number of studies have assessed the effects of the interaction of DMT with other drugs on animal behavior and physiological variables. Moore et al. (1975) studied the effects of iproniazid, chlorpromazine and methiothepin on DMT-induced changes in body temperature, pupillary dilatation, blood pressure and EEG in rabbits. In this paper, only three animals were studied and no figures are provided regarding the EEG and pupil diameter data, despite the fact that the authors mention that both variables are modified after DMT and discuss the effects of the various drugs tested on the DMT-induced changes. They conclude that iproniazid had a potentiating action in the effects of DMT, while chlorpromazine and methiothepin antagonized these actions of DMT. Regarding the effects of iproniazid, they conclude that the drug prolonged the mydriatic action of DMT while no data are provided to support this conclusion. Similarly, the authors state that iproniazid prolonged the elevated rectal temperature induced by DMT, but the data do not clearly support this if differences in baseline values are taken into account. Drug effects on blood pressure were not markedly altered by pretreatment with iproniazid. Results from chlorpromazine and methiothepin show that these drugs attenuated DMT-induced hyperthermia and increases in blood pressure. Shah and Hedden (1978) found the MAOI iproniazid prolonged the abnormal behavior induced by DMT, in contrast with the microsomal enzyme inhibitor SKF-525A and the neuroleptic chlorpromazine, which did not alter the effects of DMT administered alone. These authors also found that iproniazid increased drug levels in plasma, brain and liver. They concluded that DMT effects are due to the parent compound and not to a metabolite and that degradation by MAO is the primary metabolic route of DMT in vivo. Finally, Stoff et al. (1982) found the MAOI pargyline to potentiate DMT-induced disruption of conditioned avoidance response in rats.

5.3. DMT interactions with MAOIs and other drugs in humans

The specific question of the interaction of oral DMT with MAO inhibitors has not been evaluated in controlled clinical trials, but valuable information has been obtained by different researchers in single-subject experiments involving the self-administration of drugs. Initial efforts were directed at elucidating whether harmaline could render DMT orally active. Bigwood found that a dose of 100 mg DMT freebase (1.16 mg/kg) taken in combination with

a dose of harmine hydrochloride equivalent to 86 mg freebase (1.0 mg/kg) resulted in distinct psychedelic effects (Bigwood 1978, cited in Ott, 1999). However, given the low concentrations of harmaline relative to those of harmine in *B. caapi* and in *ayahuasca*, Ott (1999) experimented with the oral ingestion of combinations of DMT plus harmine. With this procedure he established the threshold dose of harmine necessary to render DMT orally active at 1.5 mg/kg. At this harmine dose, the threshold for DMT was found at 0.38 mg/kg and doses as high as 2.0 mg/kg were tested. The author found the intensity of effects to be enhanced with increasing doses, while the time course of effects remained the same, resembling that of his self-experiments with *ayahuasca*. In his own words: "45 minutes to an hour incubation period, with effects quickly building to a peak within the next 30 minutes and maintaining a plateau for 45 minutes to an hour, followed by about an hour of diminishing effects; the experience was usually completely over within three hours". Ott expanded these findings to the MAO inhibitor isocarboxazid, which rendered DMT orally active after pretreatment with 30 mg. Other self-experimenters have replicated these results, finding that harmaline alone in doses around 1 mg/kg lacks visionary activity and that both harmine and harmaline at 1.0-1.5 mg/kg doses render various tryptamines such as DMT, 5-MeO-DMT and diethyltryptamine orally active (for a review see Ott, 1999).

The above findings are in clear contrast with an early study in which healthy human volunteers received i.m. doses (two subjects 0.35-0.55 mg/kg and five subjects 0.65-0.83 mg/kg) of DMT following pretreatment with 100 mg of the MAOI iproniazid administered daily for four days (Sai-Halász, 1963). Subjects receiving the DMT low doses following the iproniazid pretreatment period experienced none of the typical visual effects, reporting only a feeling of "strangeness". Those receiving the high doses had a biphasic response. Immediately after drug administration the usual DMT effects were experienced, although less pronounced, and after the first 30 min, they reported a persistent feeling of "strangeness" characterized by indifference and "emotional bleakness" (Sai-Halász, 1963). Thus, while acute MAO inhibition may facilitate DMT absorption and probably reduce its elimination rate by preventing drug metabolism, chronic MAOI administration apparently leads to a reduction in the potency of subjective DMT effects. The mechanism involved remains to be clarified, although Sai-Halász (1963) proposed that enhanced serotonin levels in the CNS may be responsible for this effect.

6. Pharmacology of *B. caapi* β-carbolines in humans

These tricyclic compounds are the most abundant alkaloids in *ayahuasca* potions, and represent the chief contribution of *B. caapi* to the tea. Despite the fact that harmaline has at some stage been considered to be responsible for the psychotropic properties of *ayahuasca* (Naranjo, 1967), the capacity of the β-carbolines to interact with the 5-HT_{2A} receptor and/or their ability to elicit effects in humans analogous to those of the psychedelic tryptamines and phenylethylamines has not been clearly established. Receptor binding and drug discrimination studies have provided inconsistent results, and definitive conclusions cannot be drawn regarding whether certain doses of these drugs, which show varying affinity to the 5-HT_{2A} receptor, elicit in animals interoceptive effects similar to those of the classical psychedelics. In addition, their ability to elicit clear-cut psychedelic effects has been repeatedly contested. The question remains controversial owing to the fact that the pharmacology of β-carbolines has been poorly characterized in humans.

6.1. Subjective effects

The few data available on the subjective effects of ß-carbolines mainly stem from reports of self-experiments and from the clinical studies by Pennes and Hoch (1957), Naranjo (1967), and Slotkin (1970). Information from anecdotal reports makes the classification of the harmala alkaloids as psychedelics doubtful a priori. In their book TIHKAL, Shulgin and Shulgin (1997) cite other researchers' self-experiment reports in which harmine was described as mainly inducing dysphoric physical reactions and some psychological effects which ranged from relaxation (300 mg sublingually) and sedation (140 mg orally) to excitement and restlessness (40 mg orally), belligerency (above 40 mg orally) and "psychotic symptoms" (300-400 mg orally). These reports thus appear to be contradictory, and there seems to be no consistent dose-response pattern that could be derived from these data. In the chapter devoted to harmaline, Shulgin and Shulgin (1997) describe their own self-experiments with the drug. They report experiencing no effects with 100 mg orally, numbness and some modification of auditory perception with 150 mg orally, and visual imagery with eyes closed at 200 mg orally and also at the higher 300-500 mg dose range. At these higher doses nausea and general malaise were very prominent.

Also in self-experiments, Ott found that when 1.5 mg/kg harmine (120 mg), i.e, the threshold dose capable of rendering DMT orally active, was ingested in the absence of DMT, the drug exerted "barely perceptible sedative effects" (Ott, 1999). Based on these observations, the typical visionary effects of *ayahuasca* would appear to be related to the DMT present in the potion rather than to the psychotropic effects of harmine. Self-reports by other authors have described no "notable psychoactive or somatic effect felt" after 0.5 mg/kg harmine taken intranasally and orally (De Smet, 1985, cited in Ott, 1993) or have characterized harmine as "a mild sedative in low doses, causing unpleasant vegetative and neurological symptoms at doses above 300 mg" (Leuner and Schlichting, 1991, cited in Ott, 1993). The same applies to harmaline, which Bigwood found to be inactive after ingesting a 100 mg dose (Bigwood 1978, cited in Ott, 1993).

Pennes and Hoch (1957) administered harmine orally, subcutaneously and intravenously to psychiatric patients, mainly schizophrenics, in doses ranging from 20 to 960 mg. Harmine was described to be hallucinogenic above 150-200 mg intravenously, with 50% of the subjects reporting "visual hallucinations", or rather "hypnagogic imagery or visions", which disappeared with eyes open. Visual hallucinations occurred at medium or high dosage, and shallow euphoria was occasionally observed. The maximum administered i.v. dose was 300 mg. These researchers observed bradycardia and hypotension following i.v. harmine administration. The drug was not found to be hallucinogenic after oral (up to 960 mg) or subcutaneous administration. The authors commented that harmine produced "mental clouding" and drowsiness, in contrast with LSD and mescaline, which they had studied previously.

Naranjo (1967) published a report on the human pharmacology of the harmala alkaloids. While most of the chapter centered basically on harmaline, he also provided some data regarding harmine and THH. According to Naranjo, threshold doses capable of eliciting hallucinogenic activity are 8 mg/kg orally for harmine and 1 mg/kg i.v. or 4 mg/kg orally for harmaline. He further commented that THH appears to be even less active than harmine, with an oral dose of 300 mg *d,l*-THH eliciting subjective effects similar to those of 100 mg of harmaline. Apparently, these studies were conducted in the absence of placebo controls and the author does not provide any information in the paper as to how the intensity of effects was measured in order to establish comparisons between drugs. In the case of the comparison between harmine and THH, data were obtained from only one volunteer. Unfortunately, this

limited report remains to date the primary source of evidence of the psychedelic effects of the harmala alkaloids. In addition to methodological limitations, further doubts on the validity of Naranjo's conclusions arise from the apparent contradiction with results by Pennes and Hoch (1957). Thus, the threshold dose for oral harmine reported by Naranjo (1967), i.e., 8 mg/kg, is below the 960 mg oral dose (aprox. 12 mg/kg in a 70 kg individual) which was found by Pennes and Hoch (1957) to lack hallucinogenic effects. As mentioned above, the main focus of Naranjo's paper was harmaline, the effects of which (orally and i.v. administered) were compared with those of the more prototypical psychedelic mescaline. The study participants were apparently able to distinguish between the effects of harmaline and mescaline. Whereas mescaline induced marked modifications in the environment, this remained unchanged under harmaline. However, following harmaline administration subjects described images superimposed on the surroundings and one participant reported experiencing a true hallucination. Vibration in the visual field and light flashes were also reported. With eyes closed, subjects experienced vivid colorful dream-like images. In contrast with mescaline, auditory perception was not modified, and neither were thought processes, emotions or the rate of time passing. Naranjo (1967) concluded that harmaline appeared to be "more of a pure hallucinogen than other substances" due to its effects targeting primarily visual perception without affecting other spheres of the psyche. He globally described harmine and harmaline as showing a "highly hallucinogenic quality in the visual domain", although harmaline appeared to cause "more withdrawal and lethargy" than harmine.

Slotkin and coworkers (1970) administered i.v. doses of 0.5 mg/kg harmine to five healthy male volunteers. This dose was far below the threshold of hallucinogenic activity reported by Pennes and Hoch (1957) or the amounts administered by Naranjo (1967). Contrary to the observations by the latter, the volunteers in this study did not experience hallucinations or psychedelic effects.

6.2. Pharmacokinetics and metabolism

Slotkin and coworkers (1970) reported a rapid drop in plasma harmine concentrations following the drug's i.v. administration. Similarly to DMT, pharmacokinetic parameter values have never been assessed for these compounds in humans. Regarding metabolism, *O*-demethylation appears to be a major metabolic pathway for *β*-carbolines with a methoxy group in position 7. Harmol glucuronide and harmol sulfate have been described as the main

urinary metabolites of harmine following its i.v. administration to humans and rodents (Slotkin et al., 1970). A very recent study has found cytochrome P450 to catalyze the *O*-demethylation of harmine and harmaline, and has identified CYP2D6 and CYP1A1 as the major isoenzymes involved in the process (Yu et al., 2003). To my knowledge no data are available regarding the metabolism of THH.

6.3. Cardiovascular effects

No systematic controlled study was found in the literature on the cardiovascular effects of the harmala alkaloids. However, Pennes and Hoch (1957) and Slotkin et al (1970) have reported bradycardia and hypotension after acute i.v. administration of harmine.

6.4. Adverse effects

Harmine and harmaline appear to induce a plethora of somatic-dysphoric effects. These have become apparent both in self-experiments and in clinical studies. Thus, Leuner and Schlichting cited "unpleasant vegetative and neurological symptoms" above 300 mg harmine (Leuner and Schlichting, 1991, cited in Ott, 1993). Pennes and Hoch (1957) reported nausea, vomiting, tremor and body numbness, which were very frequent after i.v. administration and in some cases per os with doses above 300-400 mg. Naranjo (1967) reported harmaline appeared to elicit more physical effects than mescaline. Unpleasant effects were mainly paresthesias and numbness, physical discomfort, nausea, intense vomiting and dizziness. Finally, Slotkin et al. (1970) observed "bradycardia, trouble in focussing the eyes, tingling, hypotension, cold extremities and light-headedness".

7. Mechanism of action of β-carbolines

7.1. Receptor level interactions

The receptor binding profile of the β-carbolines has not been evaluated systematically until recent times. Drug displacement studies using [^{3}H]DOB as 5-HT_{2A} ligand showed that the β-carbolines in general bind with modest affinity to this receptor. An exception is harmine which shows nanomolar affinity for this site, a surprisingly similar affinity value to that of DMT (Glennon et al., 2000). These authors studied a series of β-carbolines and found the

following order of decreasing affinity for the 5-HT_{2A} site: harmine > harmaline > *l*-THH > *d*-THH > harmalol (Glennon et al., 2000). Analogous results had been obtained in a previous study using [^{3}H]ketanserin as the labeled radioligand (Grella et al., 1998). In these two studies, affinity for the 5-HT_{2C} site also decreased from the full aromatic harmine, to harmaline and THH, which showed the lowest affinity. Harmine affinity for the 5-HT_{2A} receptor was an order of magnitude greater than for the 5-HT_{2C} site. Furthermore, harmine, harmaline, THH and harmalol showed no relevant affinity for the 5-HT_{1A} receptor, the D_2 dopamine receptor or the benzodiazepine receptor. Only certain compounds with a methoxycarbonil group in position three of the fully aromaticβ-carboline (not present in *B. caapi*) showed any relevant affinity for the benzodiazepine receptor in this study (Glennon et al., 2000), in agreement with previous results by Lippke et al. (1983). Other studies for harmine and harmaline had found low micromolar affinity for the benzodiazepine receptor (Müller et al., 1981; Rommelspacher et al., 1981). A recent study found that β-carbolines show nanomolar affinity for the I_2 imidazoline receptor. Affinity was higher for harmine, intermediate for harmaline and lowest for THH (Husbands et al., 2001). Receptor affinity values for the β-carbolines are shown in Tables 7-10.

Table 7: Harmine radioligand binding data at serotonergic, dopaminergic, benzodiazepine, muscarinic cholinergic, opioid, imidazoline and adrenergic receptors.

	K_i (nM)									
	5-HT_{2A}	**5-HT_{2C}**	**5-HT_{1A}**	**D_2**	**BZD**	**Musc.**	**Opioid**	**I_2**	**I_1**	**α_2**
Husbands et al., 2001[a]								10	-	>10,000
Glennon et al., 2000[b]	397	5,340	>10,000	>10,000	>10,000	-	-	-	-	-
Grella et al., 1998[c]	230	5,340	-	-	-	-	-	-	-	-
	IC_{50} (nM)									
Husbands et al., 2001[a]	-	-	-	-	-	-	-	-	629	-
Müller et al., 1981[d]	-	-	-	69,000	134,000	5,000	7,000	-	-	-

D_2=Dopamine-2, BZD=benzodiazepine, Musc.=muscarinic cholinergic, I_1=Imidazoline-1, I_2=Imidazoline-2, α_2=α_2-adrenergic. (-) = not determined.

[a] Radioligands: [^{3}H]clonidine + rauwolscine (Imidazoline I_1), [^{3}H]2BFI (Imidazoline I_2), [^{3}H]RX821002 (α_2-adrenoceptor).

[b] Radioligands: [^{3}H]DOB (5-HT_{2A}), [^{3}H]8-OH DPAT (5-HT_{1A}), [^{3}H]Mesulergine (5-HT_{2C}), [^{3}H]N-methylspiperone (D_2), [^{3}H]RO15,1788 (BZD).

[c] Radioligands: [^{3}H]Ketanserin (5-HT_{2A}), [^{3}H]Mesulergine (5-HT_{2C}).

[d] Radioligands: [^{3}H]Spiperone (D_2),[^{3}H]Flunitrazepam (BZD), [^{3}H]Quinuclidinylbenzylate (QNB, Muscarine Cholinergic), [^{3}H]Naloxone (Opioid).

Table 8: Harmaline radioligand binding data at serotonergic, dopaminergic, benzodiazepine, muscarinic cholinergic, opioid, imidazoline and adrenergic receptors.

	K_i (nM)									
	5-HT$_{2A}$	**5-HT$_{2C}$**	**5-HT$_{1A}$**	**D$_2$**	**BZD**	**Musc.**	**Opioid**	**I$_2$**	**I$_1$**	**α_2**
Husbands et al., 2001[a]	-	-	-	-	-	-	-	22	-	>10,000
Glennon et al., 2000[b]	5,010	9,430	>10,000	>10,000	>10,000	-	-	-	-	-
Grella et al., 1998[c]	7,790	9,430	-	-	-	-	-	-	-	-
Helsley et al., 1998b[d]	42,500	-	-	-	-	-	-	-	-	-
	IC_{50} (nM)									
Husbands et al., 2001[a]	-	-	-	-	-	-	-	-	13,800	-
Müller et al., 1981[e]	-	-	-	207,000	390,000	52,000	13,000	-	-	-
Rommelspacher et al., 1981[f]	-	-	-	-	390,000	-	-	-	-	-

D_2=Dopamine-2, BZD=benzodiazepine, Musc.=muscarinic cholinergic, I_1=Imidazoline-1, I_2=Imidazoline-2, α_2=α_2-adrenergic. (-) = not determined.

[a] Radioligands: [^{3}H]clonidine + rauwolscine (Imidazoline I_1), [^{3}H]2BFI (Imidazoline I_2), [^{3}H]RX821002 (α_2-adrenoceptor).

[b] Radioligands: [^{3}H]DOB (5-HT$_{2A}$), [^{3}H]8-OH DPAT (5-HT$_{1A}$), [^{3}H]Mesulergine (5-HT$_{2C}$), [^{3}H]N-methylspiperone (D_2), [^{3}H]RO15,1788 (BZD).

[c] Radioligands: [^{3}H]Ketanserin (5-HT$_{2A}$), [^{3}H]Mesulergine (5-HT$_{2C}$).

[d] Radioligand: [^{3}H]Ketanserin (5-HT$_{2A}$).

[e] Radioligands: [^{3}H]Spiperone (D_2),[^{3}H]Flunitrazepam (BZD), [^{3}H]Quinuclidinylbenzylate (QNB, Muscarine Cholinergic), [^{3}H]Naloxone (Opioid).

[f] Radioligand: [^{3}H]Flunitrazepam (BZD)

Table 9: THH radioligand binding data at serotonergic, dopaminergic, benzodiazepine, muscarinic cholinergic, opioid, imidazoline and adrenergic receptors.

	K_i (nM)									
	5-HT$_{2A}$	**5-HT$_{2C}$**	**5-HT$_{1A}$**	**D$_2$**	**BZD**	**Musc.**	**Opioid**	**I$_2$**	**I$_1$**	**α_2**
Husbands et al., 2001[a]	-	-	-	-	-	-	-	172	-	-
Glennon et al., 2000[b]	>10,000	>10,000	>10,000	-	-	-	-	-	-	-
Grella et al., 1998[c]	>10,000	>10,000	-	-	-	-	-	-	-	-
	IC_{50} (nM)									
Husbands et al., 2001[a]	-	-	-	-	-	-	-	-	2650	-

D_2=Dopamine-2, BZD=benzodiazepine, Musc.=muscarinic cholinergic, I_1=Imidazoline-1, I_2=Imidazoline-2, α_2=α_2-adrenergic. (-) = not determined.

[a] THH enantiomer tested not specified, presumably racemic mixture. Radioligands: [^{3}H]clonidine + rauwolscine (Imidazoline I_1), [^{3}H]2BFI (Imidazoline I_2), [^{3}H]RX821002 (α_2-adrenoceptor).

[b] R(+) enantiomer of THH tested. Radioligands: [^{3}H]DOB (5-HT$_{2A}$), [^{3}H]8-OH DPAT (5-HT$_{1A}$), [^{3}H]Mesulergine (5-HT$_{2C}$), [^{3}H]N-methylspiperone (D_2), [^{3}H]RO15,1788 (BZD).

[c] R(+) enantiomer of THH tested. Radioligands: [^{3}H]Ketanserin (5-HT$_{2A}$), [^{3}H]Mesulergine (5-HT$_{2C}$).

Table 10: Harmalol radioligand binding data at serotonergic, dopaminergic, benzodiazepine, muscarinic cholinergic, opioid, imidazoline and adrenergic receptors.

	K_i (nM)									
	5-HT_{2A}	**5-HT_{2C}**	**5-HT_{1A}**	**D_2**	**BZD**	**Musc.**	**Opioid**	**I_2**	**I_1**	**α_2**
Husbands et al., 2001[a]	-	-	-	-	-	-	-	132	-	-
Glennon et al., 2000[b]	>10,000	>10,000	>10,000	-	-	-	-	-	-	-
	IC_{50} (nM)									
Husbands et al., 2001[a]	-	-	-	-	-	-	-	-	1591	-

D_2=Dopamine-2, BZD=benzodiazepine, Musc.=muscarinic cholinergic, I_1=Imidazoline-1, I_2=Imidazoline-2, α_2=α_2-adrenergic. (-) = not determined.
[a] Radioligands: [^{3}H]clonidine + rauwolscine (Imidazoline I_1), [^{3}H]2BFI (Imidazoline I_2), [^{3}H]RX821002 (α_2-adrenoceptor).
[b] Radioligands: [^{3}H]DOB (5-HT_{2A}), [^{3}H]8-OH DPAT (5-HT_{1A}), [^{3}H]Mesulergine (5-HT_{2C}), [^{3}H]N-methylspiperone (D_2), [^{3}H]RO15,1788 (BZD).

The visionary effects reported for the β-carbolines by Naranjo (1967) would well fit an interaction with the 5-HT_{2A} receptor. It is interesting, however, that Naranjo found harmaline to be more potent than harmine in humans, but it is the latter that shows the highest affinity for the 5-HT_{2A} sites in receptor binding studies. Results from drug discrimination studies and phosphoinositide hydrolysis regarding the β-carbolines are also contradictory. While DOM stimulus has been found to generalize to harmaline, it does not appear to generalize to harmine (Glennon et al., 1983b), and results for harmaline are not very consistent either. Harmaline has recently been found not to substitute for LSD in the drug-discrimination paradigm (Helsley et al., 1998a) in line with results by Nielsen and coworkers (1982) who only found a maximum of 54% appropriate responding for harmaline. In addition to these inconsistent findings, none of the β-carbolines has been found to stimulate phosphoinositide hydrolisis (Glennon et al., 2000), in clear opposition with the phenylethylamines and indolalkylamines, which behave as partial agonists in this model (see section 4.1.). These results, considered together with the equal affinity shown by harmine to agonist- and antagonist-labeled 5-HT_{2A} receptors (Glennon et al., 2000; Grella et al., 1998), question the assumption that these compounds act as agonists at this receptor.

In summary, based on the limited data available today, the question whether the β-carbolines in general activate the 5-HT_{2A} receptor remains unanswered. In addition, given the

contradictory nature of the reports on the subjective effects of the β-carbolines, the pharmacology of these compounds in humans would need to be reexamined before any final conclusions can be drawn on their potential as psychedelics.

7.2. The β-carbolines as MAO inhibitors

The one pharmacological action some β-carbolines, especially harmine and harmaline, seem to display beyond doubt is their ability to inhibit the enzyme monoamine oxidase at concentrations in the micromolar and nanomolar range. As indicated in section 4.2., MAO is a mitochondrial enzyme involved in the inactivation of biogenic amines. The enzyme actually occurs as two isoforms, A and B, found throughout the body, which catalyze the oxidative deamination of different endogenous (neurotransmitters) and exogenous substrates. Both MAO isoforms are found in high levels in the human brain but also in peripheral organs such as the liver (Saura et al., 1996). The proportion of the different isoforms in a given organ shows marked interspecific variations. Thus, while human and rat liver show high MAO-A activity (in addition to MAO-B activity), non-human primate liver is devoid of MAO-A activity (Inoue et al., 1999). Studies reporting on the potency of MAO inhibitors in general and of the β-carbolines in particular may provide different IC_{50} values depending on the MAO source tissue (richer in the A or B form) and the substrate used (which can be unspecific or show selective affinity for one of the two isoforms). Two commonly used substrates, tyramine and tryptamine have been found to be substrates of both isoenzymes A and B, while β-phenylethylamine is predominantly a substrate of MAO-B. Regarding the oxidative deamination of psychedelic tryptamines and more specifically of DMT, the existing evidence indicates that the drug is a substrate of both isoforms at low concentrations, with probably a greater affinity for MAO-B at higher concentrations (Suzuki et al., 1981).

Initial studies found that certain tetrahydro-β-carbolines could inhibit the oxidative deamination of serotonin by MAO (Freter et al., 1958). Subsequently, Undenfriend and coworkers (1958) demonstrated that harmine, harmaline and THH behaved in vitro as MAO inhibitors. In addition, harmaline was also tested and shown to be active in vivo. Furthermore, these authors also demonstrated that harmaline inhibited MAO through a competitive and reversible mechanism. These findings were replicated in subsequent studies conducted by other researchers.

Yashuhara et al. (1972) found harmine exhibited different efficiency in MAO inhibition depending on the substrate used. Thus, harmine was more effective at inhibiting serotonin oxidation than tyramine oxidation. Furthermore, in a subsequent study this author found that harmine was much more effective at inhibiting either serotonin or tyramine oxidation by brain MAO than by beef liver MAO, pointing out that different isoenzymes predominate in each organ (Yashuhara, 1974). Similarly, Fuller et al. (1972) demonstrated harmaline was far more effective at inhibiting the MAO isoform capable of degrading serotonin than the isoform degrading phenylethylamine. Thus, *β*-carbolines appear to be more effective inhibitors of MAO-A, of which serotonin is a substrate, than MAO-B.

Using tyramine as substrate, McIsaac and Estevez (1966) studied structure-activity relationshipss in a series of *β*-carbolines and found the degree of saturation of the pyridine ring to influence the potency of these compounds as MAO inhibitors. Tetrahydro-derivatives were found to be the least active, dihydro-derivatives showed intermediate potency and the fully aromatic compounds were the most active. Thus, harmine was two orders of magnitude more potent than harmaline. Harmol and harmalol were equipotent with harmine. In this study THH was not tested.

Buckholtz and Boggan (1977a) conducted a study including harmine, harmaline, THH and their *O*-demethylated analogues harmol, harmalol, and tetrahydroharmol. Structure-activity relationships were studied using tryptamine as substrate and MAO obtained from mouse brain as the MAO source. Results replicated the findings by McIsaac and Estevez in liver (1966) indicating that the aromatic and dihydro-derivatives are more potent than the tetrahydro-derivatives. In this study the hydroxylated derivatives were several orders of magnitude weaker than their methoxylated counterparts. Another interesting finding was the higher potency showed by harmaline at inhibiting oxidation of serotonin than oxidation of *β*-phenylethylamine both in vitro and in vitro, again suggesting a greater selectivity for MAO-A than for MAO-B.

In a recent in vivo study, intraperitoneal administration of harmine to rats dose-dependently increased brain levels of dopamine and reduced the levels of the deaminated monoamine metabolites of dopamine 3,4-dihydroxyphenylacetic acid (DOPAC), norepinephrine homovanillic acid (HVA) and serotonin 5-hydroxyindoleacetic acid (5-HIAA) in a similar fashion to moclobemide, confirming the MAOI effects of systemically administered harmine

(Iurlo et al., 2001). Table 11 shows IC_{50} and K_i estimates for MAO inhibition by various β-carbolines.

Table 11: IC_{50} and K_i values for MAO inhibition by β-carbolines

Harmine	**Tissue**	**Substrate**	**IC_{50}**	**Study**
	Rat liver homogenate	Serotonin	$1.0x10^{-6}$	Undenfriend et al., 1958
	Calf liver mitochondrial fraction	Tyramine	$1.5x10^{-8}$	McIsaac & Estevez, 1966
	Beef brain mitochondrial fraction	Serotonin	$3.0x10^{-7}$	Yasuhara, 1974
	Beef brain mitochondrial fraction	Tyramine	$3.0x10^{-3}$	Yasuhara, 1974
	Mouse whole brain homogenate	Tryptamine	$8.0x10^{-8}$	Buckholtz & Boggan, 1977a
	Rat liver cytosol	Tryptamine	$1.3x10^{-8}$	McKenna et al., 1984
	Tissue	**Substrate**	**K_i**	**Study**
	Pure human liver MAO-A	Kynuramine	$5.0x10^{-9}$	Kim et al., 1997
Harmaline	**Tissue**	**Substrate**	**IC_{50}**	**Study**
	Rat liver homogenate	Serotonin	$1.0x10^{-6}$	Undenfriend et al., 1958
	Calf liver mitochondrial fraction	Tyramine	$1.0x10^{-6}$	McIsaac & Estevez, 1966
	Mouse whole brain homogenate	Tryptamine	$6.0x10^{-8}$	Buckholtz & Boggan, 1977a
	Rat liver cytosol	Tryptamine	$1.6x10^{-8}$	McKenna et al., 1984
	Tissue	**Substrate**	**K_i**	**Study**
	Pure human liver MAO-A	Kynuramine	$48.0x10^{-9}$	Kim et al., 1997
THH	**Tissue**	**Substrate**	**IC_{50}**	**Study**
	Rat liver homogenate	Serotonin	$1.0x10^{-5}$	Undenfriend et al., 1958
	Mouse whole brain homogenate	Tryptamine	$1.4x10^{-5}$	Buckholtz & Boggan, 1977a
	Rat liver cytosol	Tryptamine	$1.8x10^{-6}$	McKenna et al., 1984
Harmol	**Tissue**	**Substrate**	**IC_{50}**	**Study**
	Calf liver mitochondrial fraction	Tyramine	$2.8x10^{-8}$	McIsaac & Estevez, 1966
	Mouse whole brain homogenate	Tryptamine	$5.8x10^{-6}$	Buckholtz & Boggan, 1977a
	Rat liver cytosol	Tryptamine	$5.0x10^{-7}$	McKenna et al., 1984
Harmalol	**Tissue**	**Substrate**	**IC_{50}**	**Study**
	Calf liver mitochondrial fraction	Tyramine	$2.5x10^{-8}$	McIsaac & Estevez, 1966
	Mouse whole brain homogenate	Tryptamine	$1.0x10^{-5}$	Buckholtz & Boggan, 1977a
THH-OH	**Tissue**	**Substrate**	**IC_{50}**	**Study**
	Mouse whole brain homogenate	Tryptamine	$1.0x10^{-5}$	Buckholtz & Boggan, 1977a

7.3. The β-carbolines as monoamine reuptake inhibitors

Buckholtz and Boggan (1977b) studied the effects of β-carbolines on monoamine reuptake into mouse brain synaptosomes. These researchers found that these compounds inhibited the reuptake of serotonin at micromolar concentrations. Contrary to MAOI activity, tetrahydro-derivatives were more potent than their dihydro- or aromatic counterparts (see Table 12).

Table 12: IC_{50} for monoamine reuptake inhibition by β-carbolines.

	IC_{50} (μM)			
Compound	**^{3}H-Serotonin (3.0 nM)**	**^{3}H-Serotonin (0.1 μM)**	**^{3}H-NE (0.1 μM)**	**^{3}H-DA (0.1 μM)**
Harmine	11	41	22	16
Harmaline	15	-	-	-
THH	3.4	-	-	-
Harmol	17	-	-	-
Harmalol	13	-	-	-
THH-OH	21	-	-	-

Taken from Buckholtz and Boggan (1977b). (-) = not determined.

7.4. Other pharmacological effects

As shown above, the β-carbolines demonstrate a marked variation in their receptor interaction characteristics and pharmacological activity depending on their degree of saturation of the pyridine ring and on their substituents. Besides their MAO inhibitory activity, harmine and harmaline are notorious for their tremorigenic effects (Fuentes and Longo, 1971; Kawanishi et al., 1994). β-carboline-induced tremor has been related to drug interactions with serotonergic and noradrenergic neurotransmission but more prominently with inverse agonist activity at the benzodiazepine receptor. However, as discussed above, contrary to other β-carbolines with alkyloxycarbonil substituents, harmine and harmaline display little affinity for the benzodiazepine receptor and their behavioral and neurochemical effects in animals are not counteracted by flumazenil (Auta et al., 1997; Iurlo et al., 2001). In the specific case of harmaline, tremor has been related to activation of neurons in the inferior olivary nucleus. At high doses, this effect has been found to exert excitotoxicity on Purkinje cells in the cerebellum (O'Hearn and Molliver, 1993). Very recent research has demonstrated the

involvement of glutamate NMDA receptors in the tremor-inducing effects of this drug. The noncompetitive NMDA channel blocker dizocilpine was able to suppress the tremorigenic activity of harmaline (Du and Harvey, 1997). These authors postulated that harmaline may produce tremor by acting as an inverse agonist at the MK-801 binding site in the NMDA channel (Du et al., 1997).

8. The β-carboline-DMT interaction hypothesis in *ayahuasca*

Agurell and coworkers (1968) examined the alkaloid content of *Diplopterys cabrerana* (reported as *Banisteriopsis rusbyana*), a common plant admixture to *ayahuasca*, by means of gas chromatography coupled to mass spectrometry and confirmed previous findings by Poisson (1965) that the plant contained substantial amounts of DMT. At that time, it had already been reported that DMT was inactive orally (Szára, 1957; Turner and Merlis, 1959) and the authors postulated that "The combination in yajé of monoamine oxidase inhibiting harman alkaloids with N,N-dimethyltryptamine might result in specific pharmacological effects" (Agurell et al., 1968). In the same issue of the American Journal of Pharmacy, Der Marderosian and coworkers (1968) replicated the finding and noted that "both the harman alkaloids and DMT are monoamine oxidase inhibitors", and speculated that "perhaps the strong concentration of the active principles or the presence of other unidentified substances facilitate absorption". Since these early works, the interaction hypothesis involving MAO inhibition has been widely accepted and the MAO inhibitory effects of the β-carbolines, *ayahuasca* brews and of an *ayahuasca* analogue obtained by combining pure compounds have been demonstrated in vitro (McKenna et al., 1984). However, MAO inhibition after *ayahuasca* in vivo has not been evaluated to date in humans either by assessing monoamine metabolite levels in plasma or urine or by determining platelet MAO activity.

9. Pharmacology of *ayahuasca* in humans

In 1993, McKenna, Grob, Callaway and other researchers conducted the field phase of a study evaluating the effects of *ayahuasca* in humans who regularly ingest the tea (McKenna et al., 1998). To my knowledge, this is the first attempt to systematically characterize the pharmacology of *ayahuasca* in humans. Acute effects after drug administration were assessed and also several parameters (neurochemical and neuropsychological) were evaluated in long term ritual users. The acute-effect study generated data on the pharmacokinetics of *ayahuasca*

alkaloids and provided information on the subjective, cardiovascular and neuroendocrine effect of the tea following the administration of a single dose. This was an open-label, non-placebo-controlled study in which drug-induced pharmacodynamic changes were referred to baseline values. The *ayahuasca* dose administered by Callaway and coworkers (1999) contained the following average (range) amounts of alkaloids in mg: 35.5 (28.8-43.2) for DMT, 252.3 (204.0-306.0) for harmine, 29.7 (24.0-36.0) for harmaline, and 158.8 (128.4-196.6) for THH. The findings of this study are described in the following sections.

9.1. Subjective effects

In all 15 participants, the administered *ayahuasca* dose was described as eliciting an experience in which "peak plasma levels of DMT were associated with intricate and colored eyes-closed visual imagery, complex thought processes and a general state of heightened awareness". The authors also comment that "overall perceptual, cognitive and affective processes were significantly modified while maintaining the presence of a clear sensorium" (Callaway et al., 1999). A quantitative measure of the intensity of subjective effects was also obtained by means of the Hallucinogen Rating Scale. In general terms, scores were found to be low when compared with prior data obtained after i.v. DMT administration. Thus, the administered 0.48 mg DMT/kg dose yielded scores on the Intensity, Affect, Cognition and Volition comparable to those obtained after 0.1–0.2 mg i.v. DMT/kg. Scores on the Perception scale corresponded to an i.v. dose of 0.1 mg DMT/kg and scores on the Somaesthesia scale were even lower than those of an i.v. dose of 0.05 mg DMT/kg (Grob et al., 1996).

9.2. Pharmacokinetics

Following the oral administration of *ayahuasca*, DMT plasma levels were detected in all volunteers and were high enough to calculate pharmacokinetic parameters in 12. Harmaline levels were very low and allowed the calculation of parameters for only 5 volunteers. In the case of harmine and THH, these were calculated for 14 volunteers. The calculated C_{max}, t_{max}, elimination halflife ($t_{1/2}$), clearance and apparent volume of distribution are shown below.

Table 13: Pharmacokinetic parameters of *ayahuasca* alkaloids expressed as mean (SD)

Alkaloid	C_{max} (ng/ml)	T_{max} (min)	$t_{1/2}$ (min)	Cl/F (ml/min . kg)	V_{ss}/F (l/kg)
DMT	15.8 (4.4)	107.5 (32.5)	259.4 (207.4)	221.8 (129.9)	54.8 (14.8)
Harmine	114.8 (61.7)	102.0 (58.3)	115.6 (60.1)	271.7 (180.3)	49.6 (40.4)
Harmaline	6.3 (3.1)	145.0 (66.9)	-	-	-
THH	91.0 (22.0)	174.0 (39.6)	531.9 (290.8)	63.3 (21.9)	43.5 (8.0)

Taken from Callaway et al. (1999). (-) = not determined.

9.3. Cardiovascular effects

Ayahuasca was found to increase systolic blood pressure, diastolic blood pressure and heart rate over pre-drug baseline values. These pressor and chronotropic effects were not particularly intense, with mean increases of 11 mm Hg for systolic blood pressure, 9.3 for diastolic blood pressure and a maximum mean increase of 7.4 bpm in heart rate. Systolic and diastolic blood pressure increases peaked at 40 min postadministration and returned to baseline values at 3 h postadministration. The peak increase in heart rate was observed at 20 min. Return to baseline values was observed at 4 h after dosing.

9.4. Autonomic effects

Ayahuasca administration had mydriatic effects, increasing pupil diameter 1 mm in relation to preadministration values. This increase was maximum at 3 h postadministration and returned to baseline values at 6 h. Respiratory frequency was also found to increase with *ayahuasca*. This variable fluctuated showing increases and decreases, with a maximum mean increase of 3.1 breaths per min at 90 min postadministration. Body temperature also increased reaching a maximum mean increase of 0.3 ºC at 4 h after dosing.

9.5. Neuroendocrine effects

Following *ayahuasca* administration, plasma levels of cortisol, prolactin and growth hormone increased from baseline values. Peak increases were found at 60 min for cortisol, at 120 min for prolactin and at 90 min for growth hormone. Levels returned to baseline or below baseline (cortisol) at 6 h postadministration.

9.6. Adverse effects

Callaway and coworkers (1999) comment that nausea, vomiting and diarrhea are not uncommon following *ayahuasca* consumption. In fact one of the volunteers enrolled in this study vomited 45 min after receiving an oral dose of the tea. In the course of the clinical trial, nystagmus and tremor were also observed. Other adverse events associated with acute *ayahuasca* intake reported in the scientific literature include intense fear in the course of a self-experience with the tea described by one researcher (Rivier and Lindgren, 1972). Finally Callaway and Grob (1998) have warned against possible interactions between *ayahuasca* alkaloids and serotonin reuptake inhibitors. They report a case study in which the concomitant use of both drugs led to a syndrome characterized by loss of consciousness, motor tremors and severe nausea and vomiting.

9.7. Long term effects

Callaway and coworkers (1994) examined the effects of long-term ingestion of *ayahuasca* on serotonergic neurotransmission by means of a peripheral marker. They studied the binding of [^{3}H]citalopram to the platelet serotonin transporter. The authors found an increase in the number of platelet binding sites relative to *ayahuasca*-naive individuals, and no change in the dissociation constant. No definite explanation was given for this finding, but in a later study utilizing Single Photon Emission Computerized Tomography (SPECT), one of the researchers of the study found his own levels of cerebral serotonin transporter to be increased in the prefrontal cortex after six weeks of daily dosing with THH. These findings suggest that serotonergic neurotransmission undergoes a modulation which lasts beyond the acute effects of *ayahuasca*. Furthermore, these changes appear to be related with THH and seem to be reversible, since the cerebral levels of the serotonin transporter returned to baseline levels after THH was discontinued (McKenna et al., 1998). Whether this modulation also takes place in the brains of regular *ayahuasca* users, and the therapeutic or pathological implications of these changes remain unknown.

HYPOTHESES

At the time when the present investigation was designed (end of 1996), virtually all data available on *ayahuasca* dealt only with its botany and chemistry. The only information on its effects in humans came from reports involving its self-administration by single subjects and no data were available regarding its acute administration to healthy humans in controlled studies. The following hypotheses were postulated:

1. The administration of *ayahuasca* to healthy humans would elicit dose-dependent subjective effects characteristic of psychedelics.

2. The access of *ayahuasca* alkaloids to the CNS would be measurable by means of quantitative pharmaco-electroencephalography (q-EEG).

3. Discrete brain regions would be identified by means of Low Resolution Electromagnetic Tomography (LORETA) as underlying the acute changes observed in the EEG.

4. The acute administration of *ayahuasca* would disrupt sensory and sensorimotor gating.

5. Oral administration of *ayahuasca* would lead to measurable plasma levels of the major alkaloids present in the tea, i.e., DMT, harmine, harmaline and *d*-tetrahydroharmine.

6. The in vivo inhibition of MAO elicited by the β-carbolines present in *ayahuasca* could be measured by determining the levels of neurotransmitter metabolites in urine. *Ayahuasca* administration should lead to decreases in the excretion of MAO-dependent metabolites and to increases in COMT-dependent metabolites.

7. The acute administration of *ayahuasca* would induce elevations in blood pressure and heart rate. No hypotheses were postulated regarding other somatic-dysphoric or adverse events associated to *ayahuasca* administration.

AIMS OF THE STUDY

The present investigation aimed to obtain information on the human pharmacology of *ayahuasca*. The study focussed on the effects of *ayahuasca* following its acute administration in a controlled clinical trial, i.e., incorporating a placebo and a double-blind randomized design, without addressing at this stage any possible adverse psychopathological effects derived from repeated consumption, or studying any hypothetical therapeutic properties of the tea.

Specific main objectives:

1. Measure the nature and time course of subjective effects elicited by acute *ayahuasca* administration.

2. Assess the CNS effects of *ayahuasca* by means of quantitative pharmaco-electroencephalography (q-EEG).

3. Conduct an exploratory analysis of the brain cortical regions responsible for the observed q-EEG effects by means of Low Resolution Electromagnetic Tomography (LORETA).

4. Measure the effects of *ayahuasca* on sensory and sensorimotor gating.

5. Describe the pharmacokinetics of DMT and the β-carbolines present in *ayahuasca*, after the oral administration of various doses of the preparation.

6. Measure *in vivo* the inhibition of MAO provoked by acute *ayahuasca* administration.

7. Assess the general tolerability of *ayahuasca*, i.e., the subject's vital signs after the drug's administration, and any event, either physical or psychological, regarded as unpleasant by the subject. Additionally, evaluate hematological and biochemical parameters.

Specific secondary objectives:

1. Translate into Spanish and explore the sensitivity and psychometric properties, i.e., reliability and construct validity of a recently developed self-report instrument designed to evaluate the subjective effects elicited by psychedelics: the Hallucinogen Rating Scale (HRS).

2. Develop and validate an analytical methodology to adequately quantify *ayahuasca* alkaloids in plasma in order to characterize their pharmacokinetics in humans following oral administration of the tea.

SUMMARY OF THE EXPERIMENTAL DESIGN

The steps taken in the course of the present project are described below in chronological order. First, the Spanish version of the HRS questionnaire was administered both to *ayahuasca* users and to users of psychedelics in order to explore the psychometric properties of this instrument. The next stage was the initiation of the clinical studies with a pilot study conducted to explore the general tolerability, i.e., safety of the lyophilizate, and its capacity to elicit psychotropic effects. The information obtained was used to establish the doses to be administered in the larger final study. This involved a greater number of volunteers and study variables, and the implementing of an optimal design.

Questionnaire assessment studies

Study 1

Participants: 75 users of *ayahuasca*.

Design: Volunteers answered the HRS approximately 4 hours after intake (immediate retrospective assessment).

Variables: Cronbach's alpha.

Study 2

Participants: 56 polydrug users.

Design: Volunteers answered the HRS and the ARCI recalling effects of their last psychedelic drug intake (delayed retrospective assessment).

Variables: Cronbach's alpha and correlations between HRS and ARCI scales.

Results in: *Psychometric assessment of the Hallucinogen Rating Scale*.

Pilot Clinical Study

Participants: 6 male volunteers with prior experience with *ayahuasca*.

Design: Single-blind, placebo-controlled, crossover, increasing doses: 0.5, 0.75 and 1.0 mg DMT/kg body weight.

Variables: HRS, ARCI, verbal reports.

SBP, DBP, HR, hematological and biochemical parameters.

Results in: *Subjective effects and tolerability of the South American psychoactive beverage ayahuasca in healthy volunteers.*

Final Clinical Study

Participants: 18 volunteers (15 male, 3 female) with prior experience with psychedelic drug use.

Design: Double-blind, placebo-controlled, randomized, crossover: 0.6 and 0.85 mg DMT/kg body weight.

Variables: HRS, ARCI, APZ, verbal reports.

SBP, DBP, HR, hematological and biochemical parameters.

EEG (Topography and LORETA).

PPI, P50.

Pharmacokinetics.

Urine monoamine metabolite excretion.

Results in: *Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion and pharmacokinetics.*

Topographic pharmaco-EEG mapping of the effects of the South American psychoactive beverage ayahuasca in healthy volunteers.

Effects of ayahuasca on sensory and sensorimotor gating in humans as measured by P50 suppression and prepulse inhibition of the startle reflex, respectively.

Determination of N,N-dimethyltryptamine and β-carboline alkaloids in human plasma following oral administration of Ayahuasca.

Effects of the South American psychoactive beverage Ayahuasca on regional brain electrical activity in humans: a functional neuroimaging study using low resolution electromagnetic tomography (LORETA).

Estimation of the bioavailability of DMT in ayahuasca.

Abbreviations

SBP:	Systolic blood pressure
DBP:	Diastolic blood pressure
HR:	Heart rate
LORETA:	Low Resolution Electromagnetic Tomography
HRS:	Hallucinogen Rating Scale
ARCI:	Addiction Research Center Inventory
APZ:	Altered States of Consciousness Questionnaire
EEG:	Electroencephalography
PPI:	Prepulse inhibition of the startle reflex
P50:	Suppression of the P50 auditory evoked potential in a paired-stimuli paradigm

RESULTS

Original publications

Subjective effects and tolerability of the South American psychoactive beverage Ayahuasca in healthy volunteers.

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ORIGINAL INVESTIGATION

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Subjective effects and tolerability of the South American psychoactive beverage *Ayahuasca* in healthy volunteers

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Abstract *Rationale: Ayahuasca* is a South American psychoactive beverage that contains the naturally occurring psychedelic agent *N*,*N*-dimethyltryptamine (DMT). This "tea" has been used for centuries in religious and medicinal contexts in the rain forest areas of South America and is presently gaining the attention of psychedelic users in North America and Europe. *Objectives:* In the present study, the psychological effects and tolerability of *ayahuasca* were assessed. *Methods:* Three increasing doses of encapsulated freeze-dried *ayahuasca* (0.5, 0.75, and 1.0 mg DMT/kg body weight) were administered to six healthy male volunteers with prior experience in the use of this tea, in a single-blind crossover placebo-controlled clinical trial. *Results: Ayahuasca* produced significant dose-dependent increases in five of the six subscales of the Hallucinogen Rating Scale, in the LSD, MBG, and A scales of the Addiction Research Center Inventory, and in the "liking", "good effects" and "high" visual analogue scales. Psychological effects were first noted after 30–60 min, peaked between 60–120 min, and were resolved by 240 min. The tea was well tolerated from a cardiovascular point of view, with a trend toward increase for systolic blood pressure. Modified physical sensations and nausea were the most frequently reported somatic-dysphoric effects. The overall experience was regarded as pleasant and satisfactory by five of the six volunteers, while one volunteer experienced an intensely dysphoric reaction with transient disorientation and anxiety at the medium dose and voluntarily withdrew from the study. *Conclusions: Ayahuasca* can be described as inducing changes in the perceptual, affective, cognitive, and somatic spheres, with a combination of stimulatory and visual psychoactive effects of longer duration and milder intensity than those previously reported for intravenously administered DMT.

Keywords *Ayahuasca* · DMT · Subjective effect · Tolerability · Human

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Introduction

Ayahuasca, a potent psychotropic drink that has been used for centuries for magico-religious purposes and folk medicine in the Amazon and Orinoco river basins (Dobkin de Ríos 1972; Schultes and Hofmann 1982), is becoming increasingly popular in Europe and North America as a sacramental drug (Metzner 1999). In recent years, the use of *ayahuasca* has spread outside South America, and several groups using this tea have become established in Spain and other European countries (Marshall 1997; López 1999), where the tea is reportedly used to facilitate self-knowledge and introspection. A relevant facet in expanding *ayahuasca* use can be attributed to the growing interest of the many individuals who are interested in shamanic practices, in addition to the activities of a number of Brazilian syncretic religions, particularly the *Santo Daime* and the *União do Vegetal*, that have combined Old World religious beliefs with the indigenous use of *ayahuasca*. Because this tea contains measurable amounts of *N*,*N*-dimethyltryptamine (DMT), the *ayahuasca* churches are actively working to obtain legal exemption for *ayahuasca* use within a religious context outside Brazil, the only country where it currently enjoys legal protection, analogous to the status held

by the Native American Church for the use of *peyote* (*Lophophora williamsii*, a mescaline-containing cactus) in the United States. Even though the number of users is still relatively small outside of Brazil, *ayahuasca* use has raised concerns for public health (Callaway and Grob 1998), and extensive clinical data on its somatic, psychological, and neurophysiological effects are indicated.

Ayahuasca, also known as *Daime* or *Hoasca* in Brazil, *Yajé* in Colombia, or *Natem* in Ecuador, is generally obtained by infusing the shredded stalk of the malpighiaceous vine *Banisteriopsis caapi* with the leaves of *Psychotria viridis* (Rubiaceae) or *Diplopterys cabrerana* (Malpighiaceae). *B. caapi* contributes a mixture of β-carboline alkaloids to the tea, particularly harmine, tetrahydroharmine (THH), and trace amounts of harmaline (Rivier and Lindgren 1972). *P. viridis* and *D. cabrerana* are rich in the psychedelic indole DMT (River and Lindgren 1972; Schultes and Hofmann 1980; Callaway et al. 1996).

DMT, the main psychotropic agent of *ayahuasca*, is capable of eliciting an intensely emotional dream-like experience characterized by vivid visual imagery, perceptual and cognitive changes, and profound modifications in the sense of self and reality, when administered parenterally (Strassman et al. 1994). On the molecular level, DMT has affinity for 5-HT_2 and 5-HT_{1A} binding sites, similarly to LSD (Pierce and Peroutka 1989; Deliganis et al. 1991), and is structurally similar to serotonin. Interestingly, DMT is known for its lack of psychoactivity when orally ingested, even in quantities in the order of grams (Ott 1999), due to metabolism by monoamine oxidase (MAO; Suzuki et al. 1981). The β-carbolines present in *ayahuasca*, particularly harmine and harmaline, have been found to inhibit MAO (McKenna et al. 1984), an effect that apparently allows the viable access of DMT to the systemic circulation and the central nervous system. In addition to the action of DMT on serotonin receptors, it has also been suggested that *ayahuasca*'s psychoactive effects may also be partly due to a general increase of catecholamines and serotonin (Callaway et al. 1999). This increase would be due to both the inhibited metabolic breakdown of serotonin in addition to its uptake inhibition by THH and also competition with DMT for receptor sites (Callaway et al. 1999). Thus, *ayahuasca* constitutes a very complex psychoactive preparation, acting at least through three different pharmacologic mechanisms.

In the present paper we report a single-blind placebo-controlled clinical trial conducted with *ayahuasca*, in which the subjective effects and tolerability of three different doses of *ayahuasca* were evaluated in healthy volunteers. This study is part of a wider research project designed to further characterize the pharmacologic effects of this tea.

Materials and methods

Volunteers

For ethical reasons, participation in this initial study was limited to six healthy male volunteers having previous experience with *ayahuasca*. Volunteers were contacted by word of mouth in the Barcelona area of Spain, and all had previous exposure to the "tea", but had no formal connections to any *ayahuasca* church. The volunteers were given a structured psychiatric interview (DSM-III-R) and completed the trait-anxiety scale from the state-trait anxiety inventory (Spielberger et al. 1970). Exclusion criteria included a present or past history of axis-I disorders and alcohol or other substance dependence, and high scores on trait anxiety. Following the psychiatric interview, participants underwent a complete physical examination that included a medical history, laboratory tests, ECG, and urinalysis. Mean age was 32.2 years (range: 26–44), mean weight 71.5 kg (range: 66–85), and mean height 174.3 cm (range 167–186). All volunteers had previous experience with cannabis, cocaine, psychedelics, and other illicit substances. Regarding their prior experience specifically with *ayahuasca*, volunteers 1 and 2 had previously consumed it on 10 occasions, volunteer 3 on about 60 occasions, volunteer 4 on 2 occasions, volunteer 5 on 6 occasions, and volunteer 6 on 30 occasions. 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.

Drug

A 9.6 litre batch of *ayahuasca* (*Daime*) was obtained from CEFLURIS, a Brazilian-based religious organization related to the *Santo Daime* church. The tea had the appearance of a brown-reddish suspension with a characteristic bitter-sour taste and smell, and a markedly acidic pH (3.63). In order to mask the drug in the single-blind design and establish accurate dosings, the tea underwent a freeze-drying process that yielded 611 g of a yellowish powder, which was subsequently homogenized and analyzed for alkaloid contents by an HPLC method previously described in the literature (Callaway et al. 1996). One gram of freeze-dried material contained 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline, and 11.36 mg THH. Thus, the alkaloid concentrations in the original tea were as follows: DMT 0.53 mg/ml, harmine 0.90 mg/ml, harmaline 0.06 mg/ml, and THH 0.72 mg/ml. The DMT concentration found in the tea was similar to that reported previously for a sample of *Daime* (Liwszyc et al. 1992) and several Peruvian *ayahuasca* samples (McKenna et al. 1984), and twice as great as the amount reported for a sample of *Hoasca* from the Brazilian church *União do Vegetal* (Callaway et al. 1996). Similarly, the β-carboline concentrations found in the *ayahuasca* used in the present study were also higher than those reported in the previously mentioned samples. In view of the mild psychological effects reported from the 0.48 mg DMT/kg body weight dosage (Grob et al. 1996), and considering the total amounts of DMT consumed in what have been reported as typical doses (McKenna et al. 1984; Liwszyc et al. 1992), the following experimental doses were chosen for the present study: 0.5 mg DMT/kg body weight as the low dose and 0.75 and 1.0 mg DMT/kg body weight as the medium and high dose, respectively. The freeze-dried material was encapsulated in 00 gelatin capsules containing 0.5, 0.25, or 0.125 g, and stored at –20°C under nitrogen atmosphere and protected from light until administered to the volunteers. Placebo capsules consisted of 00 gelatin capsules with 0.75 g lactose. Each volunteer received his calculated individual dose by combination of these capsules. Placebo capsules were added when necessary, so that all volunteers received 20 capsules on each experimental day.

Study design and experimental procedure

The study was carried out in a single-blind fashion. Volunteers were informed that they would receive a single oral dose of encapsulated freeze-dried *ayahuasca* (one low, one medium, and one high dose) or placebo on each of 4 experimental days. In order to avoid subjective effects related to expectancy, the volunteers were also informed that administrations would be made in a double-blind balanced fashion. For security reasons, they were actually administered in increasing doses, i.e., placebo for the first session, the low dose containing 0.5 mg DMT/kg for the second session, the medium dose containing 0.75 mg DMT/kg for the third session, and the high dose containing 1.0 mg DMT/kg for the fourth and final session, in order to control for tolerability and the possible risk in elevations of cardiovascular parameters. Two weeks prior to the beginning of the experimental sessions, volunteers abstained from any medication or illicit drug and remained drug-free throughout the 4 study weeks. Urinalysis for illicit drug use was carried out for each experimental session. Additionally, volunteers abstained from alcohol, tobacco, and caffeinated drinks 24 h prior to each experimental day. Experimental days were a week apart.

The volunteers were admitted to the research unit on 4 separate experimental days. Upon arrival at 8:00 a.m. under fasting conditions, a urine sample was collected, a cannula was inserted in the cubital vein of their right arm for drawing blood samples, and capsules were administered by approximately 9:00 a.m. with 250 ml tap water. Throughout the experimental session the volunteers remained seated in a comfortable reclining chair in a quiet and dimly lit room. The experimenter remained beside the volunteer for most of the time, and no music was used during the sessions. Four hours after administration of the capsules, the volunteers left the room, answered subjective effect questionnaires, were able to have a light meal if they wished to, and were discharged after 5 h from the administration.

Measurements

Besides the measures described below, spontaneous verbally reported effects were also recorded. Additionally, blood samples were drawn at set time points in order to establish the alkaloids' pharmacokinetic profiles (not reported here). The time points selected for the measurements described below were based on field observations of duration of *ayahuasca* effects, and on the published pharmacokinetic and pharmacodynamic data by Callaway et al. (1999).

Psychological measures

The psychological effects elicited by *ayahuasca* were measured by means of visual analogue scales (VAS) and self-report questionnaires. VAS were 100-mm horizontal lines with the following labels: "any effect" indicated any effect, either physical or psychological, that the volunteer attributed to the administered dosage; "good effects" indicated any effect the volunteer valued as good; "liking" reflecting that the volunteer liked the effects of the administered substance; "drunken" indicating any dizziness or lightheadedness; "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 treatment. The volunteers were requested to answer the VAS immediately before administration and at 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after administration.

Self-report questionnaires included Spanish adaptations of the Hallucinogen Rating Scale (HRS) and the Addiction Research Center Inventory (ARCI). The HRS version, which had been previously translated from English and validated in Spanish (Riba et al. 2000), includes six subscales: "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 (Strassman et al. 1994). The ARCI (Lamas et al. 1994) consists of five scales or groups: morphine-benzedrine group (MBG), measuring euphoria; pentobarbital-chlorpromazine-alcohol group (PCAG), measuring sedation; lysergic acid diethylamide scale (LSD), measuring somatic-dysphoric effects; and the benzedrine group (BG) and the A scale, for amphetamine, both sensitive to stimulants. The volunteers answered the ARCI immediately before drug administration and, 4 h after drug intake, they again answered the ARCI and the HRS.

Tolerability measures

Cardiovascular variables were recorded by means of a sphygmomanometer cuff (Dinamap Critikon, Tampa, Fla., USA) which was placed around the volunteer's left arm. Blood pressure [systolic (SBP) and diastolic (DBP)] and heart rate were measured immediately before administration (baseline) and at 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after intake. Somatic-dysphoric effects were recorded by means of the questionnaires previously mentioned, and as spontaneous verbal reports. Finally, after each experimental session, a blood sample was taken for laboratory testing, which included blood cell counts, plasma bilirubin, creatinine, and liver enzymes.

Statistical analysis

Values from cardiovascular measures and ARCI scores were transformed to differences from baseline and differences from preadministration scores, respectively. Transformed values, HRS scores, and mean values obtained across time points for a given treatment (i.e., cardiovascular and VAS data) were analyzed by means of a non-parametric Friedman test. When a significant effect was observed, *post hoc* comparisons were performed using the Wilcoxon test. In all tests performed, differences were considered statistically significant for P values lower than 0.05.

Results

Psychological effects

Results for the statistical analyses performed on all subjective effect variables are presented in Table 1. A significant effect of treatment was observed for all seven VAS items, all HRS subscales except "volition", and the A, MBG, and LSD scales of the ARCI. The 0.5 mg DMT/kg body weight dosage chosen in the present study as the lower dose proved to be psychoactive in five of the six volunteers and subthreshold for the sixth volunteer, who mistook it for the placebo. At this dose, the Wilcoxon test showed significant effects for all VAS items except for "high". A significant effect was also found for the HRS "somaesthesia" subscale. Finally, the ARCI questionnaire showed a significant increase in the MBG scale.

When administered at 0.75 and 1.0 mg DMT/kg body weight, *ayahuasca* was correctly identified as an active substance by all participants. All VAS items and all HRS subscales, except for "volition", discriminated between each of these two doses and the placebo. At the medium

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Table 1 Statistical analyses performed on the visual analogue scale (VAS) scores (mean values across ten time points) and on scores obtained for the Hallucinogen Rating Scale (HRS) subscales and Addiction Research Center Inventory (ARCI) (differences from pre-drug values) (n=5). *NS* Not significant, *A* amphetamine scale, *LSD* lysergic acid diethylamide scale, *BG* benzedrine group, *MBG* morphine-benzedrine group, *PCAG* pentobarbital-chlorpromazine-alcohol group

Variable	Friedman test	Wilcoxon test					
		Placebo			0.5 mg/kg		0.75 mg/kg
	P value	0.5	0.75	1.0	0.75	1.0	1.0
VAS							
Any effect	**	*	*	*	*	*	NS
Good effects	**	*	*	*	(*)	*	NS
Visions	*	*	*	*	NS	(*)	NS
Liking	**	*	*	*	(*)	*	NS
Drunken	**	*	*	*	*	*	NS
Stimulated	**	*	*	*	(*)	NS	NS
High	**	(*)	*	*	*	*	NS
HRS							
Somaesthesia	**	*	*	*	*	NS	NS
Perception	*	NS	*	*	NS	(*)	NS
Cognition	*	NS	*	*	(*)	*	NS
Volition	NS	–	–	–	–	–	–
Affect	**	(*)	*	*	*	(*)	NS
Intensity	**	(*)	*	*	*	NS	NS
ARCI							
MBG	**	*	*	*	(*)	NS	NS
BG	NS	–	–	–	–	–	–
A	**	(*)	*	*	NS	(*)	NS
LSD	*	(*)	(*)	*	NS	NS	NS
PCAG	NS	–	–	–	–	–	–

*P<0.05, **P<0.01, (*)P<0.1

dose (i.e., 0.75 mg DMT/kg) the ARCI MBG and A scales showed statistically significant differences from the placebo. At the high dose, the LSD, MGB, and A scales showed significant differences from the placebo. Regarding discrimination between the doses, five of the seven VAS items and the HRS "cognition" subscale were able to discriminate between the low and the high doses. None of the variables were able to discriminate between the medium and the high doses. Three VAS items, "any effects", "drunken", and "high", were discriminative between the low and medium doses. Discrimination between these two doses was also achieved by the HRS "somaesthesia", "affect", and "intensity" subscales.

Scores on the HRS subscales for the four experimental conditions are shown in Fig. 1. Pre- and post-treatment scores on the ARCI scales for the four experimental conditions are shown in Fig. 2. The time course of effects, as reflected by the seven VAS items, is presented in Fig. 3. The initial somatic effects of *ayahuasca* appeared between 15–30 min, which translated as increases in the "any effect" VAS. This was followed by an onset of psychological effects at around 30–60 min, which was reflected by the increases in the other six VAS items. Both somatic and psychic effects peaked between 60 and 120 min after drug intake and gradually decreased to baseline levels at approximately 240 min. It is worth noting that the "good effects" and "liking" items of the VAS remained elevated at 240 min after drug administration, when most of the perceptual, cognitive, and affective effects had disappeared. The volunteers verbally described this state as a lingering sensation of well-being after the resolution of the more intense psychotropic effects.

Tolerability

Cardiovascular effects

Mean values for SBP, DBP, and heart rate over time are presented in Fig. 4 as differences from their baseline values. All three *ayahuasca* doses produced increases in SBP and DBP when compared with placebo. Changes were not statistically significant, although a robust trend toward significance was observed for SBP (P=0.0503) at the high dose. The peak differences in SBP were 13.8 mm Hg between the high dose and placebo, 13.4 mm Hg between the medium dose and placebo, and 8.8 mm Hg between the low dose and placebo. The maximal increases in SBP were observed at 90 min after administration of all three *ayahuasca* doses. The peak differences in DBP were 10.4 mm Hg between the high dose and placebo, 9.8 mm Hg between the medium dose and placebo, and 8.6 mm Hg between the low dose and placebo. The maximal increases in DBP were observed at 60 min after administration of all three *ayahuasca* doses. Mean arterial pressures showed a 10.6 mm Hg maximum difference from placebo at 60 min. Heart rate was affected very little by *ayahuasca*. Increases above baseline values were only seen for the medium and high doses, with peak differences of 9.2 beats/min between the high dose and placebo, 8 beats/min between the medium dose and placebo, and 6.4 beats/min between the low dose and placebo at 45 min after drug administration. At no point did SBP reach 140 mm Hg, nor did heart rate reach 100 beats/min for any individual volunteer. On the other hand, two volunteers showed sporadic

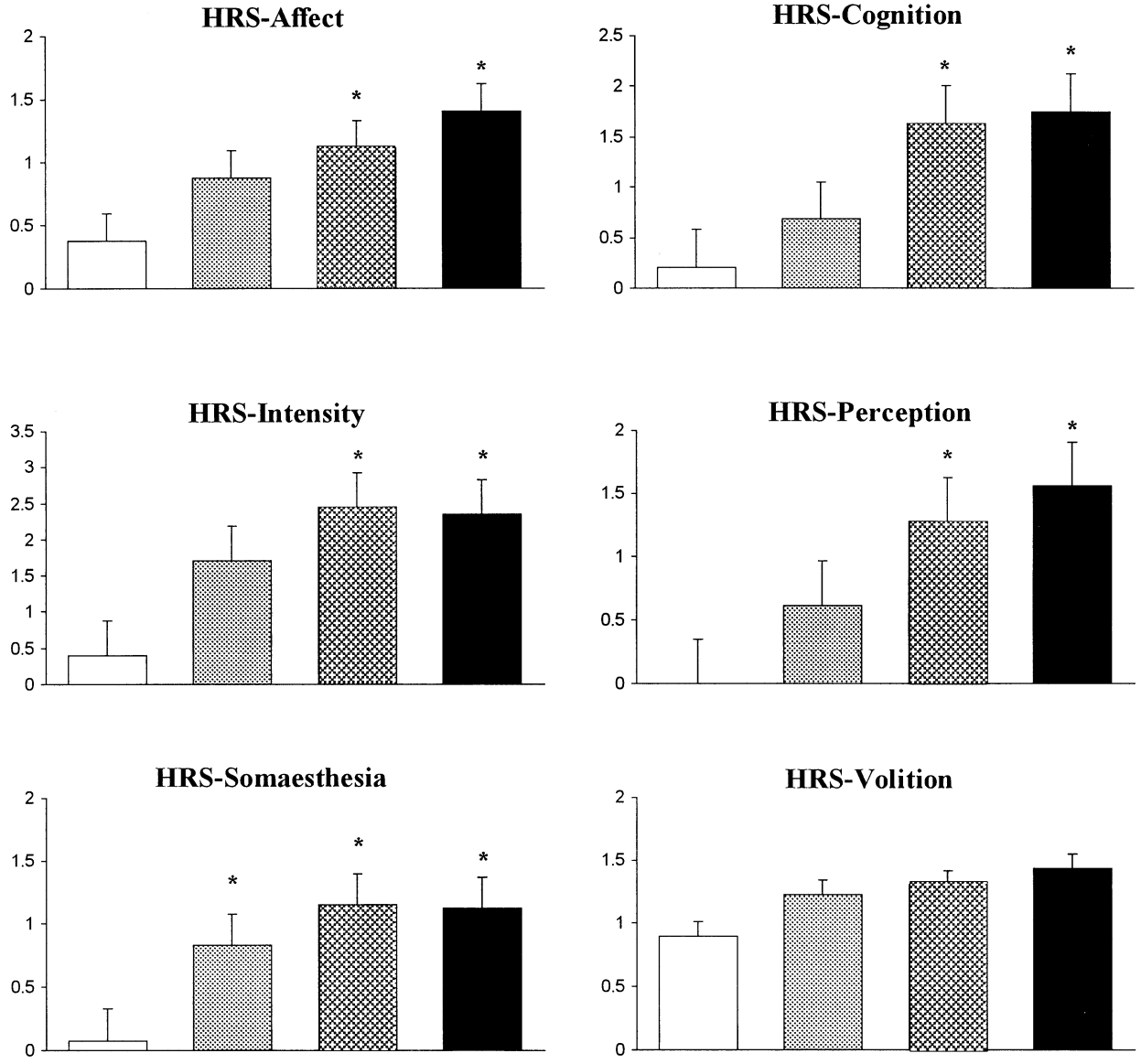

Fig. 1 Mean scores on the six Hallucinogen Rating Scale (HRS) subscales after administration of placebo (□), 0.5 mg *N*,*N*-dimethyltryptamine (DMT)/kg body weight *ayahuasca* (*lightly shaded*), 0.75 mg/kg (*shaded*), and 1.0 mg/kg (■). *Error bars* denote 1 standard error of mean (n=5). Significant differences from placebo (Wilcoxon test, P<0.05) are indicated by an *asterisk*

DBP values between 91–93 mm Hg after the medium and high doses, which lasted between 15 and 30 min.

Somatic-dysphoric effects

Table 2 lists the main somatic-dysphoric effects reported by the volunteers either spontaneously, or as positive responses to particular items in the HRS and ARCI questionnaires.

Blood analysis

No clinically relevant alterations were observed in the hematological or biochemical parameters tested after completion of each experimental session.

Verbal reports

The first effects noted by the volunteers were somatic modifications which included burning sensations in the stomach, tingling sensations, changes in perception of body temperature and skin sensitivity, and mild nausea. The onset of psychic effects was generally sudden and intense. Volunteers reported a certain degree of anxiety or fear at this initial stage, tending to decrease thereafter. Visual imagery was characteristic and dose-dependent. The images and visual modifications did not persist throughout the entire experience, but usually came and went in waves. These effects ranged from increases

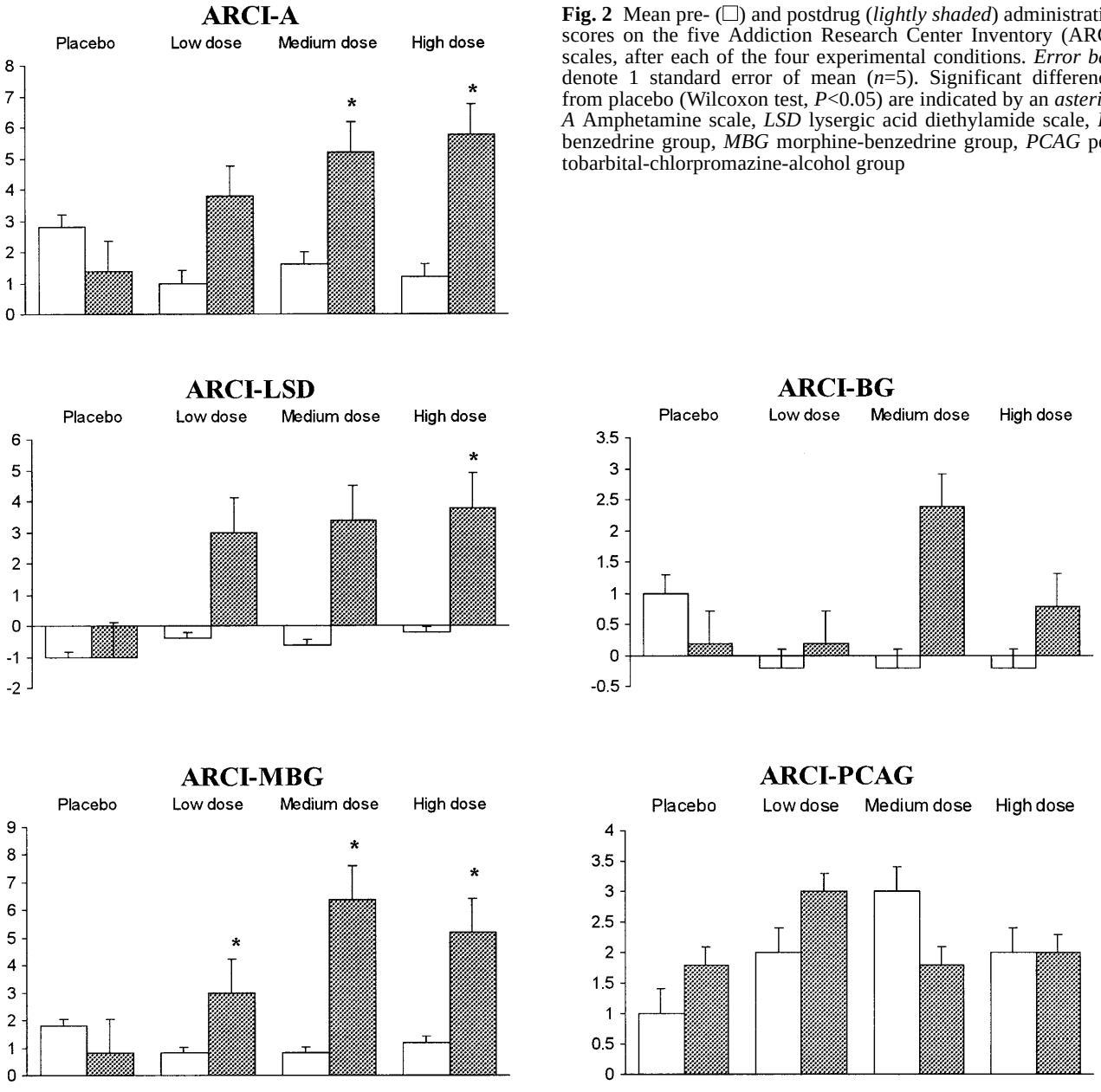

Fig. 2 Mean pre- (□) and postdrug (*lightly shaded*) administration scores on the five Addiction Research Center Inventory (ARCI) scales, after each of the four experimental conditions. *Error bars* denote 1 standard error of mean (n=5). Significant differences from placebo (Wilcoxon test, P<0.05) are indicated by an *asterisk*. *A* Amphetamine scale, *LSD* lysergic acid diethylamide scale, *BG* benzedrine group, *MBG* morphine-benzedrine group, *PCAG* pentobarbital-chlorpromazine-alcohol group

in an object's brightness and sharpness, or as vibrations in the visual field, to rapidly moving patterns, and scenes that were visible with eyes either closed or open at the medium and high doses. Changes in auditory perception were also reported and showed a dose-dependent effect. Hearing was perceived to be enhanced, with sounds becoming more clear and distinct. Although infrequent, transient auditory phenomena were reported in some subjects at the three doses. Thought processes were also modified, with the volunteers reporting an enhanced rate of thinking which generally centered on personal psychologic content. These thoughts were experienced as providing new insight into personal concerns. As the doses increased, emotional reactions were intensified, with the volunteers experiencing happiness, sadness, awe, amazement, and at times simultaneously contradictory feelings. At the medium and high doses, volunteers agreed on the similarity of the experience to dreaming. Memories were present, mostly related to recent personal matters. The sense of self and the passing of time were deeply affected at the medium and high doses. While sensations of closeness to others, happiness, and euphoria were similar at the medium and high doses, sensations of detachment from the body, oneness with the universe, and chaos, were more frequently reported with the latter. Five of the six volunteers were able to interact with the experimenter and the environment without major problems at all three doses. The sixth volunteer experienced a brief but intense disorientation state at the medium dosage. It is noteworthy that this volunteer had the least amount of experience with *ayahuasca*, having consumed it prior to the study on on-

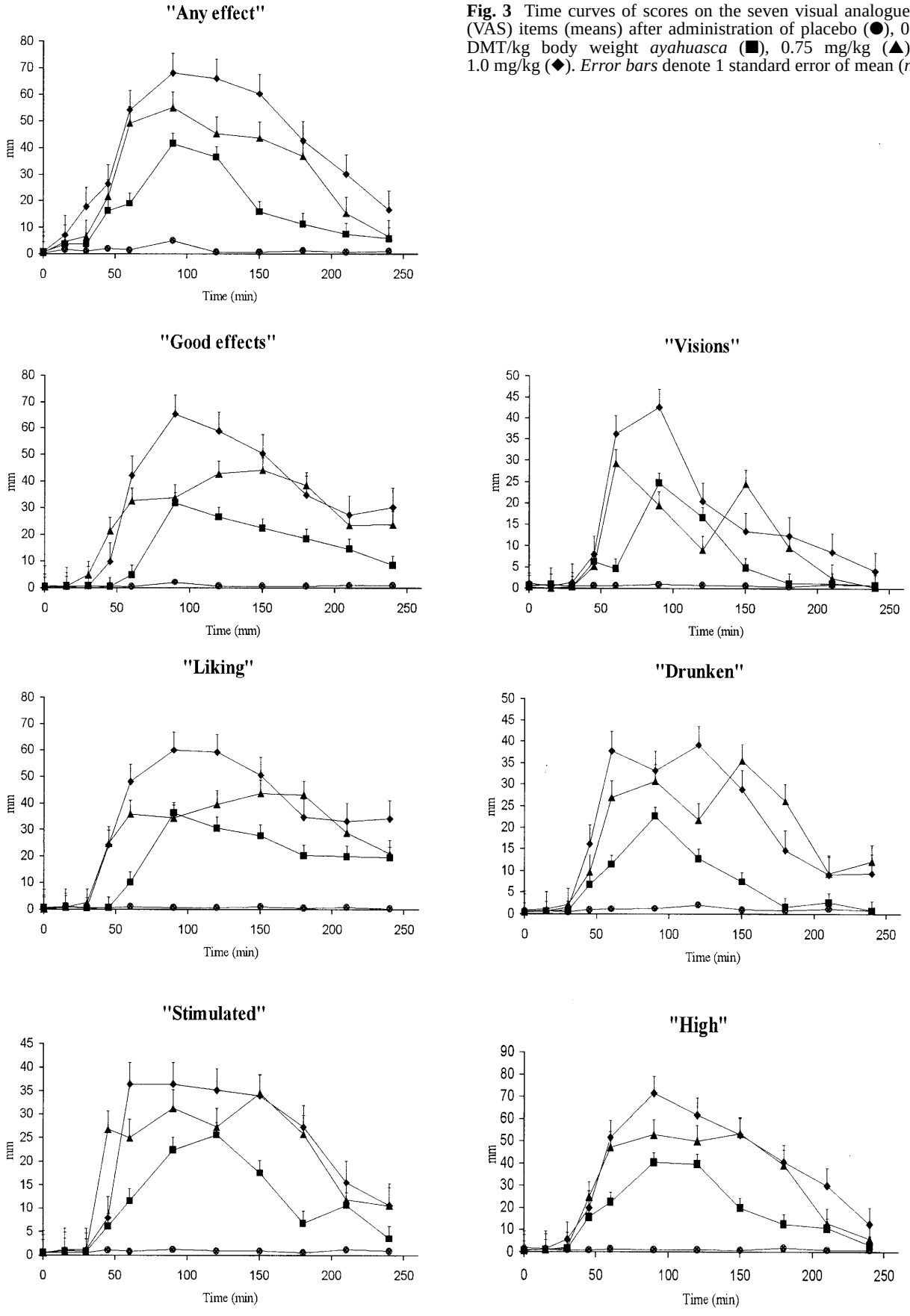

Fig. 3 Time curves of scores on the seven visual analogue scale (VAS) items (means) after administration of placebo (●), 0.5 mg DMT/kg body weight *ayahuasca* (■), 0.75 mg/kg (▲), and 1.0 mg/kg (◆). *Error bars* denote 1 standard error of mean (*n*=5)

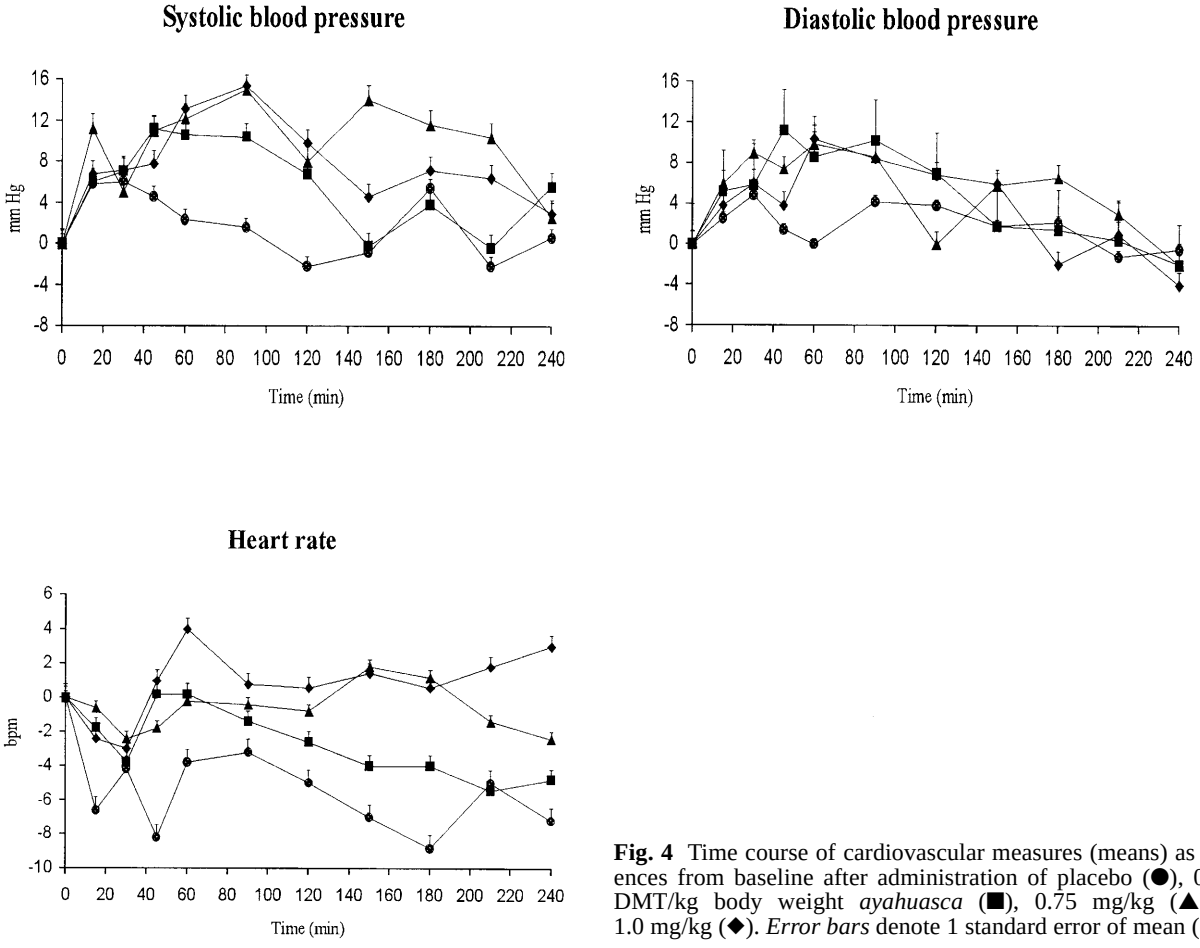

Fig. 4 Time course of cardiovascular measures (means) as differences from baseline after administration of placebo (●), 0.5 mg DMT/kg body weight *ayahuasca* (■), 0.75 mg/kg (▲), and 1.0 mg/kg (◆). *Error bars* denote 1 standard error of mean (n=5)

Table 2 Somatic-dysphoric effects spontaneously reported by the six volunteers, or as positive responses on particular items of the HRS and ARCI questionnaires on the 4 experimental days, presented as most to least frequently reported. Figures indicate the number of subjects who reported a specific effect, regardless of intensity, at the three different *ayahuasca* doses administered and placebo

	Somatic-dysphoric effect	Placebo	0.5 mg/kg	0.75 mg/kg	1.0 mg/kg
1	Body feels different[a]	1/6	5/6	6/6	5/5
2	Nausea[a]	0/6	4/6	5/6	3/5
3	Change in body temperature[a]	1/6	4/6	4/6	3/5
4	Electric/tingling feeling[a]	1/6	2/6	3/6	5/5
5	I have a disturbance in my stomach[b]	0/6	3/6	4/6	2/5
6	My hands feel clumsy[b]	1/6	2/6	3/6	3/5
7	My speech is slurred[b]	0/6	3/6	3/6	2/5
8	Urge to urinate[a]	1/6	1/6	3/6	3/5
9	Feel body shake/tremble[a]	0/6	1/6	3/6	2/5
10	Urge to move bowels[a]	0/6	2/6	0/6	3/5
11	I feel dizzy[b]	0/6	2/6	2/6	0/5
12	My head feels heavy[b]	0/6	2/6	2/6	0/5
13	Sweating[a]	0/6	1/6	2/6	1/5
14	A thrill has gone through me...[b]	0/6	0/6	1/6	0/5
15	Vomiting[c]	0/6	0/6	0/6	1/5
16	Disorientation[c]	0/6	0/6	1/6	0/5

[a]Item included in the HRS
[b]Item included in the ARCI
[c]Spontaneously reported

ly two occasions. Verbal support was sufficient to get him through this temporary crisis, but he was left with a general feeling of dissatisfaction toward the experience and withdrew from the study. Nevertheless, all volunteers, including this one, were well aware of the effects being caused by the administered drug and of their transient nature.

Discussion

The administration of *ayahuasca* to experienced healthy volunteers induced intense modifications of their conscious state, which was evaluated as dose-dependent elevations in all VAS items used, in five of the HRS subscales, and in the MBG, LSD, and A scales of the ARCI questionnaire. At no time of the study did any of the vol-

unteers lose consciousness or contact with reality. Compared with the effects of intravenous (IV) DMT (Strassman et al. 1994), clear differences were found in the intensity and duration of the experience. The slower onset and longer duration of effects seen for *ayahuasca* can be readily attributed to the oral route of administration for DMT and to the enzymatic blockade process (MAO inhibition) which mediates the drug's access to systemic circulation. Additionally, the competition between DMT and increasing levels of serotonin for available receptor sites may contribute to an overall attenuation of effects from *ayahuasca* vs IV administration of pure DMT. MAO inhibition not only allows for increased levels of serotonin and other monoamines but also temporarily blocks the immediate metabolism of DMT, thus extending its action relative to its IV administration. The cardiovascular effects observed were milder than those reported for IV DMT (Strassman and Qualls 1994). Peak increases of blood pressure and heart rate after *ayahuasca* were relatively delayed and comparable in magnitude to those brought about by a 0.1–0.2 mg/kg IV DMT dose. Our cardiovascular values are in line with those previously reported by Callaway and coworkers (1999) after an *ayahuasca* (*hoasca)* oral dose of 0.48 mg DMT/kg, though a direct comparison is not possible given the non placebo-controlled nature of this earlier study. Considering the fact that in the present study elevations were observed in cardiovascular parameter after placebo, it seems likely that the inclusion of a placebo control in the earlier study could have rendered lower increases of cardiovascular parameters for the 0.48 mg DMT/kg dose used. When compared with IV DMT, it is reasonable to assume that the reversible MAO-inhibiting properties of harmine and harmaline leads to a transient increase in endogenous monoamines, in addition to DMT's own cardiovascular effects. Nevertheless, the moderate nature of these increases could also be due to the simultaneous enhancement of vagal activity induced by decreased serotonin metabolism. Additionally, a*yahuasca* seemed to induce more somatic-dysphoric effects than IV DMT, the most frequently reported being the modifications in body feeling and nausea. These effects may be attributable to the β-carbolines present in the tea. A relationship between the nausea and other distressing effects on the digestive tract and increased 5-HT levels has been postulated (Callaway et al. 1999).

Scorings on the six HRS subscales and the nature of the effects elicited by *ayahuasca* at the present low dose resembled those reported by Strassman et al. (1994) after 0.1 mg/kg IV DMT. In both cases, somatic reactions predominated over perceptual or cognitive effects. Scores on the “affect”, “volition”, and “intensity” subscales were also close to those reported by Grob et al. (1996) after an *ayahuasca* dose equivalent to the low dose used in the present study. Except for the “perception” and “volition” subscales, which showed lower values, scores on the HRS at the medium dose were greater than those reported by Grob et al. (1996) and fell close to those described for 0.2 mg/kg IV DMT, a dosage known to be fully psychoactive for DMT (Strassman et al. 1994). These differences probably indicate less overwhelming perceptual effects and greater control over the experience after *ayahuasca*. Finally, the five volunteers who received the high dose (1.0 mg DMT/kg) identified it as being fully active and verbally described its effects as being very high in intensity. However, several subjective-effect variables showed a saturation relative to the 0.75 mg DMT/kg dose. This saturation, or ceiling effect, may indicate an “order” effect due to the exploratory nature of the study design, with doses being administered in an increasing order rather than in a randomized balanced manner. At the medium dose, scores on all HRS subscales were higher than those reported by Grob et al. (1996) in their single-dose study. The “cognition” subscale for the medium dose in the present study scored close to the value obtained by Strassman et al. (1994) at 0.4 mg/kg IV DMT, whereas scores on the other five subscales remained near those obtained after a 0.2 mg/kg IV DMT dose. Thus, not even at the 1.0 mg DMT/kg *ayahuasca* dose did the volunteers experience the overwhelming effects reported for the highest dose used in Strassman's study (0.4 mg/kg IV), probably reflecting the milder effects of DMT made orally active by means of MAO inhibition.

Results obtained for the ARCI-A scale are indicative of a subjective effect of increased activation. Despite the coexistence of marked somatic-dysphoric effects, as reflected by increases in the HRS-LSD scale, the administration of *ayahuasca* induced elevations in the ARCI-MBG scale, indicative of subjective feelings of well-being. The pleasant nature of the effects experienced by five of the six volunteers was also reflected as increases in the “good effects”, “liking”, and “high” VAS items, especially at the high dose. On the contrary, sedation ratings, as reflected by the ARCI-PCAG scale did not reach statistical significance and tended to decrease as the doses increased.

Regarding the similarities and differences of the *ayahuasca* experience with those elicited by other better characterized serotonergic psychedelics, important differences can be found in the time course of effects. A*yahuasca* effects are comparable in duration to those of psilocybin. On the other hand, mescaline and LSD are clearly longer-acting drugs, with peak effects at 3–5 h and an overall duration which can exceed 8 h (Strassman 1994). Psychological effects are difficult to compare between studies, due to the different psychometric instruments used. However, in a recent human study where the HRS was administered, psilocybin was found to induce increases in all the HRS subscales, including “volition”. This greater impairment of the subjects' capacity to interact with themselves and their surroundings was further corroborated by their verbal reports, which described sensations of loss of control and paranoid thoughts (Gouzoulis-Mayfrank et al. 1999a), neither of which were observed in the present study.

From a neurochemical perspective, data from preclinical studies strongly support the involvement of seroto-

nergic neurotransmission in the effects elicited by the classic psychedelics, which includes DMT. Such compounds containing an indole moiety bind with high affinity to both the 5-HT_{2A} and 5-HT_{1A} sites in the human brain. A close correlation has been found between psychotropic potency and binding at the 5-HT_{2A} site (Glennon et al. 1984) which is considered to be chiefly responsible for the behavioral effects elicited by these agents. The interaction with the 5-HT_{1A} site has recently been argued to modulate the intensity of the psychedelic experience (Strassman 1996). Additionally, evidence of a possible long-term modulation of serotonergic neurotransmission by *ayahuasca* has been reported in a previous study, in which an apparent upregulation of the platelet serotonin transporter was found in regular users of the tea (Callaway et al. 1994). Nevertheless, the role of dopaminergic involvement in the effects of the classic psychedelics has also been examined. A recent PET study found that the administration of psilocybin to human volunteers leads to the displacement of ^{11}C-raclopride in the striatum, an effect that may reflect an increase in dopamine release (Vollenweider et al. 1999). This secondary pro-dopaminergic activity may not be, however, the key to the perceptual and cognitive modifications induced by these agents, as in another study psilocybin's subjective effects were found to be increased rather than reverted by the D_2 receptor antagonist, haloperidol, while they were effectively counteracted by ketanserin and risperidone (Vollenweider et al. 1998).

Neuroimaging studies have revealed patterns of increased metabolism throughout the brain, and more specifically in the prefrontal cortices, particularly in the right hemisphere, in healthy volunteers after dosing with psilocybin (Vollenweider et al. 1997; Gouzoulis-Mayfrank et al. 1999b) and mescaline (Hermle et al. 1992). In this respect, recent electrophysiological studies have shown that 5-HT_{2A} receptor activation by serotonin mediates an increase of excitatory postsynaptic potentials (EPSPs) and currents (EPSCs) in pyramidal neurons of the neocortex and transitional cortex (Aghajanian and Marek 1997), an effect involving glutamate release and which is most pronounced in the medial prefrontal cortex (Marek and Aghajanian 1998a, b). These findings suggest an excitatory action of the classic psychedelics on the human frontal and parietal cortices and in the primary auditory and visual areas, which show very high densities of 5-HT_{2A} sites (Pazos et al. 1987). This excitatory effect may account for the enhancement and modifications of auditory and visual perception described by the volunteers. An analogous excitatory action on the somatosensory and visual association areas, both showing high 5-HT_{2A} densities, may also play a role in the peculiar modifications of perception brought about by *ayahuasca*. Finally, the activation of the anterior cingulate cortex (ACC), an area also showing dense serotonergic innervation and 5-HT_{2A} sites, could contribute to the emotional overtones of the *ayahuasca* experience. A recent PET study has implicated the ACC in normal emotional awareness (Lane et al. 1998), and psilocybin administration leads to increases in metabolism in this area, where 5-HT_{2A}-mediated EPSPs/EPSCs have also been recorded (Aghajanian and Marek 1997).

To summarize, *ayahuasca* induced a modified state of awareness in which stimulatory and psychedelic effects were present, and increased in a dose-dependent manner. The volunteers experienced modifications in perception and thought processes, such as rapid succession of thoughts, visions, and recollections of recent events, frequently having a marked emotional content. *Ayahuasca* was safely administered to the volunteers in this study and its effects were regarded as pleasant and desirable, except for one volunteer who experienced a dysphoric state that was characterized by transient disorientation and anxiety. Nevertheless, this adverse reaction was most likely related to the limited previous experience of that volunteer with the tea. Finally, the nature of the experience produced by *ayahuasca* resembled that of IV DMT, though it was less overwhelming, of longer duration, and displayed a greater variety of somatic-dysphoric effects. Moderate actions on blood pressure and heart rate were found and no clinically relevant changes were observed in biochemical parameters after any of the experimental sessions. Future studies will include measures of sensorimotor gating and brain imaging techniques in larger volunteer groups, using a double-blind balanced design, in order to obtain additional information on the mechanisms underlying the central effects of *ayahuasca.*

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Human Pharmacology of Ayahuasca: Subjective and Cardiovascular Effects, Monoamine Metabolite Excretion, and Pharmacokinetics

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ABSTRACT

The effects of the South American psychotropic beverage ayahuasca on subjective and cardiovascular variables and urine monoamine metabolite excretion were evaluated, together with the drug's pharmacokinetic profile, in a double-blind placebo-controlled clinical trial. This pharmacologically complex tea, commonly obtained from *Banisteriopsis caapi* and *Psychotria viridis*, combines *N,N*-dimethyltryptamine (DMT), an orally labile psychedelic agent showing 5-hydroxytryptamine$_{2A}$ agonist activity, with monoamine oxidase (MAO)-inhibiting β-carboline alkaloids (harmine, harmaline, and tetrahydroharmine). Eighteen volunteers with prior experience in the use of psychedelics received single oral doses of encapsulated freeze-dried ayahuasca (0.6 and 0.85 mg of DMT/kg of body weight) and placebo. Ayahuasca produced significant subjective effects, peaking between 1.5 and 2 h, involving perceptual modifications and increases in ratings of positive mood and activation. Diastolic blood pressure showed a significant increase at the high dose (9 mm Hg at 75 min), whereas systolic blood pressure and heart rate were moderately and nonsignificantly increased. 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. Drug administration increased urinary normetanephrine excretion, but, contrary to the typical MAO-inhibitor effect profile, deaminated monoamine metabolite levels were not decreased. This and the negligible harmine plasma levels found suggest a predominantly peripheral (gastrointestinal and liver) site of action for harmine. MAO inhibition at this level would suffice to prevent first-pass metabolism of DMT and allow its access to systemic circulation and the central nervous system.

Ayahuasca, also known by the names Daime, Yajé, Natema, and Vegetal, is a psychotropic plant tea used by shamans throughout the Amazon Basin in traditional medicine, rites of passage, and magico-religious practices (Schultes and Hofmann, 1982; Dobkin de Rios, 1984). This ancient pattern of use has given way to a more widespread and frequent consumption by members of a number of modern Brazilian-based syncretic religious groups, mainly the Santo Daime and the Uniao do Vegetal, which have incorporated the use of the beverage in their rituals (Dobkin de Rios, 1996). In recent years, groups of followers of these Brazilian religions have become established in the United States and in several European countries, including Germany, Great Britain, Holland, France, and Spain (Anonymous, 2000). As a larger number of people have come into contact with ayahuasca, the tea has begun to attract the attention of biomedical researchers (Callaway et al., 1999; Riba et al., 2001b).

Ayahuasca is obtained by infusing the pounded stems of the malpighiaceous vine *Banisteriopsis caapi* either alone or, more frequently, in combination with the leaves of *Psychotria viridis* (rubiaceae) in Brazil, Peru, and Ecuador or *Diplopterys cabrerana* (malpighiaceae), used mainly in Ecuador and Colombia (Schultes and Hofmann, 1980; McKenna et al., 1984). *P. viridis* and *D. cabrerana* are rich in the psychedelic indole *N,N*-dimethyltryptamine (DMT; Rivier and Lindgren, 1972; Schultes and Hofmann, 1980), whereas *B. caapi* contains substantial amounts of β-carboline alkaloids, mainly harmine and tetrahydroharmine (THH), and to a lesser extent harmaline and traces of harmol and harmalol (Rivier and Lindgren, 1972; McKenna et al., 1984).

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ABBREVIATIONS: DMT, *N,N*-dimethyltryptamine; THH, tetrahydroharmine; LSD, D-lysergic acid diethylamide; CNS, central nervous system; MAO, monoamine oxidase; COMT, catechol-*O*-methyltransferase; VMA, vanillylmandelic acid; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; MDMA, methylenedioxymethamphetamine; HPLC, high-performance liquid chromatography; VAS, visual analog scale(s); HRS, Hallucinogen Rating Scale; ARCI, Addiction Research Center Inventory; MBG, morphine-benzedrine group; PCAG, pentobarbital-chlorpromazine-alcohol group; BG, benzedrine group; AUC, area under the concentration-time curve; CL/F, total plasma clearance; V_z/F, apparent volume of distribution; ANOVA, analysis of variance; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

DMT is structurally related to the neurotransmitter serotonin and, like better-characterized psychedelics such as LSD and mescaline, binds to 5-hydroxytryptamine $_{2A}$ receptors in the central nervous system (CNS), where it acts as an agonist (Pierce and Peroutka, 1989; Smith et al., 1998). Studies in humans have shown that when administered parenterally, DMT provokes dramatic modifications in perception, the sense of self and reality that can be very intense but relatively short in duration (Strassman et al., 1994). The drug also exerts marked autonomic effects elevating blood pressure, heart rate, and rectal temperature, and causes mydriasis (Strassman and Qualls, 1994). Unlike the vast majority of known psychedelic phenethylamines, tryptamines, and ergolines, DMT is orally inactive (Ott, 1999), apparently due to metabolism by monoamine oxidase (MAO; Suzuki et al., 1981). Interestingly, harmine and harmaline, and, to a lesser extent, THH, are potent MAO inhibitors (Buckholtz and Boggan, 1977; McKenna et al., 1984). In 1968, Agurell and coworkers (cited in Ott, 1999, p. 172) postulated that the interaction between β-carbolines and DMT in ayahuasca "might result in specific pharmacological effects". It is now a widely accepted hypothesis that following ayahuasca ingestion, MAO inhibition brought about by harmine, given that it is more potent than THH and is present in the tea in larger amounts than harmaline (McKenna et al., 1984), prevents the enzymatic degradation of DMT, allowing its absorption. It has also been speculated that β-carbolines may contribute to the overall central effects of ayahuasca by blocking brain MAO and weakly inhibiting serotonin reuptake, which combined would lead to enhanced neurotransmitter levels and modulate the effects of DMT (Callaway et al., 1999).

In the present paper we report a double-blind placebo-controlled crossover clinical trial conducted with ayahuasca, in which subjective and cardiovascular effects, and alkaloid pharmacokinetics were assessed in a group of healthy volunteers experienced in psychedelic drug use. Additionally, urine monoamine metabolites were studied to measure in vivo the MAO-inhibitory effects of ayahuasca. In this respect, the neurotransmitters norepinephrine, epinephrine, and dopamine are physiologically degraded by MAO and catechol-*O*-methyltransferase (COMT) to produce deaminated and methylated metabolites, respectively. Serotonin, on the other hand, is exclusively metabolized by MAO to produce a deaminated compound. In vivo and in vitro studies have shown that when MAO is pharmacologically inhibited, the levels of MAO-dependent deaminated metabolites decrease and those of COMT-dependent methylated metabolites increase. In humans, MAO inhibitors decrease, after acute administration, the urinary excretion of vanillylmandelic acid (VMA), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA), the deaminated metabolites of norepinephrine/epinephrine, dopamine, and serotonin, respectively, while increasing that of metanephrine and normetanephrine, the methylated metabolites of epinephrine and norepinephrine, respectively (Pletscher, 1966; Koulu et al., 1989). Monoamine metabolites have both a CNS and a non-CNS origin, and their assessment in urine does not give information regarding the organ in which MAO was inhibited. Nevertheless, this approach can identify dose-response relationships after drug administration and allows for the study of the time course of MAO inhibition.

Materials and Methods

Volunteers

A total of 18 volunteers (15 males and 3 females) with experience in psychedelic drug use were recruited by word of mouth. Eligibility criteria required prior use of psychedelics on at least five occasions without sequelae derived thereof, i.e., psychedelic-related disorders as described in the DSM-III-R. Participants had used psychedelics from six to hundreds of times. The most commonly used psychedelic was LSD (17 of 18), followed by psilocybian mushrooms (15 of 18) and ketamine (10 of 18). The least commonly used were peyote (3 of 18), *Salvia divinorum* (3 of 18), mescaline (2 of 18), *Amanita muscaria* (2 of 18), and *Datura stramonium* (1 of 18). Although prior exposure to ayahuasca was not required for participation, two of the volunteers had ingested this tea before inclusion. Besides psychedelics, volunteers had consumed cannabis (18 of 18), cocaine (17 of 18), and MDMA (17 of 18). Volunteers were in good health, confirmed by medical history, laboratory tests, ECG, and urinalysis. Prior to physical examination, volunteers were interviewed by a psychiatrist (structured interview for DSM-III-R) and completed the trait-anxiety scale from the State-Trait Anxiety Inventory (Spielberger et al., 1970). Exclusion criteria included current or previous history of psychiatric disorder and/or family history of Axis-I psychiatric disorder in first degree relatives, alcohol or other substance dependence, and high scores on trait anxiety (over 1 standard deviation above normative mean). Participants had a mean age of 25.7 years (range 19–38), mean weight 66.47 kg (range 50.7–79.5), and mean height 175.11 cm (range 158–188). 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, 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

To administer ayahuasca in accurate dosings and masked in a double-blind, double-dummy design, a 9.6-liter batch of Brazilian Daime was subjected to a freeze-drying process that yielded 611 g of powder, which was subsequently homogenized and analyzed. The DMT content was determined by HPLC, as described by Callaway et al. (1996), and the β-carbolines were determined according to a modified version of the method described therein. One gram of freeze-dried material contained 8.33 mg of DMT, 14.13 mg of harmine, 0.96 mg of harmaline, and 11.36 mg of THH, which corresponded to the following alkaloid concentrations in the original tea: DMT, 0.53 mg/ml; harmine, 0.90 mg/ml; harmaline, 0.06 mg/ml; and THH, 0.72 mg/ml. The ayahuasca doses administered to the volunteers in the present study were chosen based on tolerability and subjective effect data gathered in a previous study (Riba et al., 2001b). The low and the high dose contained, per kilogram of body weight: 0.6/0.85 mg of DMT, 1.0/1.4 mg of harmine, 0.07/0.09 mg of harmaline, and 0.82/1.16 mg of THH. The average (range) alkaloid content in milligrams administered in each dose (low dose/high dose) was: 39.8 (30.4–47.9)/57.4 (43.7–67.7) for DMT, 67.4 (51.6–81.2)/95.8 (74.2–114.8) for harmine, 4.6 (3.5–5.5)/6.5 (5.0–7.8) for harmaline, and 54.2 (41.5–65.3)/77.0 (59.6–92.3) for THH. The calculated individual dose for each volunteer was administered by combining 00 gelatin capsules containing different amounts of freeze-dried ayahuasca, i.e., 0.5 g, 0.25 g, or 0.125 g, and placebo capsules containing 0.75 g of lactose. Placebo capsules were added when necessary, so that all volunteers took the same number of capsules on each experimental day. It is interesting to note that although the amount of DMT administered with the present low dose was similar to that administered in the only other published study on the human pharmacology of ayahuasca (Callaway et al., 1999), the amounts of β-car-

bolines administered in this work were much lower. This was due to the different alkaloid proportions present in the tea samples used in each study. Thus, the average amounts (range) in milligrams administered by Callaway et al. (1999) were: 35.5 (28.8–43.2) for DMT, 252.3 (204.0–306.0) for harmine, 29.7 (24.0–36.0) for harmaline, and 158.8 (128.4–196.6) for THH.

Study Design

Each volunteer participated in four experimental sessions at least 1 week apart. Volunteers were informed that on each experimental day they would randomly receive a single oral dose of encapsulated freeze-dried ayahuasca (one low and one high dose), a placebo, and a random repetition of one of the three mentioned treatments. In actual fact, they all received a placebo on the first experimental day in a single-blind fashion, followed by one of the three treatments from days 2 to 4 in a double-blind balanced fashion, according to a randomization table. The first nonrandomized placebo was administered to familiarize the volunteers with the experimental setting and to minimize the stress associated with the experimental interventions. Volunteers were requested to abstain from any medication or illicit drug use 2 weeks before the beginning of the experimental sessions until the completion of the study. Volunteers also abstained from alcohol, tobacco, and caffeinated drinks 24 h before each experimental day. Urinalysis for illicit drug use was performed for each experimental session. The volunteers were admitted to the research unit on four separate experimental days. Upon arrival at 8:00 AM under fasting conditions, a cannula was inserted in the cubital vein of their right arm for drawing blood samples, and capsules were administered at approximately 10:00 AM with 250 ml of tap water. Throughout the experimental session, the volunteers remained seated in a comfortable reclining chair in a quiet, dimly lit room. At 4 h after administration of the capsules, when the most prominent subjective effects associated with the drug had disappeared, the volunteers had a meal. The last experimental time point was at 8 h, and volunteers were discharged approximately 9 h after administration.

Study Methods

Subjective Effect Measures. The subjective effects elicited by ayahuasca were measured by means of visual analog scales (VAS) and self-report questionnaires. VAS were 100-mm horizontal lines with the following labels: "any effect," indicating any effect, either physical or psychological, that the volunteer attributed to the administered drug; "good effects," indicating any effect the volunteer valued as good; "liking," reflecting that the volunteer liked the effects of the administered substance; "drunken," indicating any dizziness or lightheadedness; "stimulated," indicating any increases in thought speed and/or content, or any increases in associations and/or insights; "visions," indicating modifications in visual perception, including any variations in object shape, brightness, or color and any illusion, abstract or elaborate, seen with either eyes closed or open; and "high," which reflected any positive psychological effect the volunteer attributed to the drug. Except for the "visions" item, the other VAS items administered had been used in human studies by other researchers assessing the subjective effects of a variety of psychoactive drugs (Farré et al., 1993, 1998; Camí et al., 2000). The volunteers were requested to answer the VAS immediately before administration (baseline) and at 15, 30, 45, 60, and 75 min, and 1.5, 2, 2.5, 3, 3.5, 4, 6, and 8 h after administration.

Self-report questionnaires included the Hallucinogen Rating Scale (HRS) and the Addiction Research Center Inventory (ARCI). The HRS (Strassman et al., 1994) measures psychedelic-induced subjective effects and includes six 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 (Riba et al., 2001a). The range of scores for all HRS scales is 0 to 4. The short version of the ARCI (Martin et al., 1971) consists of five scales or groups: MBG, morphine-benzedrine group, measuring euphoria and positive mood; PCAG, pentobarbital-chlorpromazine-alcohol group, measuring sedation; LSD, 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. Both the A and BG scales are sensitive to psychostimulants. 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 et al. (1994). Volunteers answered the ARCI immediately before drug administration and 4 h after drug intake, whereas the HRS was only answered at 4 h postadministration.

Cardiovascular Measures. Systolic and diastolic blood pressure and heart rate were measured with the volunteer seated, immediately before administration (baseline), and at 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, and 240 min after intake using a sphygmomanometer cuff (Dinamap; Critikon, Tampa, FL) placed around the volunteer's left arm. No measurements were made after 240 min, the time point when subjects had their meal and after which they were allowed to move and leave the room.

Urine Samples. Urine was collected in fractions of 0 to 8 h, 8 to 16 h, and 16 to 24 h in plastic containers with 3 ml of 6 N HCl and kept in the refrigerator during the 0- to 24-h collection period. Volunteers took home the two plastic containers corresponding to the 8- to 16-h and 16- to 24-h periods. Volume of each fraction was recorded and pH was adjusted to 2 to 4 with 6 N HCl, and two 50-ml aliquots were frozen at −20°C and stored at −80°C until analysis. The following monoamine metabolites, VMA, HVA, 5-HIAA, metanephrine, and normetanephrine were quantified by means of HPLC with coulometric detection following previously validated procedures (Soldin and Hill, 1980; Parker et al., 1986; Gamache et al., 1993). The limit of quantification was 3 mg/l for VMA, HVA, and 5-HIAA, 0.05 mg/l for metanephrine, and 0.10 mg/l for normetanephrine.

Blood Samples. Blood samples (10-ml EDTA tubes) were drawn at baseline, 30, 60, 90, 120, and 150 min, and 3, 4, 6, 8, and 24 h after administration for analysis of DMT, harmine, harmaline, and THH concentrations in plasma and those of the *O*-demethylated metabolites harmol and harmalol. Samples were centrifuged at 2000 rpm for 10 min at 4°C and plasma was immediately frozen at −20°C. The frozen plasma samples were stored at −80°C until analysis. DMT was quantified by gas chromatography with nitrogen-phosphorus detection and the β-carbolines by means of HPLC with fluorescence detection following previously reported methods (Yritia et al., 2002). The limit of quantification was 1.6 ng/ml for DMT, 0.5 ng/ml for harmine, 0.3 ng/ml for harmaline, 1.0 ng/ml for THH, and 0.3 ng/ml for harmol and harmalol. The intraday and interday coefficients of variation were lower than 10.9% and 13.4%, respectively, for all determined compounds.

Pharmacokinetic Analysis

After quantification of the different compounds in plasma, the following pharmacokinetic parameters were calculated using a noncompartmental approach by means of WinNonlin software (version 3.0; Pharsight, Mountain View, CA): maximum concentration (C_{max}), time taken to reach the maximum concentration (T_{max}), and area under the concentration-time curve from 0 to 8 h (AUC_{0-8h}), calculated by means of the trapezoidal rule. AUC was extrapolated to infinity ($AUC_{0-\infty}$) by addition of the residual area calculated by the last plasma concentration/terminal elimination rate constant. Terminal half-life ($t_{1/2\lambda z} = \ln 2/\lambda_z$) was obtained by linear regression analysis of the terminal log-linear portion of the plasma-concentration curve. Clearance (CL/F) was determined as dose/$AUC_{0-\infty}$. Appar-

ent volume of distribution (V_z/F) was calculated as dose/($\lambda_z \cdot AUC_{0-\infty}$). The $AUC_{0-\infty}$ normalized by dose ($AUC_{0-\infty}$/D) was also calculated. All data are expressed as mean ± S.D. except for T_{max}, where median and range are given.

Statistics

Prior to statistical analysis, ARCI scores were transformed to differences from preadministration values, and the following parameters were calculated for VAS items: peak effect (maximum absolute change from baseline values), time taken to reach the maximum effect (t_{max}), and the 8-h area under the curve (AUC_{0-8h}) of effect versus time calculated by the trapezoidal rule. For cardiovascular variables, peak effect (maximum absolute change from baseline values) and the 4-h area under the curve (AUC_{0-4h}) of effect versus time were calculated. The obtained parameters, transformed ARCI scores, and raw HRS scores were analyzed by means of a one-way repeated measures ANOVA with drug (placebo, ayahuasca low dose, ayahuasca high dose) as factor. When a significant effect was observed, post hoc comparisons were performed using Tukey's multiple comparisons test. The time course of subjective effects was explored using repeated measures two-way ANOVAs with drug and time (13 time points) as factors. When a drug by time interaction was significant, multiple comparisons were performed at each time point by means of Tukey's test.

Monoamine metabolite levels in urine were analyzed by means of a one-way repeated measures ANOVA with drug (placebo, ayahuasca low dose, ayahuasca high dose) as factor. When a significant effect was observed, post hoc comparisons were performed using Tukey's test. The time course of effects was explored using repeated measures two-way ANOVAs with drug and time (three time points) as factors. Pharmacokinetic parameter comparisons between doses were performed by means of Student's t test, except for T_{max}, which was compared by means of a nonparametric Wilcoxon test.

To explore possible differences in the time-to-peak of DMT plasma concentrations and time-to-peak of subjective effects (for each of the administered VAS), nonparametric Wilcoxon tests were performed comparing T_{max} for DMT and t_{max} for each VAS. These tests were performed for data obtained after each of the two administered ayahuasca doses. In all tests performed, differences were considered statistically significant for p values lower than 0.05.

Results

Subjective Effects. Subjective effects results are shown in Tables 1 and 2 and Figs. 1 and 2. Ayahuasca administration induced significant increases in all six HRS scales, both after the low and the high dose, except for Volition, which showed statistically significant differences from placebo only after the 0.85 mg of DMT/kg dose. The ARCI questionnaire showed statistically significant dose-dependent increases after ayahuasca in measures of stimulatory effects (A scale), euphoria (MBG scale), and somatic symptoms (LSD scale).

TABLE 1

Results of the statistical analyses performed on raw HRS scores, transformed ARCI scores (differences from predrug values), VAS measures (peak values and AUC_{0-8h}), and cardiovascular parameters (peak values and AUC_{0-4h})

For all measures $n = 18$.

Variable		ANOVA (2,34)		Tukey's Multiple Comparison Test		
				Placebo		Low Dose/High
		F	p Value	Low Dose	High Dose	
HRS						
Affect		29.35	<0.001	**	**	**
Cognition		31.66	<0.001	*	**	**
Somaesthesia		39.62	<0.001	**	**	**
Perception		38.76	<0.001	**	**	**
Volition		4.68	0.016	N.S.	*	N.S.
Intensity		77.35	<0.001	**	**	**
ARCI						
A		23.10	<0.001	*	**	**
BG		3.62	0.058			
LSD		10.05	<0.001	*	**	N.S.
MBG		11.22	<0.001	N.S.	**	N.S.
PCAG		0.91	0.412			
VAS						
Any Effect	Peak	39.62	<0.001	**	**	**
	AUC	18.06	<0.001	*	**	**
Good Effects	Peak	26.64	<0.001	**	**	*
	AUC	18.69	<0.001	**	**	*
Liking	Peak	29.82	<0.001	**	**	*
	AUC	15.10	<0.001	**	**	N.S.
Visions	Peak	16.28	<0.001	**	**	*
	AUC	7.25	0.002	N.S.	**	N.S.
Drunken	Peak	6.26	0.005	N.S.	**	N.S.
	AUC	4.83	0.014	N.S.	*	N.S.
Stimulated	Peak	16.62	<0.001	**	**	*
	AUC	11.57	<0.001	N.S.	**	*
High	Peak	33.97	<0.001	**	**	**
	AUC	22.33	<0.001	*	**	**
Cardiovascular						
SBP	Peak	2.91	0.068			
	AUC	1.90	0.166			
DBP	Peak	15.54	<0.001	**	**	N.S.
	AUC	5.59	0.008	*	*	N.S.
HR	Peak	1.79	0.183			
	AUC	3.12	0.057			

* $p < 0.05$; ** $p < 0.01$.

TABLE 2

Positive responses on particular items of the HRS questionnaire given by at least 75% of the 18 volunteers after the high ayahuasca dose

Each column indicates the number of subjects who reported the effect, regardless of intensity, at the two different ayahuasca doses administered and placebo. The letter in parentheses indicates the HRS scale in which the item belongs.

	Item	Placebo	0.6 mg/kg	0.85 mg/kg
1	High (I)	1/18	15/18	17/18
2	Body feels different (S)	4/18	12/18	17/18
3	Visual effects (P)	2/18	10/18	17/18
4	A “rush” (S)	0/18	9/18	17/18
5	Change in rate of time passing (C)	2/18	12/18	16/18
6	Eyes open visual field vibrating or jiggling (P)	2/18	10/18	15/18
7	Electric/tingling feeling (S)	1/18	9/18	15/18
8	Change in quality of thinking (C)	2/18	8/18	15/18
9	Change in visual distinctiveness of objects in room (P)	4/18	7/18	15/18
10	Sounds in room sound different (P)	2/18	5/18	15/18
11	Urge to close eyes (V)	5/18	8/18	14/18
12	Change in distinctiveness of sounds (P)	2/18	7/18	14/18
13	Change in rate of thinking (C)	1/18	7/18	14/18
14	Excited (A)	1/18	7/18	14/18

A, Affect; C, Cognition; I, Intensity; P, Perception; S, Somaesthesia; V, Volition.

Scores on the BG and PCAG scales were not significantly different from placebo.

Scorings on all seven VAS items showed significant drug effects (peak values and AUC) and significant drug by time interactions. Initial effects appeared between 30 and 45 min, reflected as rises in the VAS any effect item, and were followed by a prominent increase at around 60 min, as indicated by steep rises in all seven VAS items. In general terms, the maximum scorings were observed between 90 and 120 min after drug administration. A gradual return to baseline levels followed thereafter and was complete at 360 min. Regarding effect magnitude, the largest scores were obtained for the VAS any effect, liking, and high, followed by VAS good effects, visions, and stimulated items. The least modified VAS after ayahuasca administration was the drunken item.

More qualitative information on the nature of the effects brought about by ayahuasca is provided in Table 2, which lists the most frequently reported positive responses to specific items of the HRS questionnaire.

Cardiovascular Effects. Mean values for systolic (SBP) and diastolic blood pressure (DBP) and heart rate (HR) over time are presented in Fig. 3, and results of the statistical analysis performed are shown in Table 1.

Ayahuasca administration produced only moderate elevations of cardiovascular parameters. Statistically significant changes relative to placebo were only found for DBP, both for peak values and AUC. The largest difference in DBP between the low dose and placebo was 9 mm Hg and occurred at 75 min after dosing. Between the high dose and placebo, differences of 10 and 9 mm Hg were observed at 15 and 75 min, respectively. A maximal increase of 7 mm Hg from baseline values was observed at 60 min for the low dose. After the high dose, a maximal increase of 9 mm Hg was observed at 15 min. For SBP, the largest differences with placebo were observed at 75 min and corresponded to 4 and 6 mm Hg increases for the low and high dose, respectively. Similarly, the maximal increase in SBP relative to baseline values was observed at 75 min and corresponded to 6 and 8 mm Hg for the low and high dose, respectively. Finally, HR showed the largest differences with placebo at 60 min and corresponded to 5 and 4 beats/min increases for the low and the high ayahuasca doses, respectively. The maximal increase from baseline values observed for HR was 4 beats/min and occurred at 60 min after administration of both the low and high ayahuasca doses.

Only two volunteers showed SBP values equal to or above 140 mm Hg at any time point: volunteer 1 at 75 and 90 min (140 mm Hg) after receiving the low dose, and at 60 (146 mm Hg) and 75 min (140 mm Hg) after receiving the high dose; and volunteer 6 as early as 15 min after administration of the high ayahuasca dose (146 mm Hg). Two volunteers showed DBP above 90 mm Hg: volunteer 1 at 30 min (93 mm Hg) after the low dose, and at 15 min (96 mm Hg) after the high dose; and volunteer 15 at 120 and 150 min (95 and 92 mm Hg, respectively) after administration of the high dose. Regarding HR, volunteer 1 also showed values above 100 beats/min (101 beats/min) at 60 min after the high dose.

Urine Monoamine Metabolites. Urine samples were successfully collected for 15 of the 18 volunteers enrolled in the study, and results are given for this subgroup only. Statistical analyses showed a significant effect of drug only for normetanephrine. No significant drug by time interaction was found for any of the metabolites studied. In view of this, the total monoamine metabolite amounts excreted during the 0- to 24-h period after placebo and the two ayahuasca doses are presented in Table 3. As shown therein, rather than the expected decreases in deaminated metabolites (VMA, HVA, 5-HIAA), drug administration increased the excretion of these compounds nonsignificantly. Similarly, levels of the *O*-methylated metabolites metanephrine and normetanephrine increased with dose, although only the latter showed statistically significant differences with placebo.

Pharmacokinetics. The time course of plasma concentrations and the calculated pharmacokinetic parameters for DMT, harmaline, THH, harmol, and harmalol are shown in Fig. 4 and Table 4. The graphs correspond to 15 of the total 18 participants enrolled in the study. To avoid the miscalculation of pharmacokinetic parameters, data from three volunteers were excluded from the analysis due to vomiting occurring after administration of the low dose (volunteer 6) and the high dose (volunteers 4 and 18). An additional subject (volunteer 12) was excluded from the calculation of harmalol parameters. Plasma levels for this volunteer after the high dose showed a plateau between 6 and 24 h, precluding parameter assessment.

As shown in Table 4, C_{max} and AUC values increased with

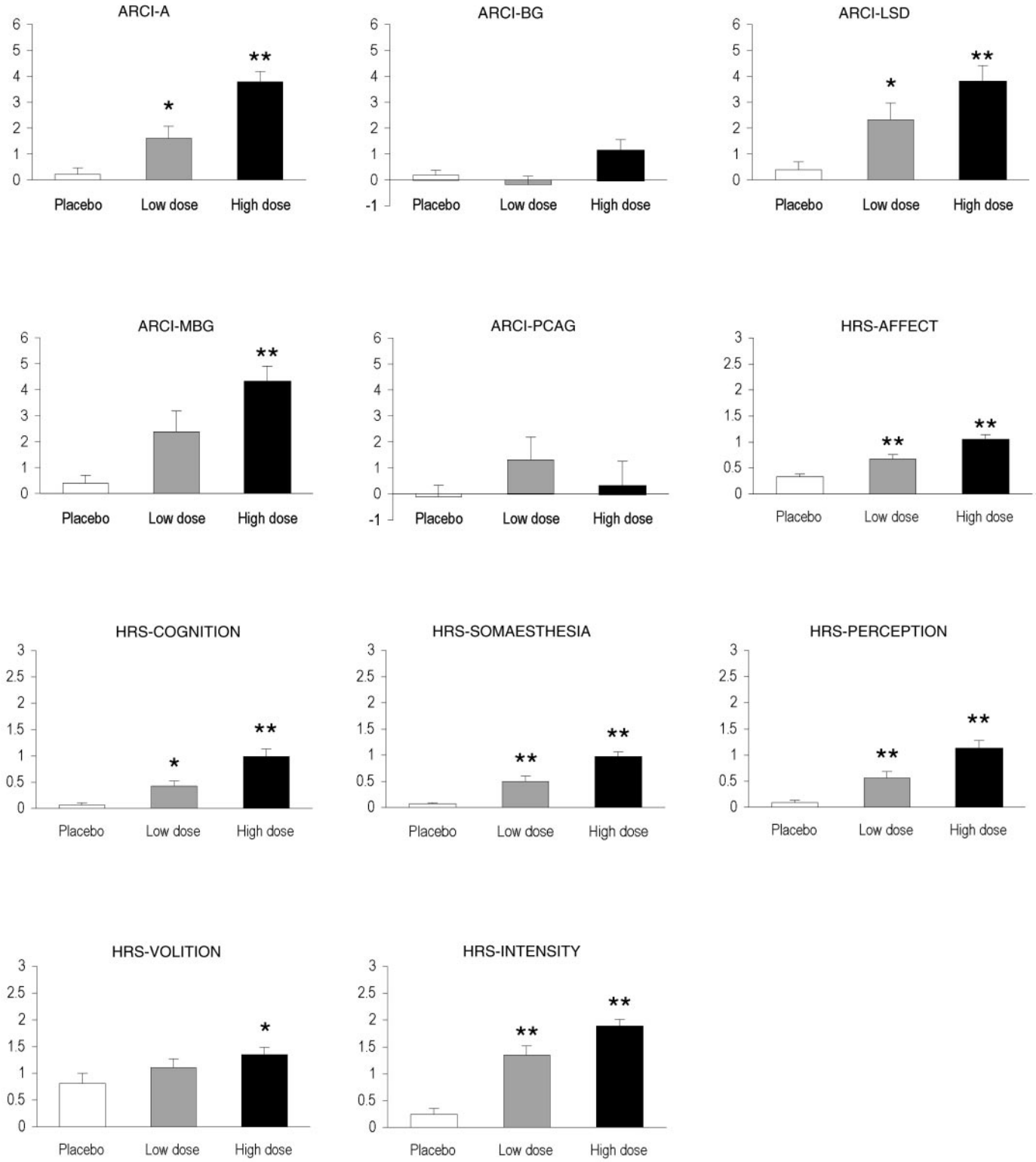

Fig. 1. Mean scores on the ARCI and HRS scales after administration of placebo (white), 0.6 mg of DMT/kg of body weight ayahuasca (shaded), and 0.85 mg of DMT/kg of body weight ayahuasca (black). Error bars denote 1 S.E.M. ($n = 18$). Significant differences from placebo are indicated by one ($p < 0.05$) or two ($p < 0.01$) asterisks.

dose for all measured compounds. DMT showed a T_{max} of 1.5 h (median) after both the low and high doses. Nevertheless, the upper end of the range of T_{max} values increased with dose, and the Wilcoxon test indicated a statistically significant difference between doses. A larger T_{max} after the high ayahuasca dose is evident also in the DMT concentration-time curve included in Fig. 4. Both harmaline and THH plasma concentrations peaked later than DMT, and their T_{max} values were larger after the high relative to the low ayahuasca dose. An unexpected finding was the absence of

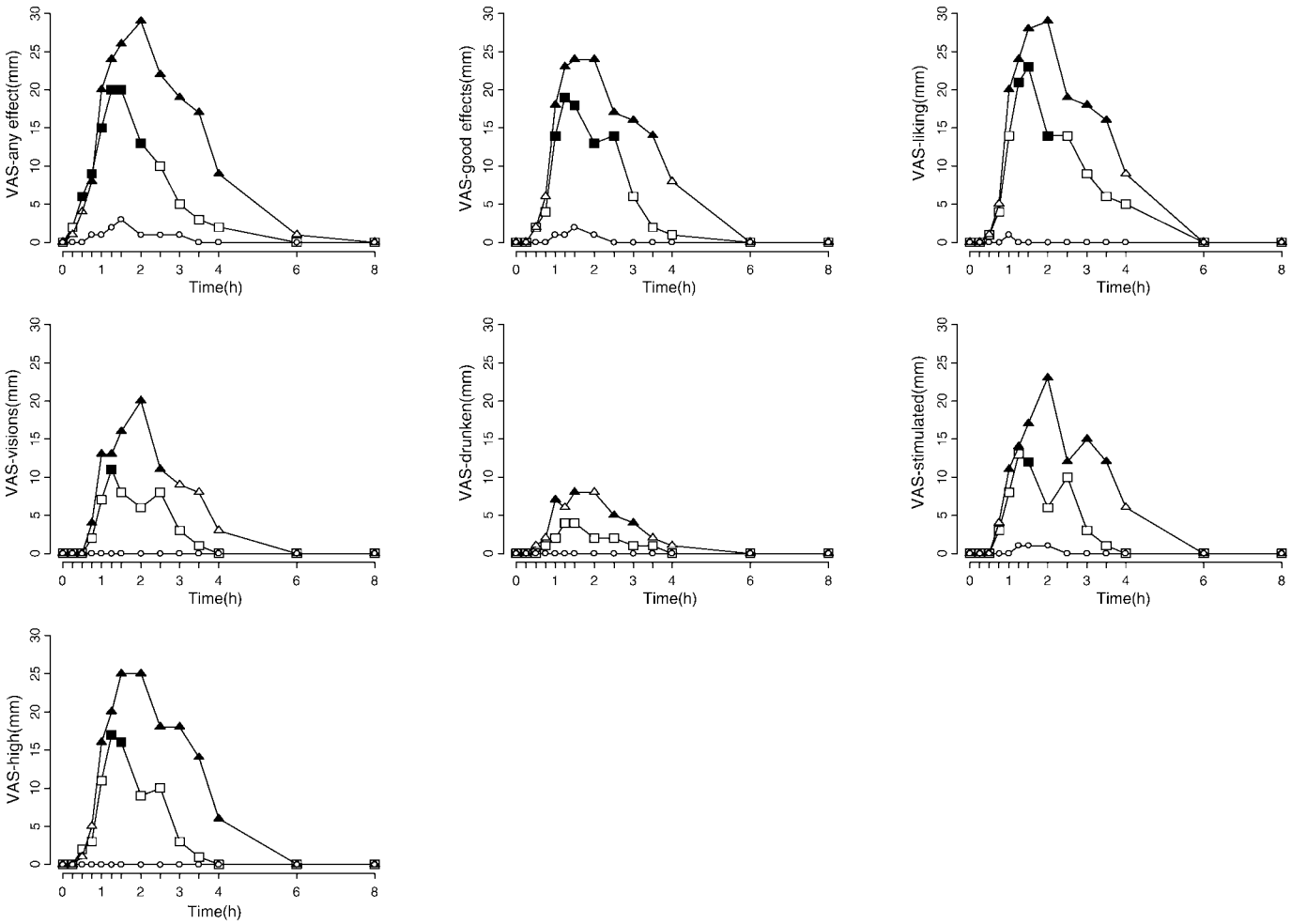

Fig. 2. Time curves of scores on the seven VAS items (means from 18 volunteers) after administration of placebo (circle), 0.6 mg of DMT/kg of body weight ayahuasca (square), and 0.85 mg of DMT/kg of body weight ayahuasca (triangle). Filled symbols indicate a significant difference from placebo.

measurable harmine plasma levels except for a few time points in 4 of 18 volunteers, precluding the calculation of pharmacokinetic parameters for this alkaloid.

Interestingly, all volunteers showed measurable levels of harmol, the *O*-demethylated analog of harmine. Plasma concentrations showed dose-dependent increases and peaked at 1.5 and 2 h after the low and high doses, respectively. Harmalol, the *O*-demethylated analog of harmaline, could also be quantified. Maximum concentrations were attained later than for harmaline, with T_{max} observed at 2.5 and 2.75 h after the low and high dose, respectively.

The AUC normalized by dose was calculated for each parent alkaloid, and these values were compared between doses by means of a paired Student's *t* test. A statistically significant difference was found for DMT, suggesting a possible nonproportional increase of plasma levels between doses. In line with this possibility, mean V_z/F and CL/F values calculated for DMT decreased with dose. These decreases were statistically significant for V_z/F and showed a tendency for CL/F ($t(14) = 1.94$, $p = 0.073$).

In support of a parallel evolution of DMT plasma levels and subjective effects, no significant differences were found between DMT T_{max} values and any of the seven VAS t_{max} values at any of the two administered ayahuasca doses.

Discussion

The psychotropic effects of ayahuasca could be demonstrated in a group of experienced psychedelic users who, in their vast majority, had reported no prior exposure to the tea. Oral administration of the freeze-dried material induced feelings of increased activation (ARCI-A, VAS-stimulated), euphoria and well being (ARCI-MBG, VAS-high, VAS-liking, VAS-good effects), and somatic effects (ARCI-LSD), in addition to perceptual modifications (HRS-Perception, VAS-visions) and changes in thought content (HRS-Cognition) and increased emotional lability (HRS-Affect). Increases in VAS-high have been observed after a great variety of drugs including MDMA (Camí et al., 2000), cocaine (Farré et al., 1993), and the sedative flunitrazepam (Farré et al., 1998). The VAS-stimulated item reflects more specifically the effects of psychostimulants such as amphetamine and MDMA (Camí et al., 2000). Increases in VAS-drunken, which was the least modified VAS item by ayahuasca, have been observed mainly after sedatives, such as flunitrazepam (Farré et al., 1998), and alcohol (Farré et al., 1993), but also after 125 mg of MDMA (Camí et al., 2000). Regarding the HRS, our findings are in line with results by other researchers who have demonstrated statistically significant increases in all HRS

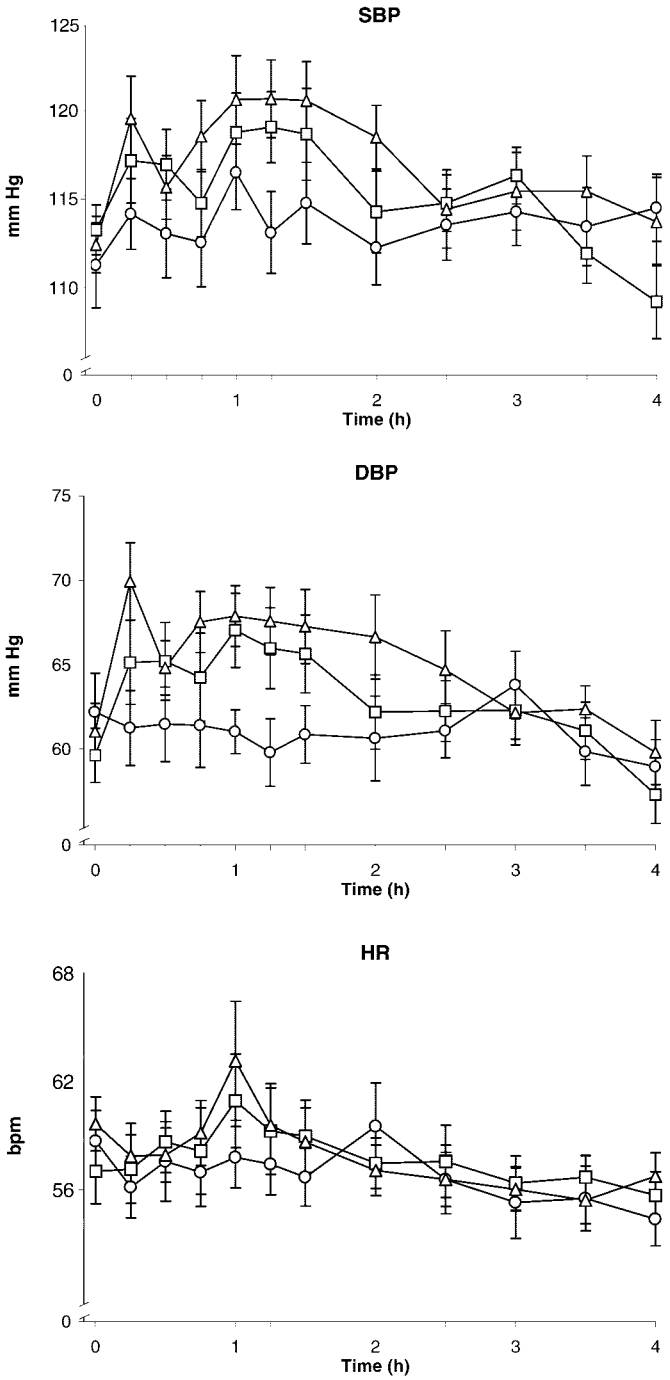

Fig. 3. Time course of cardiovascular measures (means from 18 volunteers) after administration of placebo (circle), 0.6 mg of DMT/kg of body weight ayahuasca (square), and 0.85 mg of DMT/kg of body weight ayahuasca (triangle). Error bars denote ± 1 S.E.M. (n = 18).

scales after the administration of various psychedelics, such as i.v. DMT and oral psilocybin (Strassman et al., 1994; Gouzoulis-Mayfrank et al., 1999). However, ayahuasca differed from these drugs in the time course of effects. The overall duration was longer than that of i.v. DMT, but shorter than that of mescaline or LSD (Strassman, 1994). Finally, regarding the ARCI questionnaire, increases in the ARCI-A, ARCI-BG, and ARCI-MBG scales are a common feature of psychostimulants (Martin et al., 1971; Lamas et al., 1994). However, in contrast, with drugs like amphetamine, methamphetamine, ephedrine, and methylphenidate (Martin et al., 1971), ayahuasca did not induce significant increases in the ARCI-BG scale, a measure of subjectively perceived improvement in intellectual efficiency. The coexistence of drug-induced stimulation with a wide range of modifications in the sensorium places ayahuasca among the psychedelics, a drug class which shares arousing properties with psychostimulants (Brawley and Duffield, 1972).

The present results on the subjective effects induced by ayahuasca in a clinical research setting replicate those obtained in a preliminary study involving a smaller sample of volunteers with prior experience with ayahuasca, and with a single-blind nonrandomized design (Riba et al., 2001b). In the previous study, statistically significant increases were observed in all HRS items, except volition, and in the ARCI-MBG, ARCI-LSD, and ARCI-A scales. In the present study, however, scores on these 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. Several factors such as sample size, study design, and prior exposure to ayahuasca could account for these differences. Scores on the HRS items at the present low dose were also lower than those reported by Grob et al. (1996), except for the somaesthesia and perception items, after the administration of an equivalent ayahuasca dose, in terms of DMT content, to a group of experienced long-term ritual users. Nevertheless, scores on all HRS items after the present high dose were higher than those reported by these researchers. Compared with i.v. DMT as described by Strassman et al. (1994), ayahuasca evokes effects of milder intensity, which show a slower onset and a longer overall duration. Scorings on the six HRS scales after the present high dose fell between those reported after 0.1 and 0.2 mg/kg i.v. DMT.

In our previous study on ayahuasca (Riba et al., 2001b), we failed to observe statistically significant modifications of cardiovascular parameters in a five-subject sample. In the present work, only modifications in DBP reached statistical significance. Increases in DBP, SBP, and HR were milder than those reported for other more prototypical sympathomimetics, such as amphetamine or MDMA, at doses showing psychotropic properties (Mas et al., 1999; de la Torre et al., 2000). DBP increases from baseline values after both ayahuasca doses were somewhat lower than the elevations from baseline values reported by Callaway et al. (1999) after an ayahuasca dose containing 0.48 mg of DMT/kg but larger amounts of β-carbolines.

The time course of DMT plasma concentrations closely paralleled that of subjective effects. The steep rise in DMT plasma levels observed at 1 h coincided with an analogous rise in VAS scores, and peak DMT concentrations and peak effects were obtained between 1.5 and 2 h. In the present study, quantifiable plasma levels were observed for DMT and THH. T_{max} values for DMT and THH were similar to those reported by Callaway et al. (1999). However, C_{max} values for DMT and THH in the present study were lower than expected, even after taking into account the smaller amounts administered in the case of THH. This could be due to a lower alkaloid bioavailability from the lyophilizate compared with the aqueous solution administered by Callaway et al. (1999). The calculated V_z/F values are similar in both studies, but Callaway et al. (1999) reported higher $t_{1/2}$ and lower CL/F values. In the case of DMT, these differences may be associ-

TABLE 3

Urinary excretion of monoamine metabolites pooled from 0 to 24 h after placebo, 0.6 mg, and 0.85 mg of DMT/kg of body weight ayahuasca

Figures indicate mean values (95% confidence interval), expressed in micromoles, from 15 volunteers.

Metabolite	ANOVA		Placebo	Tukey's Multiple Comparison test		
				Placebo		Low Dose/High
	F	*p* Value		Low Dose	High Dose	
VMA	0.61	0.552	20.21 (15.58–32.91)	22.54 (15.11–33.41)	23.31 (14.76–33.30)	
HVA	0.17	0.843	30.32 (23.22–49.14)	32.73 (20.83–46.90)	34.36 (19.44–45.72)	
5-HIAA	1.21	0.313	35.73 (27.11–57.53)	37.30 (28.64–60.55)	43.07 (34.25–71.63)	
MN	1.94	0.163	0.52 (0.41–0.87)	0.56 (0.46–0.96)	0.62 (0.51–1.06)	
NMN	12.56	<0.001	1.06 (0.86–1.79)	1.18 (1.03–2.09)	1.40** (1.22–2.48)	*

MN, metanephrine; NMN, normetanephrine.

* $p < 0.05$; ** $p < 0.01$.

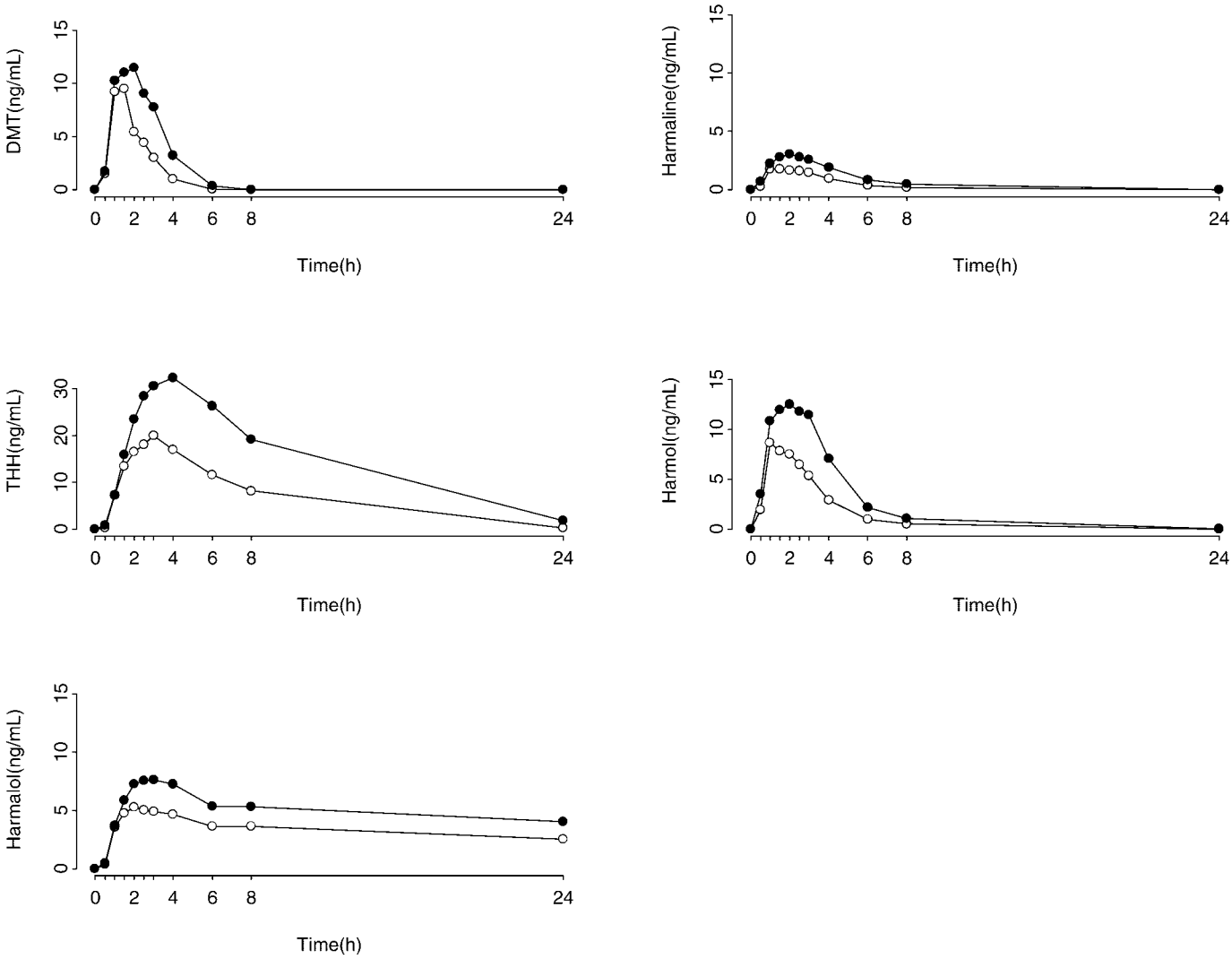

Fig. 4. Plasma concentration-time curves ($n = 15$) for three of the four main alkaloids present in ayahuasca (DMT, harmaline, and THH) and the *O*-demethylated analogs of harmine (harmol) and harmaline (harmalol). Open circles, low 0.6 mg of DMT/kg dose of ayahuasca; filled circles, high 0.85 mg DMT/kg dose of ayahuasca.

ated with the lower levels of harmala alkaloids present in our ayahuasca and the consequent lower degree of MAO inhibition. In addition to these interstudy differences, it is interesting to note that the normalized AUC calculated for DMT in the present study showed a statistically significant increase between the low and the high ayahuasca doses. This is suggestive of a nonlinear increment of DMT levels following the administration of increasing doses of ayahuasca. Considering that both V_z/F and CL/F decreased in a similar proportion between doses, these data could be interpreted as indicating a greater DMT bioavailability following the high dose, probably related to the higher amounts of harmala alkaloids ingested, leading to more effective MAO inhibition.

Another relevant difference from the study by Callaway et al. (1999) is the lack of measurable concentrations of harmine in plasma and the presence of significant levels of harmol and

TABLE 4

Pharmacokinetic parameters for DMT, harmaline, THH, harmol, and harmalol calculated for each of the two administered ayahuasca doses

Values indicate mean (S.D.), except for T_{max}, where median (range) is given. Fifteen volunteers were included in the analysis except for harmalol, where parameters were calculated from 14 volunteers.

	C_{max}	T_{max}	AUC_{0-8h}	$AUC_{0-\infty}$	$AUC_{0-\infty}/D$	$t_{1/2\lambda z}$	CL/F	V_z/F
	ng/ml	*h*	*ng/ml · h*$^{-1}$	*ng/ml · h*$^{-1}$		*h*	*l/h*	*liters*
Low Dose								
DMT	12.14* (9.09)	1.5* (1–2.5)	18.84* (10.67)	21.55* (9.93)	0.0005* (0.0003)	1.07 (0.58)	2281.41 (1054.7)	3509.86* (2158.08)
Harmaline	2.48* (1.28)	1.5 (1–3)	7.02* (4.02)	8.13* (4.39)	0.0017 (0.0009)	2.01 (0.56)	745.76 (379.68)	2040.75* (1044.47)
THH	23.06* (11.45)	2.5* (1.5–3)	100.83* (58.20)	172.07* (123.75)	0.0030 (0.0021)	4.78 (3.45)	559.84 (408.74)	3069.87 (2551.81)
Harmol	10.95* (6.04)	1.5 (1–2.5)	27.08* (12.51)	28.33* (12.78)		1.64 (0.29)		
Harmalol	6.74* (3.52)	2.5* (1–4)	31.14* (15.91)	206.93 (165.97)		30.33 (20.53)		
High Dose								
DMT	17.44 (10.49)	1.5 (1–4)	33.17 (14.68)	38.33 (17.53)	0.0007 (0.0003)	1.06 (0.77)	1812.65 (803.66)	2505.97 (1529.11)
Harmaline	4.32 (2.43)	2 (1–4)	12.80 (5.75)	14.87 (7.34)	0.0023 (0.0012)	1.95 (0.81)	596.78 (370.42)	1439.23 (567.18)
THH	39.40 (20.63)	3 (1.5–6)	180.89 (106.51)	351.89 (255.44)	0.0046 (0.0034)	4.68 (1.52)	364.94 (291.34)	2072.70 (1044.60)
Harmol	17.57 (7.72)	2 (1–3)	49.97 (16.88)	52.27 (17.30)		1.49 (0.28)		
Harmalol	9.59 (4.17)	2.75 (1.5–4)	46.79 (20.60)	333.54 (304.94)		48.64 (77.09)		

* $p < 0.05$.

harmalol. Differences in ayahuasca harmine content alone cannot entirely explain the absence of this alkaloid in plasma, considering that THH was present in the lyophilizate in amounts similar to those of harmine and was later measurable in plasma. Thus, harmine was either not absorbed in the gastrointestinal tract or was extensively degraded by first-pass metabolism before reaching systemic circulation. The presence of harmol in plasma would support the second hypothesis. Harmol glucuronide and harmol sulfate have been described as the main urine metabolites of harmine following its i.v. administration in humans (Slotkin et al., 1970). A very recent study has found cytochrome P450 to catalyze the *O*-demethylation of harmine and harmaline, and has identified CYP2D6 and CYP1A1 as the major isoenzymes involved in the process (Yu et al., 2003). Nevertheless, we cannot conclude that harmine was completely metabolized to render harmol, because very small amounts of harmol and harmalol have been detected in *B. caapi* and ayahuasca (Rivier and Lindgren, 1972; McKenna et al., 1984). Thus, it cannot be entirely ruled out that at least part of the amounts found in plasma could have been ingested with the tea.

The low plasma levels found for harmine in the present study could explain the absence of a clear-cut MAO inhibitor effect on the urinary excretion of monoamine metabolites. The acute administration of a MAO-A inhibitor induces a decrease in the levels of oxidized deaminated monoamine metabolites and an increase in the levels of COMT-dependent methylated compounds (Pletscher, 1966; Koulu et al., 1989). Whereas in the present study normetanephrine, a methylated breakdown product of norepinephrine, showed statistically significant increases after dosing with ayahuasca, the levels of the deaminated metabolites measured, i.e., VMA, HVA, and 5-HIAA, did not show decreases but, rather, were nonsignificantly increased. It is thus unclear whether the observed neurotransmitter metabolite profile was secondary to MAO inhibition. An alternative explanation would be an increase in norepinephrine release induced by DMT, which would fit well the observed sympathomimetic properties of this compound. However, this assumption is not supported by the limited available evidence from related compounds. Results obtained in two studies involving LSD administration to humans found no drug effects on monoamine metabolite excretion (Hollister and Moore, 1967; Messiha and Grof, 1973), and to our knowledge, no data are available on the effects of parenteral DMT on these measures. In any case, MAO inhibition by ayahuasca alkaloids effectively facilitated the access of DMT to systemic circulation but may have been insufficiently potent or insufficiently prolonged to modify the profile of deaminated monoamine metabolites in the 8-h urine collection periods used.

To conclude, the present findings indicate that following ayahuasca administration to humans, measurable DMT plasma levels are obtained together with distinct psychedelic effects. Psychoactivity is attained with negligible levels of circulating harmine. These results and the lack of a clear-cut systemic MAO inhibitor effect are suggestive of a harmine-DMT interaction predominantly taking place in the gastrointestinal tract and possibly in the liver. Harmine effects at a peripheral level would appear to suffice to prevent first-pass metabolism of DMT and allow its access to the CNS in amounts able to evoke psychotropic effects.

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Topographic pharmaco-EEG mapping of the effects of the South American psychoactive beverage Ayahuasca in healthy volunteers.

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Topographic pharmaco-EEG mapping of the effects of the South American psychoactive beverage *ayahuasca* in healthy volunteers

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Aims *Ayahuasca* is a traditional South American psychoactive beverage used in Amazonian shamanism, and in the religious ceremonies of Brazilian-based syncretic religious groups with followers in the US and several European countries. This tea contains measurable amounts of the psychotropic indole *N*,*N*-dimethyltryptamine (DMT), and β-carboline alkaloids with MAO-inhibiting properties. In a previous report we described a profile of stimulant and psychedelic effects for *ayahuasca* as measured by subjective report self-assessment instruments. In the present study the cerebral bioavailability and time-course of effects of *ayahuasca* were assessed in humans by means of topographic quantitative-electroencephalography (q-EEG), a noninvasive method measuring drug-induced variations in brain electrical activity.

Methods Two doses (one low and one high) of encapsulated freeze-dried *ayahuasca*, equivalent to 0.6 and 0.85 mg DMT kg^{-1} body weight, were administered to 18 healthy volunteers with previous experience in psychedelic drug use in a double-blind crossover placebo-controlled clinical trial. Nineteen-lead recordings were undertaken from baseline to 8 h after administration. Subjective effects were measured by means of the Hallucinogen Rating Scale (HRS).

Results *Ayahuasca* induced a pattern of psychoactive effects which resulted in significant dose-dependent increases in all subscales of the HRS, and in significant and dose-dependent modifications of brain electrical activity. Absolute power decreased in all frequency bands, most prominently in the theta band. Mean absolute power decreases (95% CI) at a representative lead (P3) 90 min after the high dose were -20.20 ± 15.23 μV^2 and -2.70 ± 2.21 μV^2 for total power and theta power, respectively. Relative power decreased in the delta (-1.20 ± 1.31% after 120 min at P3) and theta (-3.30 ± 2.59% after 120 min at P3) bands, and increased in the beta band, most prominently in the faster beta-3 (1.00 ± 0.88% after 90 min at P3) and beta-4 (0.30 ± 0.24% after 90 min at P3) subbands. Finally, an increase was also seen for the centroid of the total activity and its deviation. EEG modifications began as early as 15–30 min, reached a peak between 45 and 120 min and decreased thereafter to return to baseline levels at 4–6 h after administration.

Conclusions The central effects of *ayahuasca* could be objectively measured by means of q-EEG, showing a time pattern which closely paralleled that of previously reported subjective effects. The modifications seen for the individual q-EEG variables were in line with those previously described for other serotonergic psychedelics and share some features with the profile of effects shown by pro-serotonergic and pro-dopaminergic drugs. The q-EEG profile supports the role of 5-HT_2 and dopamine D_2-receptor agonism in mediating the effects of *ayahuasca* on the central nervous system.

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Introduction

Ayahuasca is the Quechuan name for both the Amazon woody vine *Banisteriopsis caapi* (Malpighiaceae) and the sacred psychoactive beverage obtained from it. The beverage, also known by the names *Yajé*, *Natema*, *Santo Daime* and *Vegetal*, has been used throughout the Amazon Basin by shamans and healers since pre-Columbian times for medicinal purposes and as a means to contact the supernatural [1, 2]. More recently, syncretic religions combining the use of *ayahuasca* with Christian beliefs, particularly the *Santo Daime* and the *União do Vegetal*, have been established in Brazil, where they enjoy legal protection. Outside Brazil, smaller groups of followers have begun to consume the tea in the United States and in several European countries, including Germany, Great Britain, Holland, France and Spain [3]. Even though the number of users is still relatively small, adverse reactions associated with the simultaneous use of *ayahuasca* and other centrally active drugs have raised concern for public health [4], and extensive clinical data on its somatic, psychological and neurophysiological effects are warranted.

Banisteriopsis caapi, the basic ingredient of the beverage, is seldom found alone in *ayahuasca*. The tea is generally obtained by infusing the stems of the vine together with the leaves of other plants, namely *Psychotria viridis* (Rubiaceae) or *Diplopterys cabrerana* (Malpighiaceae) [5]. Chemical analyses have shown that *B. caapi* contains notable amounts of β-carboline alkaloids, mainly harmine and tetrahydroharmine (THH), followed by harmaline and trace amounts of harmol [5, 6]. *P. viridis* and *D. cabrerana* also contain indole alkaloids, mainly the potent short-acting psychedelic agent *N*,*N*-dimethyltryptamine (DMT) [5, 7].

This combination of *B. caapi* and *P. viridis* in a single oral preparation is a remarkable achievement of empirical ethnopharmacological knowledge, as psychoactivity arises from combining the pharmacodynamic actions of the β-carbolines and of DMT. Similarly to other indole and phenethylamine psychedelics such as LSD and mescaline [8], DMT shows affinity for the 5-$HT_{2A/2C}$ receptor sites in the central nervous system (CNS), where it displays agonist activity [9]. However, unlike most psychedelics, DMT is *a priori* only active when parenterally administered, because the oral ingestion of the drug alone leads to its metabolic breakdown by the enzyme monoamine oxidase (MAO) [10]. Interestingly, harmine and harmaline, and to a lesser extent THH, are potent MAO inhibitors [6]. Thus, it is widely accepted that the MAO-inhibiting action of the β-carbolines present in the tea allows the viable access of DMT to the systemic circulation and the CNS. In addition to facilitating a direct agonist action of DMT at the 5-$HT_{2A/2C}$ sites, the MAO-inhibiting properties of the β-carbolines may contribute to the overall effects of *ayahuasca*, firstly, by prolonging the effects of DMT due to its decreased metabolism, and secondly, by simultaneously enhancing the levels of endogenous catecholamines and serotonin [11].

In a previous study conducted to characterize the tolerability and psychological effect profile of *ayahuasca* [12], this tea was found to induce a pattern of psychostimulant and psychedelic effects, which qualitatively resembled those of other classical serotonergic agents, such as psilocybin, and parenteral DMT [13, 14]. *Ayahuasca* was able to induce dose-dependent perceptual, cognitive and affective modifications, with a milder intensity and longer duration than those previously described for intravenous DMT [14], but with an overall duration shorter than that of better characterized psychedelics such as LSD or mescaline [15].

The aim of the present study was to assess the central actions of *ayahuasca* by means of quantitative-electroencephalography (q-EEG), an objective noninvasive method used to evaluate drug effects on the CNS with high temporal resolution [16]. We intended thus to demonstrate its cerebral bioavailability and subsequent psychoactivity by means other than subjective self-report instruments, and implementing a double-blind randomised placebo-controlled design. Recordings of brain electrical activity were carried out before and at different time points after the administration of two different doses of encapsulated freeze-dried *ayahuasca* to a group of healthy volunteers with previous experience in the use of psychedelics.

Methods

Volunteers

Eighteen healthy volunteers (15 males and three females) with no current or previous history of neurological or psychiatric disorder and no family history of Axis-I psychiatric disorder in first degree relatives were included in the study. Eligibility criteria included prior experience with psychedelic drugs at least on five occasions without sequelae derived therefrom. The volunteers were given a structured psychiatric interview (DSM-III-R) and completed the trait-anxiety scale from the State-Trait Anxiety Inventory [17]. Exclusion criteria included a present or past history of Axis-I disorders and alcohol or other substance dependence, and high scores on trait anxiety. Volunteers were given a complete physical examination that included medical history, laboratory tests, ECG and urinalysis. All volunteers gave their written informed consent to participate. Mean age was 25.7 years (range: 19–38), mean weight 66.47 kg (range: 50.7–79.5) and

mean height 175.11 cm (range: 158–188). In addition to their prior intake of psychedelics, all volunteers had previous experience with cannabis and cocaine. Although prior exposure to *ayahuasca* was not required for participation, two of the volunteers had ingested this tea before inclusion. 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.

Drug

The *ayahuasca* doses administered to the volunteers in the present study as the low and the high dose were the equivalent to 0.6 and 0.85 mg DMT kg^{-1} body weight. These doses were chosen based on tolerability and subjective effect data gathered in a previous study [12]. The *ayahuasca* was not administered in its original liquid form, but as a liophilizate. The DMT contents in the liophilizate had been determined by h.p.l.c., as described by Callaway and coworkers [18], and the β-carboline constituents following a modification of the method described therein. The concentrations found were: 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline and 11.36 mg THH per gram of freeze-dried material. These alkaloid contents corresponded to the following concentrations in the original tea: DMT 0.53 mg ml^{-1}, harmine 0.90 mg ml^{-1}, harmaline 0.06 mg ml^{-1} and THH 0.72 mg ml^{-1}. The calculated individual dose for each volunteer was administered by combining 00 gelatin capsules containing 0.5 g, 0.25 g or 0.125 g of freeze-dried *ayahuasca* and placebo capsules containing 0.75 g lactose. Placebo capsules were added when necessary, so that all volunteers took the same number of capsules on each experimental day.

Study design and experimental procedure

The volunteers participated in four experimental sessions. Volunteers were informed that they would randomly receive on each experimental day a single oral dose of encapsulated freeze-dried *ayahuasca* (one low and one high dose), a placebo and a random repetition of one of the three mentioned treatments. In actual fact they all received a placebo on the first experimental day in a single-blind fashion, followed by one of the three treatments from days 2 to 4 in a double-blind balanced fashion, according to a randomization table. The first nonrandomized placebo was administered in order to familiarize the volunteers with the experimental setting and to minimize the stress associated with the experimental interventions. Two weeks prior to the beginning of the experimental sessions, volunteers were requested to abstain from any medication or illicit drug until the completion of the study. Volunteers also abstained from alcohol, tobacco and caffeinated drinks 24 h prior to each experimental day. Urinalysis for illicit drug use was performed for each experimental session and was found negative for amphetamines, cocaine, opioids, benzodiazepines and alcohol. A 7 day washout period was established between experimental days.

On each experimental day participants arrived in the laboratory in the morning under fasting conditions. EEG electrodes were placed on the scalp and treatment capsules were administered at approximately 10.00 h with 250 ml tap water. EEG recordings were obtained at baseline and at regular intervals after treatment administration. The experimental sessions were undertaken in a quiet and dimly lit room with the volunteers seated in a reclining chair. The experimenter remained outside the room during the EEG recordings. At 4 h after administration of the capsules, when the most prominent subjective effects associated with the drug had disappeared, the volunteers answered subjective effect questionnaires, and had a meal. The last recording was performed at 8 h and volunteers were discharged approximately 9 h after drug administration.

Measurements

EEG acquisition and analysis

EEG recordings were obtained through 19 electrodes placed on the scalp according to the international 10/20 system on the following locations: Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1 and O2, referenced to averaged mastoids by means of a Neuroscan SYNAMPS amplifier. Additionally, vertical and horizontal electrooculograms (EOG) were recorded. Vigilance controlled EEG (V-EEG) for 3 min with eyes closed was recorded at −15 (PRE-1), baseline (PRE-2), +15, +30, +45, +60, +90, +120, +150, +180, +210, +240, +360 and +480 min from drug administration. During the V-EEG recordings, the experimenter tried to keep the volunteers alert; as soon as drowsiness patterns appeared in the EEG they were aroused by acoustic stimulation. The EEG signal was recorded using high-pass and low-pass filters of 0.3 Hz and 30 Hz, respectively, and digitized online with a sampling frequency of 100 Hz.

A two-step artefact processing procedure was used. It included ocular artifact minimization based on regression analysis in the time domain, as described by Semlitsch *et al.* [19], and automatic artifact rejection based on a time and frequency domain approach as described by Anderer *et al.* [20]. Subsequently, validity of the artifact processing procedure was visually inspected [21].

After recomputation to average reference, spectral analysis was performed for artefact-free 5 s epochs, resulting in a frequency resolution of 0.2 Hz. The spectral density curves for all artifact-free EEG epochs were averaged for a particular experimental situation. These mean spectral curves, containing data from 1.3 to 30 Hz, were quantified into 34 target variables: total power, absolute and relative power in 11 different frequency bands (delta [1.3–3.5 Hz], theta [3.5–7.5 Hz], alpha-1 [7.5–10.5 Hz], alpha-2 [10.5–13 Hz], beta-1 [13–16 Hz], beta-2 [16–20 Hz], beta-3 [20–25 Hz], beta-4 [25–30 Hz], combined delta-theta, alpha and beta), the dominant frequency in Hz, absolute and relative power of the dominant frequency, the centre-of-gravity frequency (centroids) and the frequency variability (centroid deviations) of the combined delta-theta, alpha and beta bands as well as of the total activity. Additionally, the vigilance alpha/delta-theta index was also calculated.

Topographic maps were computed by cubic interpolation of the values of the four nearest electrodes.

Subjective ratings

Volunteers were requested to answer the Hallucinogen Rating Scale (HRS), a self-report questionnaire specifically developed to quantify different aspects of psychedelic-induced subjective effects. The questionnaire includes six subscales: *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 [14]. In the present study a Spanish adaptation of the questionnaire was used [22].

Statistical analysis

EEG recordings

Statistical analysis of EEG recordings was performed following the IPEG (International Pharmaco-EEG Group) guideline on statistical design and analysis of pharmacodynamic trials [23]. Accordingly, the inferential strategy of descriptive data analysis (DDA) [24], as proposed for application to the mapping situation [25], was applied. In short, descriptive tests, preferably of simple null hypotheses such as equality of two treatment effects, are performed at all observation times, locations and measurements (variables). A nominal α-level for each test is chosen at 5%, and all P values lower than 0.05 are clearly distinguished in the graphical demonstration of the results. Therefore, the formal P value is calculated for each test, leading to certain pattern of P values in the whole data structure, of which the 'small' P values are indicative of areas of potentially true drug-effect-differences. Rather than considering these P values (should they be smaller than α) as a decision criterion for rejecting local null hypotheses (a procedure which would not be indicated in the absence of an α-correction measure), in DDA these patterns of small P values are analysed in a descriptive way in order to interpret results. This interpretation should be done not just by looking at the P values alone but by simultaneously taking into account the biomedical expectations based on the structure of the study. Therefore, the calculated P values and their pharmacologically sound patterns are used as 'judgement criteria'. Statistics included multivariate methods such as Hotelling T^2 to test overall differences between drugs, and paired t-tests to evaluate changes and interdrug differences in detail at different hours post-administration. According to the experimental design used, pharmacologically sound patterns of P values <0.05 would be those showing: (a) spatial clustering (b) time courses, and (c) dose dependencies. These results were displayed as significance probability maps. Additionally, dose/treatment-effect and time-effect relationships were explored by means of a multivariate, nonparametric approach [20]. Friedman tests and multiple Wilcoxon tests based on sign-adjusted changes in 28 V-EEG variables were applied. In all tests performed (parametric and nonparametric) PRE-2-values were considered as the predrug baseline, and comparisons were conducted with the randomized placebo.

Subjective ratings

HRS scores were analysed by means of a one-way analysis of variance (ANOVA) with repeated measures, with treatment (randomized placebo, *ayahuasca* low dose, *ayahuasca* high dose) as factor. Greenhouse-Geisser epsilon was used to correct possible violations of the sphericity assumption and to reduce Type I errors. Differences were considered statistically significant for $P<0.05$. When ANOVA showed significant differences between treatments, pairwise comparisons were carried out by means of t-tests, followed by Bonferroni correction.

Results

EEG recordings

(1) Pharmaco-EEG maps: multivariate analysis

In order to test the hypothesis that *ayahuasca* exerts significant central effects which induce modifications in brain electrical activity as compared with placebo, a multiple analysis of variance (MANOVA) with repeated measures was performed for V-EEG for each of the 19 electrodes. Treatment (randomized placebo, *ayahuasca*),

time (PRE-2, post) and the following set of variables: log-transformed absolute power values in the delta, theta, alpha-1, alpha-2, beta-1, beta-2, beta-3 and beta-4 frequency bands were considered in the MANOVA. Hotelling T^2 values were used in the significance probability maps to indicate differences between *ayahuasca*-induced and placebo-induced changes in brain electrical activity from baseline through 8 h after drug administration.

As shown in Figure 1, *ayahuasca* administration induced dose-dependent central effects as measured by the derived EEG variables, which were greater and longer lasting after the high dose. Thus, after the low 0.6 mg DMT kg^{-1} body weight dose, statistically significant differences with placebo were obtained only at isolated electrode locations between 45 min and 2.5 h postadministration. After the high 0.85 mg DMT kg^{-1} body weight dose, however, EEG changes were found over extensive scalp areas. 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. At the peak of the pharmacodynamic effects, variations in brain electrical activity were measured all over the scalp, with the greatest intensity in the central and right temporo-occipital electrodes.

(2) Pharmaco-EEG maps: univariate analysis

Topographic brain maps based on *t*-tests are described to show detailed drug-induced changes in the individual EEG variables.

Total power As shown in Figure 2, *ayahuasca* produced a significant and dose-dependent reduction in total power in electrodes located all over the scalp, with a temporal peak at 90 min after administration of the high dose. Both the centroid of the total activity and its deviation showed significant and dose-dependent increases peaking at 120 and 90 min, respectively.

Slow activity The effects of *ayahuasca* on slow activity are shown in Figure 3. Absolute power of the combined delta-theta activity was decreased in a dose-dependent manner after dosing with *ayahuasca*, with the peak

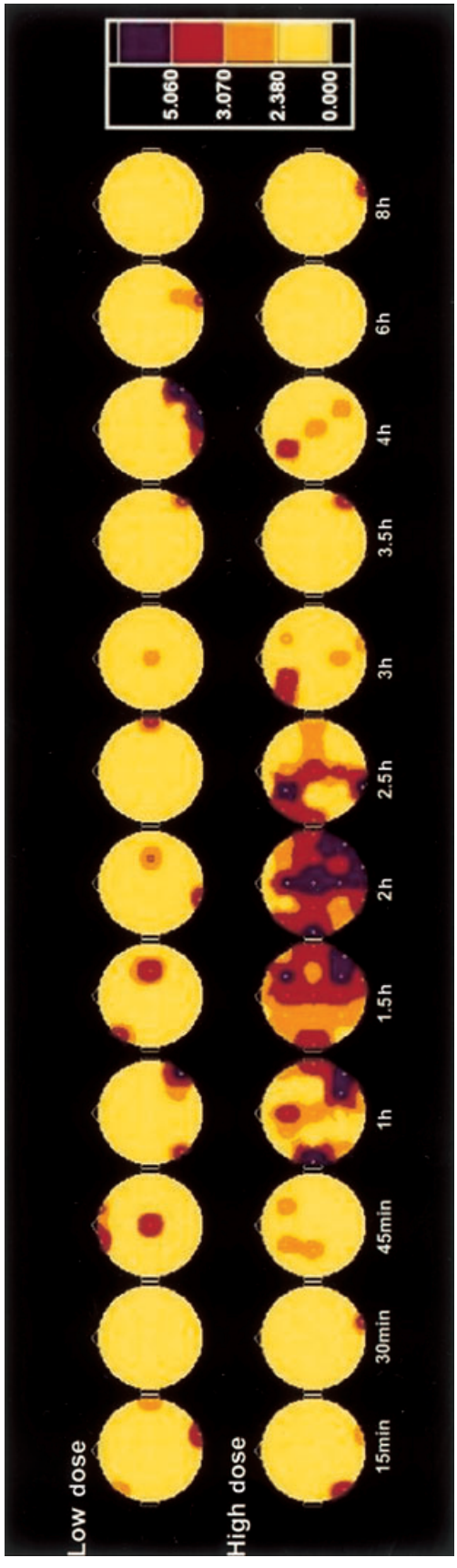
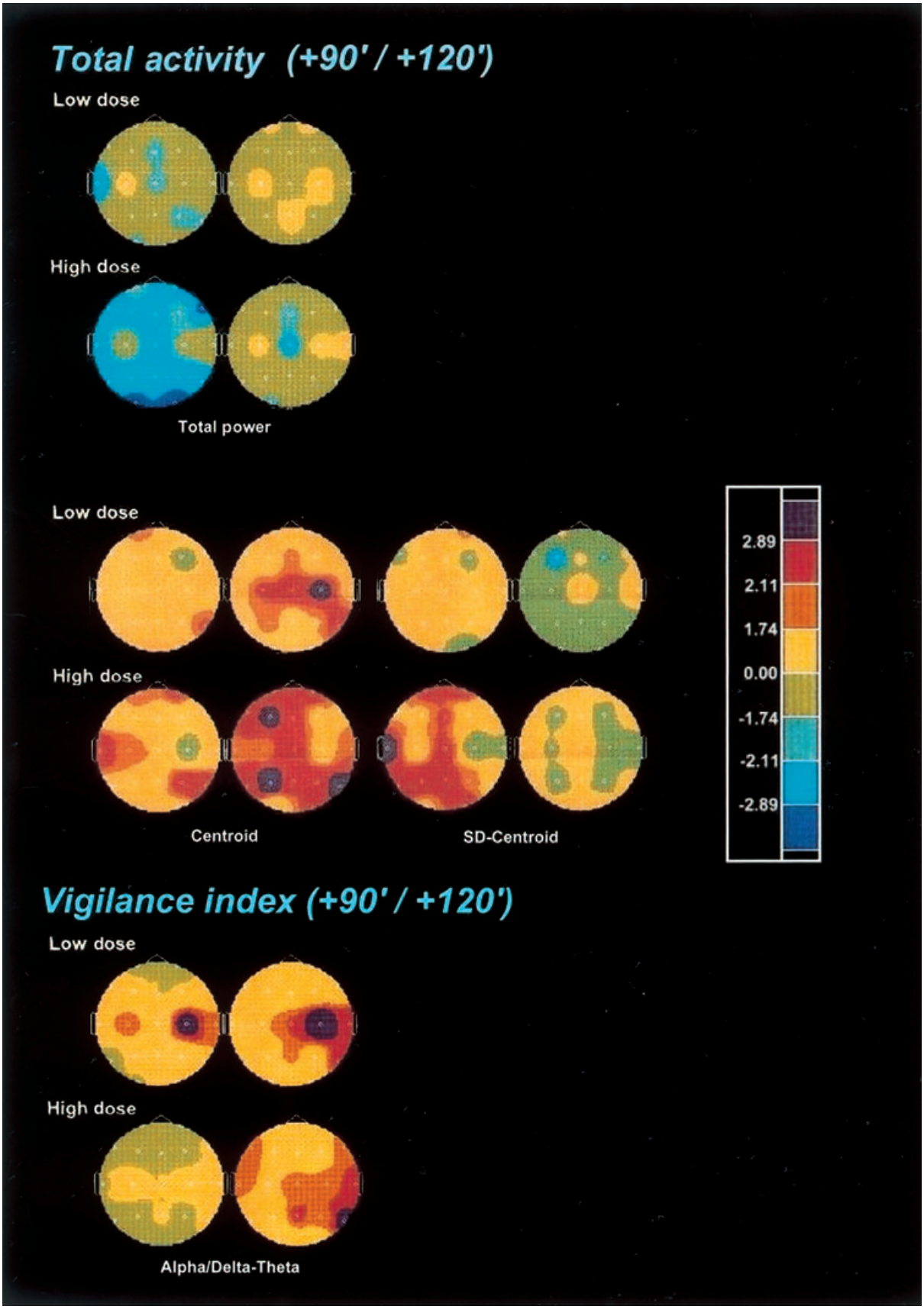


Figure 1 Significance probability maps showing differences between *ayahuasca*-induced and placebo-induced central effects at 12 time points *vs* baseline values (PRE-2) after low (upper row) and high (lower row) doses of *ayahuasca* ($n=18$). The vertex view shows the nose at the top, the left ear to the left, the right ear to the right. Electrode positions are indicated by white dots. Maps are based on Hotelling T^2 obtained from multivariate tests in repeated measures ANOVAs on eight logarithmically transformed absolute power values in delta, theta, alpha-1, alpha-2, beta-1, beta-2, beta-3 and beta-4 frequency bands. The colour key shows T^2 values with hot/red colours indicating significant differences: $T^2>2.38=P<0.10$, $>3.07=P<0.05$ and $>5.06=P<0.01$.



decreases at 90 min for the low dose and between 90 and 120 min for the high dose. When examined separately, both the delta and theta frequency bands showed decreases in absolute power. However, the most dramatic decreases were found in the theta band, an effect which showed a dose-dependent pattern and peaked between 90 and 120 min.

Relative power of the combined delta-theta bands was also dose-dependently decreased, with the peak reductions at 120 min. Decreases in relative power were marginal for the delta band, while they were prominent and dose-dependent for the theta band. These reductions in relative power were maximal at 120 min, showing a widespread distribution all over the scalp.

The centroid of the combined delta-theta activity showed a significant though modest deceleration, with a significant increase of its deviation. Nevertheless, although dose-dependent, the deceleration of the centroid was not uniformly distributed over the scalp, showing the greatest decreases at C3, T4 and O1 at the high *ayahuasca* dose at 90 min after administration. At the high dose, the significant increase seen for the deviation of the centroid was obtained at 120 min and was restricted to the Pz and P3 leads.

Alpha activity The effects of *ayahuasca* on alpha activity are shown in Figure 4. Absolute alpha activity was significantly and dose-dependently decreased after *ayahuasca*. The decreases were more prominent at the high dose in the left-temporal and centro-parieto-occipital electrodes. The maximal decrease was observed at 90 min after administration. When separately examined, the alpha-2 band showed more significant and more widely distributed decreases than the alpha-1 band. Differently from the maximal total alpha and alpha-1 power decreases, the reductions in absolute power for the alpha-2 band peaked at 60 min after administration (not shown).

Relative alpha activity was significantly increased at 120 min after administration, showing an inverse dose-reponse pattern, with maximal increase after the low dose. While this increase was consistently observed in the alpha-1 sub-band, in the alpha-2 sub-band a decrease which reached the highest significance at 60 min after the intake was seen (not shown).

No consistent pattern of changes was observed after *ayahuasca* in the dominant frequency within the alpha band (not shown). A tendency towards statistical significance was seen in the absolute power of the dominant frequency (predominantly decreases) which reached significance marginally in some electrode sites between 45 and 120 min after administration of the high dose. Conversely, relative power of the dominant frequency did show statistically significant increases after the low and the high *ayahuasca* doses at 120 min after administration. Finally, no consistent drug-induced effects were found either for the centroid of the alpha activity or its deviation.

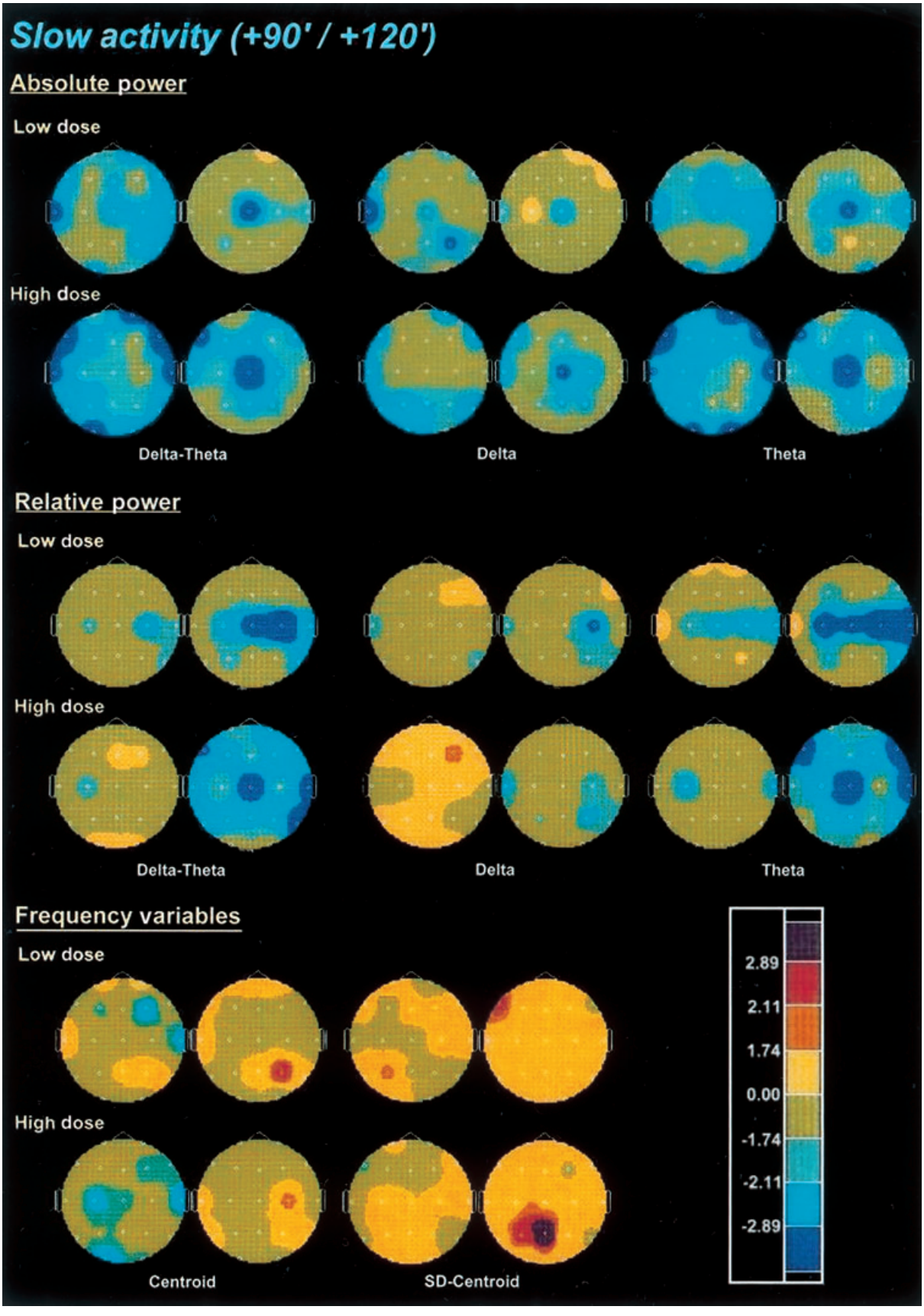
Fast activity The effects of *ayahuasca* on fast activity are shown in Figure 5. The absolute power of global beta activity was dose-dependently decreased by *ayahuasca*, with a maximal decrement at 90 min after administration. When split between the four frequency subbands, absolute power decreases were found to be more intense in the beta-1 range, with power decreases becoming less prominent as one moved to beta-2, beta-3 and beta-4. Peak decreases were observed at 90 min after administration, except for beta-3 which were more prominent at 45 min (not shown).

As far as relative power in the beta frequency range is concerned, statistically significant increases were found, these being more intense and longer-lasting at the high relative to the low *ayahuasca* dose. The maximal increments were obtained between 45 and 90 min after administration. Compared with absolute power values, the examination of relative power in the individual beta subbands rendered an inverse pattern of variation. Thus, relative power increases were marginally significant for the beta-1 band, became more widespread over the scalp for beta-2, more significant for beta-3 and were maximal for beta-4. Increases in the relative power of the beta-4 frequencies showed a predominant central and parieto-temporal distribution. Statistical significance for relative power increases for beta-2, beta-3 and beta-4 was obtained between 45 and 120 min after administration, with the maximal increase at 90 min.

The centroid of the beta frequency range showed a statistically significant and dose-dependent shift toward the higher values after *ayahuasca*, which also peaked at 90 min after administration. The deviation of the centroid was not significantly modified by the drug.

Table 1 lists 95% confidence intervals for changes in absolute (μV^2) and relative (%) power in all frequency bands at 90 and 120 min following the administration of

Figure 2 Significance probability maps showing differences between *ayahuasca*-induced and placebo-induced changes in total power and frequency variables of the EEG total activity (1.3–30 Hz), and in the alpha/delta-theta vigilance index, after low (upper rows) and high (lower rows) doses of *ayahuasca* ($n=18$) at 90 min (left) and 120 min (right) after administration *vs* baseline values (PRE-2). The vertex view shows the nose at the top, the left ear to the left, the right ear to the right. Electrode positions are indicated by white dots. Eight-colour scale represents drug-induced changes as compared with placebo based on *t*-values: lilac, increase at $P<0.01$; red, increase at $P<0.05$; ochre, increase at $P<0.10$; pale yellow, trend towards increase; pale green, trend towards decrease; bright green, decrease at $P<0.10$; light blue, decrease at $P<0.05$; dark blue, decrease at $P<0.01$.



the low and high *ayahuasca* doses in a single representative electrode (P3).

Vigilance index: alpha/delta-theta The alpha/delta-theta ratio (Figure 2) was also calculated for each of the recorded time points. This index showed a significant increase, relative to placebo, both after the low and the high *ayahuasca* doses between 90 and 150 min, with the maximal increase at 120 min.

(3) Non-parametric multilead EEG analysis

Dose/treatment-effect relationships were calculated using Friedman and multiple Wilcoxon tests of sign-adjusted changes from PRE-2-values in 28 V-EEG variables obtained in the 19 leads. As shown in Table 2, based on the rank-sums, administered at the low dose *ayahuasca* could only be differentiated from randomised placebo at 45 min and 60 min after dosing. At the high dose, however, statistically significant differences were found from 45 min through 120 min after administration. Pairwise comparisons considering the total rank-sum showed statistically significant differences between randomised placebo and each of the *ayahuasca* doses, and between the low and high *ayahuasca* doses.

Time-effect relationships were calculated using Friedman and multiple Wilcoxon tests for randomised placebo-corrected sign-adjusted changes from PRE-2-values in 28 V-EEG variables obtained in the 19 leads, as shown in Figure 6. After *ayahuasca* administration, changes on EEG variables were seen as early as 15–30 min, followed by a steep increase at 45 min in rank-sum values. At the high dose, *ayahuasca* showed the pharmacodynamic peak between 45 and 90 min, with rank-sum values gradually decreasing thereafter and approaching baseline at 4–6 h after administration. At the low dose, an analogous curve was found, with the pharmacodynamic peak between 45 and 90 min having an analogous subsequent decrease to that of the high dose. Compared to baseline values, at the low dose increases in rank-sum values did not reach statistical significance at any of the time points evaluated. At the high dose, statistically significant differences were found at 45, 60 and 90 min after administration.

Subjective ratings

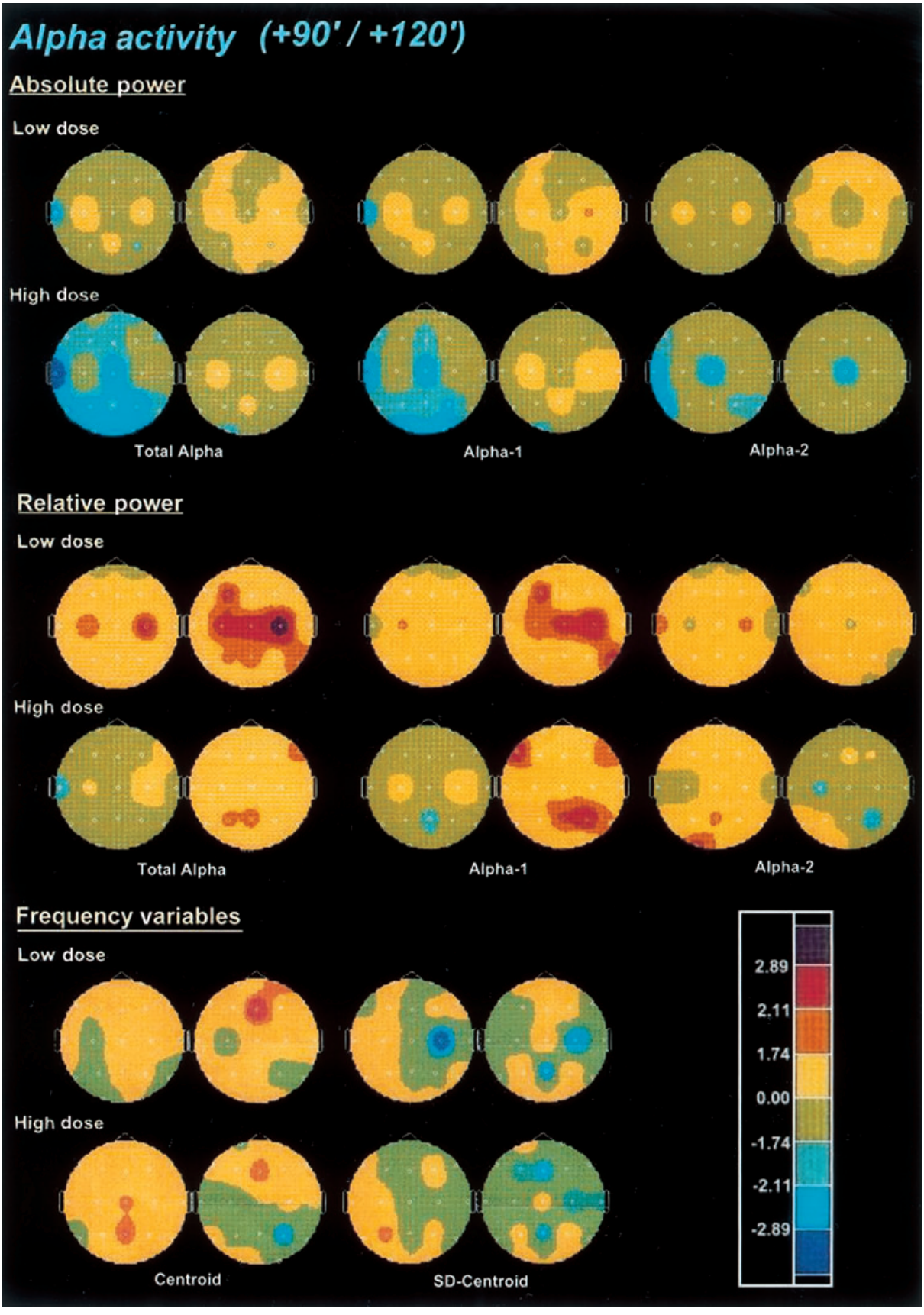
As shown in Table 3, *ayahuasca* induced significant dose-dependent increases in all subscales of the HRS, an instrument specifically designed to quantify the effects of psychedelic drugs. *Ayahuasca* was thus capable of inducing a modified state of awareness in which a psychedelic profile was prominent. At the low dose, all HRS subscales showed statistically significant increases relative to placebo, except for Volition, a measure of impairment in the capacity of the volunteer to interact with his/herself and his/her surroundings. This subscale however, reached statistical significance at the high dose, indicating that of the six aspects measured by the HRS, this was the least modified by *ayahuasca*. Qualitatively, the profile of effects induced by *ayahuasca* included paresthesias and perceptual modifications of predominantly visual, and to a lower extent, auditive nature. This coexisted with more elaborated modifications in thought, associations and emotion, in a global experience described as similar to dreaming activity.

Discussion

The administration of *ayahuasca* to a group of healthy volunteers induced a dose-dependent pattern of subjective effects typical of the psychedelics, replicating the profile obtained in a previous study [12]. In addition to results obtained by means of self-assessment instruments, the implementation of q-EEG demonstrated a significant effect of *ayahuasca*, as compared with placebo, on the human CNS. These effects consisted of an overall decrease in absolute power for all the frequency bands evaluated, and an acceleration of the centre-of-gravity frequency. Absolute power decreases were most prominent in theta, delta and slow beta bands, while the alpha and fast beta rhythms were less intensely affected. Relative power was found to be significantly decreased in the theta, and to a lower extent, delta band. In the alpha band, relative power showed an increase, predominantly in the alpha-1 subband, and significant increases were also obtained in relative power in the beta frequency band. These increases in relative fast activity were most prominent in the beta-3 and beta-4 subbands. Additionally, the alpha/delta-theta ratio, an index of activation, was found to be increased after *ayahuasca*.

The evaluation of the plots of the rank-sums of changes measured at the 19 leads over time showed the first increases between 15 and 30 min, which were followed by a steep rise at 45 min, reaching the maximum effects between 45 and 90 min EEG measures gradually declined thereafter to reach baseline values around 4–6 h after administration. Most remarkably, these objectively measured effects of the drug on the spontaneous brain electrical

Figure 3 Significance probability maps showing differences between *ayahuasca*-induced and placebo-induced changes in absolute power, relative power and frequency variables of the combined slow activity (1.3–7.5 Hz), delta (1.3–3.5 Hz) and theta (3.5–7.5 Hz) frequency bands after low (upper rows) and high (lower rows) doses of *ayahuasca* ($n = 18$), at 90 min (left) and 120 min (right) after administration *vs* baseline values (PRE-2). For technical description of the maps and explanation of the colour key see Figure 2.



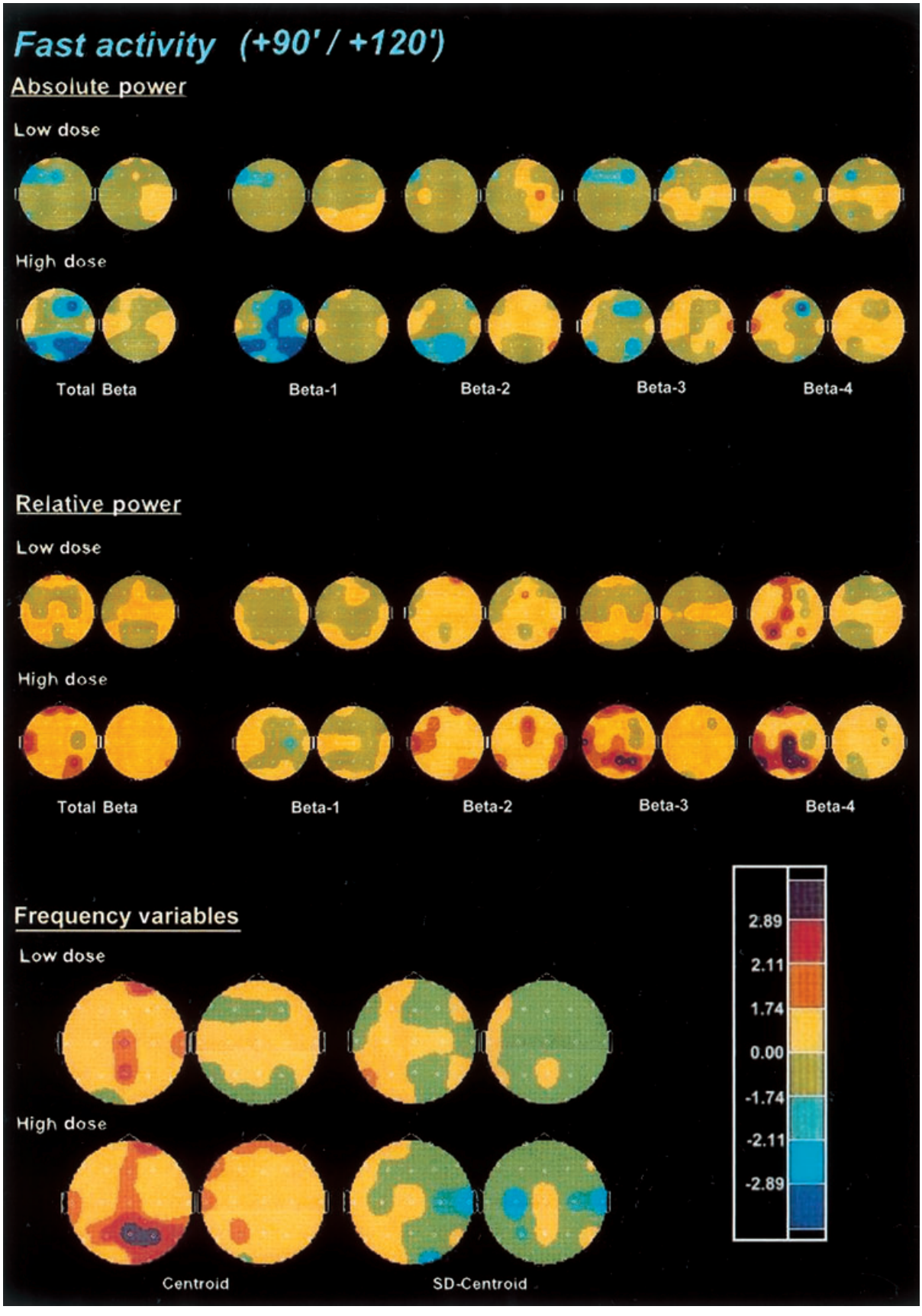
activity closely paralleled the time course of subjectively experienced effects, measured by means of self-report visual analogue scales, as previously reported [12].

To our knowledge, only one previous study has addressed the evaluation of EEG activity in humans after the ingestion of *ayahuasca*. A recent article reported the evaluation of the EEG effects of *ayahuasca* in a group of nine subjects in field conditions [26]. In the cited study, EEG recordings were obtained in the course of a ritual *Daime* session in Brazil. The study was conducted in the absence of a placebo control, and only with an approximate knowledge of the ingested *ayahuasca* dose, this being on average 0.67 mg DMT kg^{-1} body weight. These investigators reported significant changes after *ayahuasca* in relation to baseline values only in the 36–44 Hz band. Given that this frequency range was not evaluated in the present study, it is impossible to establish a comparison with the results obtained in the aforementioned study. Nevertheless, Don *et al.* also reported a pattern of changes in the classical frequency bands which did not reach statistical significance but which bore similarities to that observed in the present study. These nonsignificant variations included a 'slight increase in beta', and a 'slight decrease in theta and alpha'.

The changes in brain electrical activity observed in the present study are difficult to relate entirely to any pharmaco-EEG profile characteristic of the main psychotropic drug groups. Even a direct comparison with other psychedelics is far from easy. Virtually no studies have been conducted in the last 30 years regarding the effects of these drugs on the human EEG. The quantitative approach to the effects of psychedelics on the human EEG was absent at the time they attracted the greatest interest from psychiatry and psychopharmacology researchers in the 1950s and 1960s. Most of the information available from the early research conducted with these compounds is essentially qualitative. In these studies only marginal changes were described after the administration of psilocybin, mescaline or LSD on the visually inspected EEG trace, reporting at most an increase in fast rhythms and an overall decrease in signal amplitude [27]. Itil and coworkers, however, conducted a number of studies combining visual inspection and power spectrum analysis of the recordings obtained after administering anticholinergic compounds with true hallucinogenic properties, such as atropine, and serotonergic psychedelics like LSD. These researchers found almost opposite EEG patterns for these two groups of compounds. While atropine caused the alpha rhythm to disappear and the predominance of low-voltage slow waves, they concluded that the most characteristic effects of LSD were a reduction of theta activity and slow waves in general, as well as an increase in fast activity [27, 28]. In line with these observations, in the present study both absolute and relative power of slow activity decreased after *ayahuasca*, specially in the theta band. With regard to fast activity, while absolute power was decreased following *ayahuasca* administration, a marked enhancing effect was obtained for relative power. The milder increases were found for the slower beta-1 and beta-2 sub-bands and the most intense in the faster beta-3 and beta-4 sub-bands.

Ayahuasca shares the decremental effects seen on delta and theta power with both psychostimulants, such as amphetamine and methylphenidate, and serotonin releasers such as fenfluramine [29, 30]. Interestingly, psychostimulants act predominantly enhancing dopaminergic neurotransmission, in contrast with the serotonergic properties of psychedelics. However, a recent neuroimaging study in humans has shown that dopamine release takes place in the basal ganglia and the ventral striatum after the administration of psilocybin to humans [31], pointing to a role of dopaminergic neurotransmission in the effects of the classical psychedelics. Additional similarities are also to be found between the relative beta-3 and beta-4 band enhancing properties found for *ayahuasca*, and the analogous effect obtained after tricyclic antidepressants, which characterizes the group [29]. Drugs belonging to this pharmacological class inhibit the re-uptake of monoamines, which leads to increased levels of these endogenous compounds in the synapse [32]. (+)-Fenfluramine and the selective serotonin reuptake inhibitor fluoxetine also lead to increases in relative beta power [30, 33], an effect which is also shared by antidepressants showing MAOI properties [34]. It is consequently reasonable to assume that the blocking effects of the β-carbolines on MAO may have led to increased levels of monoamines, due to the blockade of their metabolism, which in turn may have contributed to the relative beta-promoting effect of *ayahuasca*. Regarding slow activity, the theta-dampening activity of psychostimulants and psychedelics is diametrically opposed to the theta-enhancing action of the classical neuroleptics such as haloperidol and chlorpromazine [30, 35]. This theta-enhancing action has also been observed in drugs with a mixed anti-D_2 and anti-5-HT_2 profile, such as risperidone [36], or the more selective 5-HT_2 blocker ketanserin [37], suggesting a

Figure 4 Significance probability maps showing differences between *ayahuasca*-induced and placebo-induced changes in absolute power, relative power and frequency variables of total alpha activity (7.5–13 Hz), alpha-1 (7.5–10.5 Hz), and alpha-2 (10.5–13 Hz) frequency bands after low (upper rows) and high (lower rows) doses of *ayahuasca* ($n = 18$), at 90 min (left) and 120 min (right) after administration *vs* baseline values (PRE-2). For technical description of the maps and explanation of the colour key see Figure 2.



pro-dopaminergic and pro-serotonergic activity for *ayahuasca*.

DMT, the main psychotropic agent in *ayahuasca*, not only binds to the 5-$HT_{2A/2C}$ receptors, located mainly at a postsynaptic level, but also shows affinity for the 5-HT_{1A} sites, which in certain brain regions correspond predominantly to somatodendritic autoreceptors [38]. Thus, DMT probably displays agonist activity also at the 5-HT_{1A} sites, a pattern shared by other indole psychedelics, in contrast with the phenethylamines like mescaline, which interact only with the 5-$HT_{2A/2C}$ receptors [39]. The pharmaco-EEG profile of drugs displaying selective agonist or partial agonist activity at the 5-HT_{1A} site has been described, allowing a more detailed discussion on the probable biochemical mechanisms involved in the EEG effects of *ayahuasca*. Indeed buspirone, a partial 5-HT_{1A} agonist, has been shown to produce marked increases in theta power, in the absence of other relevant EEG modifications [40]. As an opposed pattern was seen for the theta band after *ayahuasca*, one could postulate that 5-HT_{1A} agonism does not seem to be the predominant contribution at a molecular level to the EEG effects of *ayahuasca*. This is consistent with data from a previous study, in which increases in the intensity of the psychological effects elicited by intravenous DMT following blockade of the 5-HT_{1A} sites by pindolol were reported [41]. The observed increases suggest both that agonism at the 5-HT_{1A} site is not essential to obtain a psychedelic effect profile, and that a decreased binding of DMT at the 5-HT_{1A} sites leads to an increase in the amount of DMT available to interact with the 5-HT_2 receptors, and consequently to the enhanced subjective effects experienced by the volunteers. Thus, the present q-EEG findings would rather support a preponderant involvement of the 5-HT_2 receptor in the genesis of the central effects of the beverage.

To sum up, the cerebral bioavailability and psychoactivity of *ayahuasca* could be objectively measured by means of q-EEG, which evidenced a clear dose-dependent effect at the doses administered. Remarkably, the time

Table 1 95% confidence intervals for changes in absolute (μV^2) and relative (%) power in all frequency bands at 90 and 120 min, following the administration of the low 0.6 mg DMT kg^{-1} body weight, and high 0.85 mg DMT kg^{-1} body weight *ayahuasca* doses, in a single representative electrode (P3). All changes *vs* baseline (PRE-2) and randomized placebo. Data from 18 volunteers, showing mean change ±1.96 s.e.mean.

	Low dose		*High dose*	
	90 min	*120 min*	*90 min*	*120 min*
Absolute power (μV^2)				
Total power (1.3–30 Hz)	−5.70±18.62	−5.60±13.72	−20.20±15.23★	−8.30±18.07
Delta (1.3–3.5 Hz)	−1.20±1.57	−1.30±1.82	−1.40±1.10★	−1.70±1.84
Theta (3.5–7.5 Hz)	−1.10±2.70	−1.70±1.45★	−2.70±2.21★	−2.00±2.45
Alpha-1 (7.5–10.5 Hz)	−0.40±7.84	−3.00±8.41	−11.30±11.07★	−1.70±11.11
Alpha-2 (10.5–13 Hz)	−2.00±3.58	0.70±2.74	−2.60±3.65	−2.00±4.90
Beta-1 (13–16 Hz)	−0.30±0.53	0.01±1.96	−0.80±0.49★	−0.40±0.71
Beta-2 (16–20 Hz)	−0.50±0.82	−0.20±0.57	−1.00±0.98★	−0.30±0.84
Beta-3 (20–25 Hz)	−0.20±0.35	0.10±0.65	−0.40±0.53	−0.10±0.49
Beta-4 (25–30 Hz)	0.01±1.96	−0.10±0.12	−0.01±0.10	−0.01±0.06
Relative power (%)				
Delta (1.3–3.5 Hz)	−1.20±3.35	−1.80±2.70	0.50±1.63	−1.20±1.31
Theta (3.5–7.5 Hz)	−1.30±3.65	−3.20±2.98★	−1.40±2.12	−3.30±2.59★
Alpha-1 (7.5–10.5 Hz)	1.70±6.66	3.10±5.06	−2.70±5.88	4.40±5.39
Alpha-2 (10.5–13 Hz)	0.20±3.92	1.90±2.86	2.00±3.57	0.10±1.96
Beta-1 (13–16 Hz)	−0.20±0.65	0.01±1.96	−0.20±0.78	−0.40±0.61
Beta-2 (16–20 Hz)	0.30±0.59	0.10±0.39	0.40±0.57	0.30±0.53
Beta-3 (20–25 Hz)	0.20±0.49	−0.10±0.65	1.00±0.88★	0.20±0.65
Beta-4 (25–30 Hz)	0.20±0.14★	−0.10±0.16	0.30±0.24★	−0.10±0.27

Statistically significant differences *vs* placebo (★$P<0.05$) obtained after Student's *t*-test are indicated.

Figure 5 Significance probability maps showing differences between *ayahuasca*-induced and placebo-induced changes in absolute power, relative power and frequency variables of the combined fast activity (13–30 Hz), beta-1 (13–16 Hz), beta-2 (16–20 Hz), beta-3 (20–25) and beta-4 (25–30) frequency bands after low (upper rows) and high (lower rows) doses of *ayahuasca* ($n=18$), at 90 min (left) and 120 min (right) after administration *vs* baseline values (PRE-2). For technical description of the maps and explanation of the colour key see Figure 2.

Table 2 Dose/treatment-effect relationships after single oral doses of randomized placebo (A), low dose 0.6 mg DMT kg^{-1} body weight *ayahuasca* (B), high dose 0.85 mg DMT kg^{-1} body weight *ayahuasca* (C), and non-randomized placebo administered on the first (adaptation) experimental session (D). Data from 18 volunteers, based on sign-adjusted changes in 28 V-EEG variables (rank-sums, means of 19 electrodes, differences from PRE-2 baseline values).

Time (min)	*Randomized placebo (A)*	*Low dose (B)*	*High dose (C)*	*Adaptation placebo (D)*	χ^2	*Multiple Wilcoxon*
15	71.8	69.3	69.9	69.0	0.1	
30	63.5	76.8	79.9	59.8	6.3	
45	51.4	76.7	92.6	59.4	22.3**	A : B*, A : C**, D : C**
60	53.1	85.6	85.9	55.4	21.5**	A : B**, A : C** D : B**, D : C**
90	55.7	77.8	94.8	51.7	26.3**	A : C** D : B*, D : C**
120	62.0	72.5	90.3	55.2	15.2**	A : C* D : C*
150	62.1	74.8	86.3	56.8	11.3**	A : C(*) D : C*
180	65.9	74.7	74.4	64.9	1.5	
210	75.1	60.8	73.4	70.7	2.7	
240	76.3	62.1	71.4	70.3	2.6	
360	80.6	62.0	75.1	62.4	5.9	
480	70.2	62.7	83.7	63.3	5.9	
Total	787.7	855.8	977.7	738.9	57.7**	A : B*, C** D : B**, C** B : C**

(*) $= P<0.1$; *$P<0.05$; **$P<0.01$.

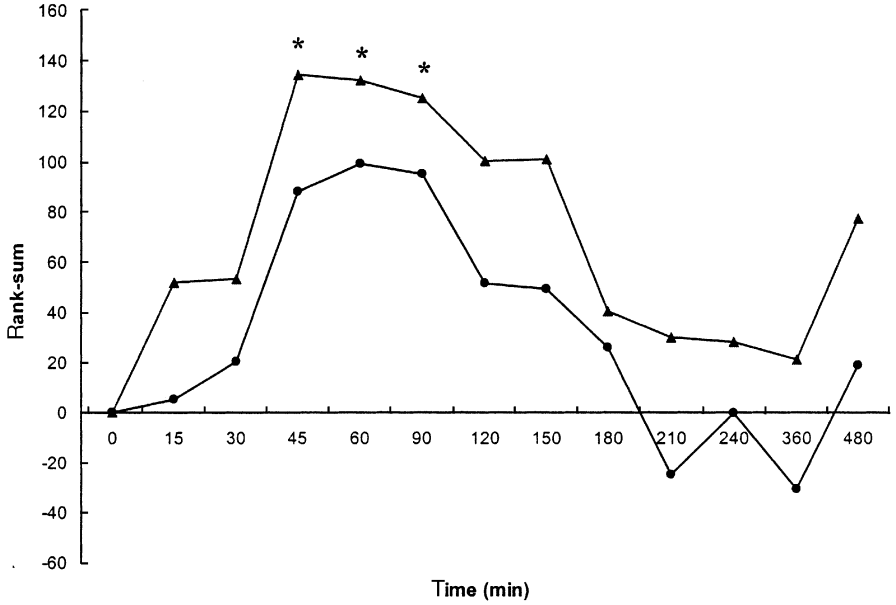


Figure 6 Time-effect relationships after single oral doses of 0.6 mg DMT kg^{-1} body weight *ayahuasca* (low dose) [●], and 0.85 mg DMT kg^{-1} body weight *ayahuasca* (high dose) [▲]. Plots show differences from baseline values (PRE-2) of sign-adjusted changes in 28 V-EEG variables (rank-sums, means of 19 electrodes, randomized placebo-corrected) from 18 volunteers. An asterisk indicates significant differences form baseline values obtained by means of multiple Wilcoxon.

pattern obtained for EEG effects closely paralleled that of previously reported subjective effects. The global reduction in total power and the shift toward higher frequencies after *ayahuasca* are in line with older reports on the classical serotonergic psychedelics, which described an amplitude reduction and a suppresion of slow activity in the human EEG. Finally, the detailed assessment of *ayahuasca* effects on the different EEG variables indicated common features

Table 3 Means (s.d.) of the scores obtained for the HRS questionnaire subscales ($n=18$) after single oral doses of randomized placebo, low dose 0.6 mg DMT kg^{-1} body weight *ayahuasca* and high dose 0.85 mg DMT kg^{-1} body weight *ayahuasca*, and results of the statistical analyses performed. Student's *t*-tests were followed by Bonferroni correction.

	ANOVA		*Student's* t-*test* vs *Placebo*		vs *Low dose*
Variable	P *value*	*Placebo*	*Low dose*	*High dose*	*High dose*
HRS					
Somaesthesia	***	0.07 (0.10)	0.50 (0.41)**	0.97 (0.40)**	**
Perception	***	0.09 (0.19)	0.55 (0.49)**	1.10 (0.67)**	**
Cognition	***	0.06 (0.16)	0.4 (0.45)**	0.96 (0.59)**	**
Volition	*	0.81 (0.79)	1.11(0.69)	1.35 (0.61)*	NS
Affect	***	0.32 (0.21)	0.65 (0.36)**	1.02 (0.38)**	**
Intensity	***	0.24 (0.45)	1.32 (0.73)**	1.85 (0.51)**	**

*$P<0.05$; **$P<0.01$; ***$P<0.001$; NS = not significant.

with the profile shown by pro-dopaminergic and pro-serotonergic drugs, and supports the involvement of serotonergic 5-HT_2 and dopaminergic D_2-receptor agonism in the central effects of *ayahuasca*.

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Effects of Ayahuasca on sensory and sensorimotor gating in humans as measured by P50 suppression and prepulse inhibition of the startle reflex, respectively.

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ORIGINAL INVESTIGATION

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Effects of *ayahuasca* on sensory and sensorimotor gating in humans as measured by P50 suppression and prepulse inhibition of the startle reflex, respectively

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Abstract *Rationale: Ayahuasca*, a South American psychotropic plant tea, combines the psychedelic agent and 5-$HT_{2A/2C}$ agonist *N*,*N*-dimethyltryptamine (DMT) with β-carboline alkaloids showing monoamine oxidase-inhibiting properties. Current human research with psychedelics and entactogens has explored the possibility that drugs displaying agonist activity at the 5-$HT_{2A/2C}$ sites temporally disrupt inhibitory neural mechanisms thought to intervene in the normal filtering of information. Suppression of the P50 auditory evoked potential (AEP) and prepulse inhibition of startle (PPI) are considered operational measures of sensory (P50 suppression) and sensorimotor (PPI) gating. Contrary to findings in lower animals, unexpected increases in sensorimotor gating have been found in humans following the administration of the serotonergic psychedelic psilocybin and the serotonin releaser 3,4-methylenedioxymethamphetamine (MDMA). In addition, to our knowledge P50 suppression has not been assessed previously in humans following the administration of a 5-$HT_{2A/2C}$ agonist. *Objectives:* To assess the effects of the acute administration of *ayahuasca* on P50 suppression and PPI in humans, in order to evaluate the drug's modulatory actions on these measures of sensory and sensorimotor gating. *Methods:* Eighteen healthy volunteers with prior experience of psychedelic drug use participated in a clinical trial in which placebo or *ayahuasca* doses (0.6 mg and 0.85 mg DMT/kg body weight) were administered according to a double-blind, cross-over balanced design. P50 and startle reflex (pulse-alone and 60 ms, 120 ms, 240 ms and 2000 ms prepulse-to-pulse intervals) recordings were undertaken at 1.5 h and 2 h after drug intake, respectively. *Results: Ayahuasca* produced diverging effects on each of the two gating measures evaluated. Whereas significant dose-dependent reductions of P50 suppression were observed after *ayahuasca*, no significant effects were found on the startle response, its habituation rate, or on PPI at any of the prepulse-to-pulse intervals studied. *Conclusion:* The present findings 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.

Keywords *Ayahuasca* · DMT · Psychedelics · Prepulse inhibition of startle · P50 suppression · Sensory gating · Sensorimotor gating · Human

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Introduction

Ayahuasca is a powerful psychotropic plant concoction, which contains the serotonergic psychedelic agent *N*,*N*-dimethyltryptamine (DMT) (Rivier and Lindgren 1972; Schultes and Hofmann 1980). This beverage, which is the shamanic inebriant par excellance in the Upper Amazon River Basin (Schultes and Hofmann 1982; Dobkin de Rios 1984), is obtained by infusing the stems of the woody vine *Banisteriopsis caapi* (malpighiaceae) together with the leaves of *Psychotria viridis* (rubiaceae) or *Diplopterys cabrerana* (malpighiaceae). *Banisteriopsis caapi*'s chief contribution to the infusion is a series of β-carboline alkaloids, namely harmine, tetrahydroharmine and, to a lesser degree, harmaline, while *Psychotria viridis* and *Diplopterys cabrerana* contribute varying amounts of DMT (Rivier and Lindgren 1972; Schultes and Hofmann 1980).

When administered parenterally, DMT is a potent ultra-short-acting psychedelic agent (Strassman et al. 1994), which binds to the 5-$HT_{2A/2C}$ receptor sites in the central nervous system (CNS), where it acts as an agonist

(Pierce and Peroutka 1989; Smith et al. 1998). Interestingly, this compound is entirely inactive after oral ingestion (Ott 1999), probably due to metabolic breakdown by gut and liver monoamine oxidase (MAO) (Suzuki et al. 1981). However, the β-carboline alkaloids present in *ayahuasca* display MAO inhibitory properties (McKenna et al. 1984). By combining both plants in a single oral preparation, the extensive first-pass effect on DMT can be diminished thanks to the reversible inhibition of MAO elicited by the β-carbolines, thus enabling DMT to reach the systemic circulation and the CNS.

Ayahuasca has attracted the interest of biomedical researchers as its use has spread in recent years, reaching the urban areas of South America, Europe, and North America, where it is used in the context of divination, traditional medicine, and syncretic religions (Dobkin de Rios 1996a, 1996b; Anonymous 2000). In previous studies we found that in a clinical setting *ayahuasca* was able to induce dose-dependent perceptual cognitive and affective modifications characteristic of the psychedelics, as measured by self-report, subjective-effect measures (Riba et al. 2001a) and a pattern of changes in spontaneous brain electrical activity analogous to that caused by other drugs displaying agonist activity at the 5-HT_2 and D_2 receptor sites (Riba et al. 2002).

Recently, the disruptive activity of psychedelics on the "gating" of sensory information has been postulated (Vollenweider 1994). This hypothesis is based on the assumption of the existence of brain mechanisms directed at filtering out, under normal conditions, the flow of sensory information reaching consciousness. Decreases in gating had been initially proposed as an underlying deficit common to a number of neuropsychiatric disorders, where a sensory overflow is postulated (Braff et al. 2001). According to this model, serotonergic psychedelics, dopaminergic agonists, and *N*-methyl-D-aspartate (NMDA) antagonists would interact with brain structures involved in the gating mechanisms, temporarily decreasing their functionality and giving rise to the characteristic perceptual and cognitive effects elicited by these agents (Vollenweider 1994).

Two neurophysiological measures have been developed to evaluate the functionality of neural gating mechanisms: suppression of the P50 auditory evoked potential (AEP) and prepulse inhibition of the startle reflex (PPI). The P50 AEP is a midlatency potential appearing about 50 ms after the presentation of an auditory stimulus (Picton et al. 1974). The consecutive administration of two identical stimuli, conditioning (C) and testing (T) stimuli, at a certain inter-stimulus interval, typically 500 ms, leads to a decrease in the amplitude of the second P50 wave (Adler et al. 1982). The amplitude decrement seen for the T stimulus is thought to obey active inhibitory mechanisms triggered by the C stimulus (Freedman et al. 1983). P50 suppression is regarded as a measure of sensory gating, and its neural substrates have been located in the hippocampus, in the mesial temporal lobe (Adler et al. 1998).

The second operational measure, PPI, is based on the inhibitory effect of a weak sensory stimulus (the prepulse) on the motor response caused by a stronger startle reflex-eliciting stimulus. The startle reflex is a brainstem reflex occurring after the presentation of intense and sudden sensory stimuli. PPI is obtained when the startling stimulus is preceded 15–400 ms by the prepulse, and it manifests as a decrease in the intensity of the reflex (Blumenthal 1999). In contrast to P50, PPI is considered a measure of sensorimotor gating, given that the response measured is the motor output to the presented stimulus. While the neural circuit mediating the startle reflex is located in the brainstem, PPI is regulated by descending projections from areas in the forebrain. These areas are interconnected in a complex circuitry combining excitatory and inhibitory synapses (Swerdlow et al. 2001).

Pharmacological challenge studies in humans have shown dopaminergic agents to disrupt PPI and P50 suppression (Adler et al. 1994a; Hutchinson and Swift 1999; Light et al. 1999), while unexpected increases in PPI have been observed after the administration of serotonergic psychedelics/entactogens, such as psilocybin and 3,4-methylenedioxymethamphetamine (MDMA) (Gouzoulis-Mayfrank et al. 1998; Vollenweider et al. 1999). To our knowledge no study has been carried out to date on the influence of serotonergic psychedelics/entactogens on the human P50 suppression paradigm.

The aim of the present study was to evaluate both P50 suppression and PPI in a single group of healthy volunteers after the acute administration of *ayahuasca* and to assess a possible differential drug modulation of these two measures.

Materials and methods

Volunteers

Eighteen healthy volunteers (15 males and 3 females) with no current or previous history of neurological or psychiatric disorder and no family history of axis-I psychiatric disorder in first degree relatives were included in the study. Eligibility criteria included prior experience with psychedelic drugs on at least five occasions without sequelae derived thereof. The volunteers were given a structured psychiatric interview [Diagnostic and Statistical Manual of Mental Disorders (DSM)-III-R] and completed the trait-anxiety scale from the State-Trait Anxiety Inventory (Spielberger et al. 1970). Exclusion criteria included a present or past history of axis-I disorders and alcohol or other substance dependence, and high scores on trait anxiety. Volunteers were given a complete physical examination that included a medical history, laboratory tests, electrocardiogram (ECG), and urinalysis. Mean age was 25.7 years (range 19–38 years), mean weight 66.47 kg (range 50.7–79.5 years) and mean height 175.11 cm (range 158–188 cm). In addition to their prior intake of psychedelics, all volunteers had previous experience with cannabis and cocaine. Although prior exposure specifically to *ayahuasca* was not required for participation, two of the volunteers had ingested the beverage before inclusion in this study. 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 psycho-

logical effects of psychedelics and their possible adverse effects, as reported in the psychiatric literature. All volunteers gave their written informed consent to participate.

Drug

Two *ayahuasca* doses containing 0.6 mg and 0.85 mg DMT/kg body weight were chosen as the low and high doses, respectively, based on tolerability and subjective effects assessed in a previous study (Riba et al. 2001a). The *ayahuasca* was not administered in its original liquid form, but as a liophilizate. The freeze-dried homogenized material was obtained from a 9.6-l batch of *Daime* obtained from Cefluris, a Brazilian-based religious organization related to the *Santo Daime* church. The DMT contents had been determined by means of high-performance liquid chromatography (HPLC), as described by Callaway and coworkers (1996), and the β-carbolines according to a modified version of the method described therein. As reported in a previous paper, the 9.6-l batch yielded 611 g freeze-dried powder, containing 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline, and 11.36 mg THH per gram. These alkaloid contents corresponded to the following concentrations in the original tea: DMT 0.53 mg/ml, harmine 0.90 mg/ml, harmaline 0.06 mg/ml, and THH 0.72 mg/ml (Riba et al. 2001a). The calculated individual dose for each volunteer was administered by combining 00 gelatin capsules containing 0.5, 0.25, or 0.125 g freeze-dried *ayahuasca* and placebo capsules containing 0.75 g lactose. Placebo capsules were added when necessary, so that all volunteers took the same number of capsules on each experimental day.

Study design and experimental procedure

The volunteers participated in four experimental sessions. Volunteers were informed that they would randomly receive on each experimental day a single oral dose of encapsulated freeze-dried *ayahuasca* (one low and one high dose) or placebo and a random repetition of one of the three mentioned treatments. In actual fact, they all received a placebo on the first experimental day in a single-blind fashion, followed by one of the three treatments from day 2 to day 4 in a double-blind balanced fashion, according to a randomization table. The first non-randomized placebo was administered in order to familiarize the volunteers with the experimental setting and to minimize the stress associated with the experimental interventions. The data obtained during the first session was not included in the statistical analysis performed and is not reported. Two weeks prior to the beginning of the experimental sessions, volunteers abstained from any medication or illicit drug and remained drug free throughout the four study weeks. Urinalysis for illicit drug use was made for each experimental session. Additionally, volunteers abstained from alcohol, tobacco, and caffeinated drinks 24 h prior to each experimental day. There was a 7-day washout period between experimental days.

On each experimental day, participants arrived at the laboratory in the morning under fasting conditions, and capsules were administered by approximately 10,00 hours with 250 ml tap water. The P50 and PPI sessions were begun at 1.5 h and 2 h after drug administration, respectively, coinciding with the peak of subjective effects (Riba et al. 2001a). The recordings were undertaken in a quiet room with the volunteers seated in a reclining chair. The experimenter remained in the neighboring room for the entire time of the recordings and monitored volunteers for alertness. Four hours after administration of the capsules, the volunteers answered subjective-effect questionnaires and had a meal. They remained in the research unit throughout the afternoon and were discharged approximately 9 h after administration.

Measurements

P50 elicitation and recording

One hundred and twenty pairs of auditory stimuli were delivered by means of air earphones. Auditory stimuli were 75-dB [A], 1000-Hz pure-tone pips of 4-ms duration, with a 500-ms inter-stimulus separation and a constant interval between pairs of 8 s. No background noise was presented during the session. Electroencephalogram (EEG) recordings were obtained by means of nineteen electrodes placed on the scalp according to the international 10/20 system, plus leads for horizontal and vertical eye-movement monitoring. All scalp electrodes were referenced to the averaged mastoids. Impedance was kept below 5 kΩ. Throughout the entire recording session, volunteers remained with eyes open with sight on a fixation point. High- and low-pass filters were set at 0.1 Hz and 100 Hz, respectively. The digitation rate was 250 Hz. The continuous recordings were epoched at an interval between 100 ms pre-stimulus and 1000 ms post-stimulus and baseline corrected (–100, 0). This was followed by rejection of any trial showing an activity exceeding ±75 μV. All artifact-free epochs were averaged to obtain the average AEP including the first or C stimulus and the second or T stimulus. The obtained averages were re-filtered between 10 Hz and 50 Hz to facilitate P50 identification (Jerger et al. 1992). P50 identification and scoring was carried out on average individual waveforms at Cz as described by Adler et al. (1994b). The C peak was identified as the greatest positivity between 40 ms and 80 ms after stimulus presentation. If more than one peak of equal amplitude was detected, the later one was selected. Peak amplitude was assessed as the difference between this peak and the preceding negative N40 trough. In cases where no N40 could be identified, the P50 amplitude was measured to pre-stimulus baseline (Cardenas et al. 1997). The T peak was identified in the same way, with the further constraint that it had to appear at a latency between ±10 ms of the latency value found to the P50 wave to the C stimulus (Adler et al. 1994b).

Startle reflex elicitation and recording

Startle stimuli were 1-KHz pure tones of 116 dB [A], with a 50-ms duration and an instantaneous rise/fall time. Acoustic stimuli were presented binaurally through air headphones. Prepulses were non-startling 1-KHz pure tones of 80 dB [A] and a 20-ms duration. No background noise was presented during the session. The electromyogram (EMG) signal was recorded bipolarly from the orbicularis oculi muscle by means of two 0.5-cm diameter silver surface disc electrodes, placed 1 cm below and 1 cm medial from the external canthus of the right eye (Fridlund and Carcioppo 1986). Two electrodes placed above and below the left eye were used to control spontaneous and voluntary blinking. The ground electrode was placed on the forehead. Impedance level was maintained below 5 KΩ. Amplifier filters were set at 10 Hz (high pass) and 500 Hz (low pass). The EMG signal was digitized at a 1000-Hz rate.

Each startle sequence was initiated with an acclimation phase comprising five pulse-alone startle stimuli, which were not used later in the calculation of PPI. These were followed by three blocks of trials comprising pulse-alone trials and prepulsed trials at the following prepulse-to-pulse intervals: 60, 120, 240, and 2000 ms. Each block included three pulse-alone trials and three prepulsed trials at each of the four intervals used. Thus, 45+5 startle stimuli were delivered in the course of a startle reflex recording session. The mean inter-trial interval was 20 s (range 10–29 s). Four different sequences of stimuli were used throughout the study, each subject receiving a different sequence on each experimental day. The order of the sequences was varied according to a randomization table and was counterbalanced across subjects. The order of presentation of each trial type was pseudo-random and varied across blocks and across sequences.

The recorded EMG signal was full-wave rectified off-line and smoothed using a five-point moving average filter. Peak eye-blink amplitude was defined as the highest point in the EMG response

within a time window of 120 ms after stimulus administration. Baseline EMG was computed as the mean EMG in the 30-ms preceding stimulus onset. Reactivity was defined as blink magnitude in the pulse-alone trials. Trials in which the apparent response had an onset latency of less than 20 ms after stimulus administration and/or a rise time greater than 95 ms were rejected. In those trials in which no response was detected, amplitude was scored as 0 μV. Epochs were screened and rejected if artifacts were present.

Subjective ratings

Volunteers were requested to answer two questionnaires measuring psychedelic-induced subjective effects. The first questionnaire was the Hallucinogen Rating Scale (HRS) (Strassman et al. 1994). The HRS includes six subscales: *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 *intensity*, which reflects the strength of the overall experience. In the present study, a Spanish version of the questionnaire was used (Riba et al. 2001b).

The second questionnaire administered was a Spanish version of the Altered States of Consciousness Questionnaire ("Ausser-gewöhnliche Psychische Zustände", APZ) developed by Dittrich (1998). It includes 72 items distributed in three subscales: *oceanic boundlessness* ("Ozeanische Selbstentgrenzung", OSE), measuring changes in the sense of time, derealization and depersonalization phenomena subjectively experienced as positive; *dread of ego-dissolution* ("Angstvolle IchAuflösung", AIA), measuring thought disorder and decreased body and thought control associated with arousal and anxiety; and *visionary restructuralization* ("Visionäre Umstrukturierung", VUS), referring to visual phenomena, such as illusions, hallucinations and synesthesia and to changes in the significance of objects. This instrument has been extensively used in studies involving the administration of psychedelics to humans. Volunteers were requested to answer the HRS and the APZ 4 h after drug intake.

Statistical analysis

P50 auditory evoked potential

Three measures related to response amplitude were derived from average waveforms at Cz for each subject and drug condition: P50 AEP amplitude values after the C and T stimuli, difference amplitude calculated as C–T, and finally percentage suppression calculated as [1–(T/C)]×100. Latency to peak after the C stimulus was also assessed. Amplitude values for the C stimulus were analyzed by means of a repeated-measures one-way analysis of variance (ANOVA) with drug as factor, in order to test for drug actions on the amplitude of the C trial. A repeated-measures, two-way ANOVA was subsequently performed, with drug and stimulus type (C vs T) as factors on amplitude values. Finally, repeated-measures, one-way ANOVAs with drug as factor were performed on difference amplitude, percentage suppression, and latency to peak values.

Startle reflex measures

Blink magnitude values were obtained from the recordings and averaged for each trial type (i.e., nine trials for each of the five trial types: pulse-alone, 60 ms prepulse-to-pulse indicated as PP60, 120 ms prepulse-to-pulse indicated as PP120, 240 ms prepulse-to-pulse indicated as PP240 and 2000 ms prepulse-to-pulse indicated as PP2000). The following variables were calculated: reactivity (magnitude of the startle response in the pulse-alone trials), magnitude of the startle response in the prepulsed trials (PP60, PP120, PP240, and PP2000), percentage PPI (PP60, PP120, PP240, PP2000), and percentage habituation. Percentage PPI for each prepulse condition was calculated as follows: [1–(prepulsed trial magnitude/pulse-alone magnitude)]×100. Percentage habituation was calculated as the difference of the averaged magnitude of pulse-alone trials in the first block minus the averaged magnitude of pulse-alone trials in the third block divided by magnitude in the first block and multiplied by 100 (i.e., %Hab = [(first block–third block)/first block]×100).

Reactivity was analyzed by means of a repeated-measures, two-way ANOVA with drug and block as factors. Percentage habituation was analyzed by means of a repeated-measures, one-way ANOVA with drug as factor. Magnitude of the startle response in the prepulsed conditions was analyzed by means of a repeated-measures, two-way ANOVA with drug and prepulse condition as factors. Finally, PPI data were subjected also to a repeated-measures, two-way ANOVA with drug and prepulse condition as factors.

Subjective reports

Scores on HRS and APZ subscales were analyzed by means of a one-way, ANOVA with repeated measures, with drug as factor. In all ANOVAs performed, Greenhouse-Geisser epsilon was used to correct possible violations of the sphericity assumption and to reduce type-I errors. *P* values after correction are shown. When ANOVA showed statistically significant differences between drug conditions, pair-wise comparisons were carried out by means of *t*-tests. Results were considered statistically significant for $P<0.05$.

Correlations

The Pearson's *r* was used to evaluate correlations between drug-induced changes in neurophysiological measures and in subjective-effect scores, and also between drug-induced changes in PPI and in P50 measures.

Results

Usable recordings of both PPI and P50 in all three experimental sessions for a given volunteer were obtained for 15 of the total 18 volunteers enrolled in the study. The results presented below were obtained from analysis of data corresponding to this subgroup of 15 volunteers (13 males and 2 females).

P50 auditory evoked potential

Figure 1 shows grand average AEP waveforms at the Cz site after the C and T stimuli for the three drug conditions. Figure 2 presents mean P50 amplitude values for C and T, difference amplitude values (C–T), and percentage suppression [1–(T/C)]×100, under the three drug conditions. Amplitude values of the P50 response after the C stimulus showed a decrease with dose, which did not reach statistical significance in the ANOVA ($F_{2,28}=2.57$, $P=0.10$, $\varepsilon=0.906$). Mean P50 amplitude (μV)±SEM for the C stimulus under the three drug conditions was 2.93±0.42 for placebo, 2.56±0.28 for the low dose, and 2.05±0.22 for the high dose. The two-way ANOVA with drug and stimulus type (C vs T) as factors showed the following results: whereas no significant main effect of

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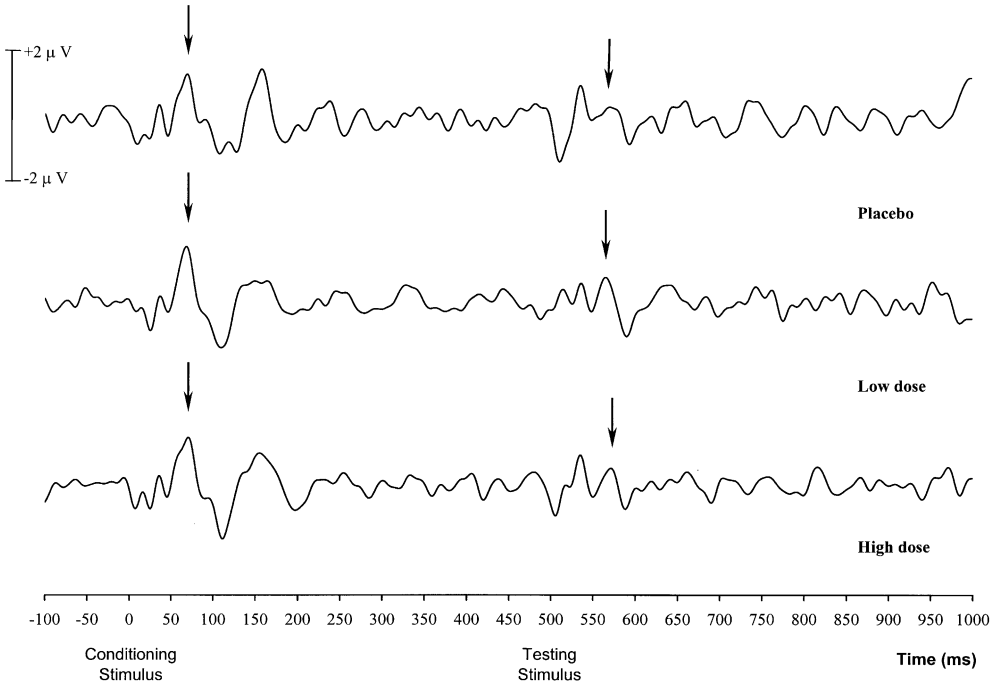

Fig. 1 Grand average band-pass filtered (10–50 Hz) auditory evoked potential (AEP) waveforms at the Cz site under the three drug conditions (n=15). The P50 component after the conditioning and testing stimuli are indicated with *arrowheads*

drug was seen on the overall amplitude of the P50 response ($F_{2,28}$=0.80), significant effects of stimulus type ($F_{1,14}$=38.49, P<0.001; linear contrast $F_{1,14}$=38.49, P<0.001; mean amplitude ±SEM: 2.53±0.21 μV for the C stimulus, 1.36±0.13 μV for the T stimulus), and the interaction drug × stimulus type ($F_{2,28}$=4.96, P<0.05, ε=0.856; linear contrast $F_{1,14}$=6.70, P<0.05) were obtained. An analogous significant effect was obtained for the difference amplitude variable (C–T), pointing out that *ayahuasca* reduced the P50 amplitude response difference to the C and T stimuli ($F_{2,28}$=4.96, P<0.05, ε=0.856; linear contrast $F_{1,14}$=6.70, P<0.05; mean difference amplitude ±SEM under the three drug conditions: 2.12±0.42 μV for placebo, 0.93±0.34 μV for the low dose, and 0.52±0.31 μV for the high dose). Pair-wise comparisons showed statistically significant differences from placebo both at the low (t_{14}=2.29, P<0.05) and the high (t_{14}=2.59, P<0.05) *ayahuasca* doses for difference amplitudes. A significant drug effect on percentage suppression was observed after *ayahuasca* ($F_{2,28}$=4.78, P<0.05, ε=0.844; linear contrast $F_{1,14}$=7.93, P<0.05; mean percentage suppression ±SEM under the three drug conditions: 71.86±8.41 for placebo, 24.57±17.17 for the low dose, and 6.00±18.10 for the high dose). Pair-wise comparisons showed statistically significant differences from placebo both at the low (t_{14}=2.83, P<0.05) and the high (t_{14}=2.82, P<0.05) *ayahuasca* doses for percentage suppression.

Finally, latency to peak of the P50 wave after the C stimulus decreased non-significantly after *ayahuasca* ($F_{2,28}$=2.76, P<0.1, ε=0.844; mean latency to peak ±SEM under the three drug conditions was 70.13± 1.91 ms for placebo, 68.53±1.17 ms for the low dose, and 65.20±2.14 ms for the high dose).

Startle reflex measures

Startle reactivity under the three drug conditions was analyzed by means of a two-way ANOVA with drug (placebo, *ayahuasca* low dose, *ayahuasca* high dose) and block of trials (first, middle and last block of the recording session) as factors. Figure 3, upper panel, shows pulse-alone startle magnitude values for each block of trials under the three drug conditions. A robust decrease of startle magnitude was observed as the recording session progressed, as evidenced by a significant effect of block ($F_{2,28}$=12.91, P<0.01, ε=0.687; linear contrast $F_{1,14}$=15.98, P<0.01; mean magnitude ±SEM for the first block was 104.96±19.63 μV, second block 66.97±13.39 μV, and third block 50.16±11.33 μV) in the ANOVA. Although mean magnitude values increased after the *ayahuasca* high dose, no significant effect of drug was seen in the ANOVA ($F_{2,28}$=1.97; mean magnitude ±SEM was 68.13±17.58 μV for placebo, 59.62±11.27 μV for the low dose, and 94.35±21.61 μV for the high dose). Finally, no significant drug × block interaction was observed ($F_{4,56}$=0.86). Similarly, a one-way ANOVA with drug as factor revealed no significant effect in percentage habituation ($F_{2,28}$=0.49; percentage habituation ±SEM was 41.74±13.25 for placebo, 37.64±11.51 for the low dose, and 36.65±46.06 for the high dose).

The effects of *ayahuasca* on global startle magnitude in the pulse-alone trials and in the prepulsed trials at the different prepulse-to-pulse intervals are shown in Fig. 3, lower panel. A two-way ANOVA with drug and prepulse condition as factors revealed a main effect of prepulse condition ($F_{3,42}$=15.02, P<0.001, ε=0.509; linear contrast $F_{1,14}$=18.95, P<0.01; mean magnitude ±SEM at the

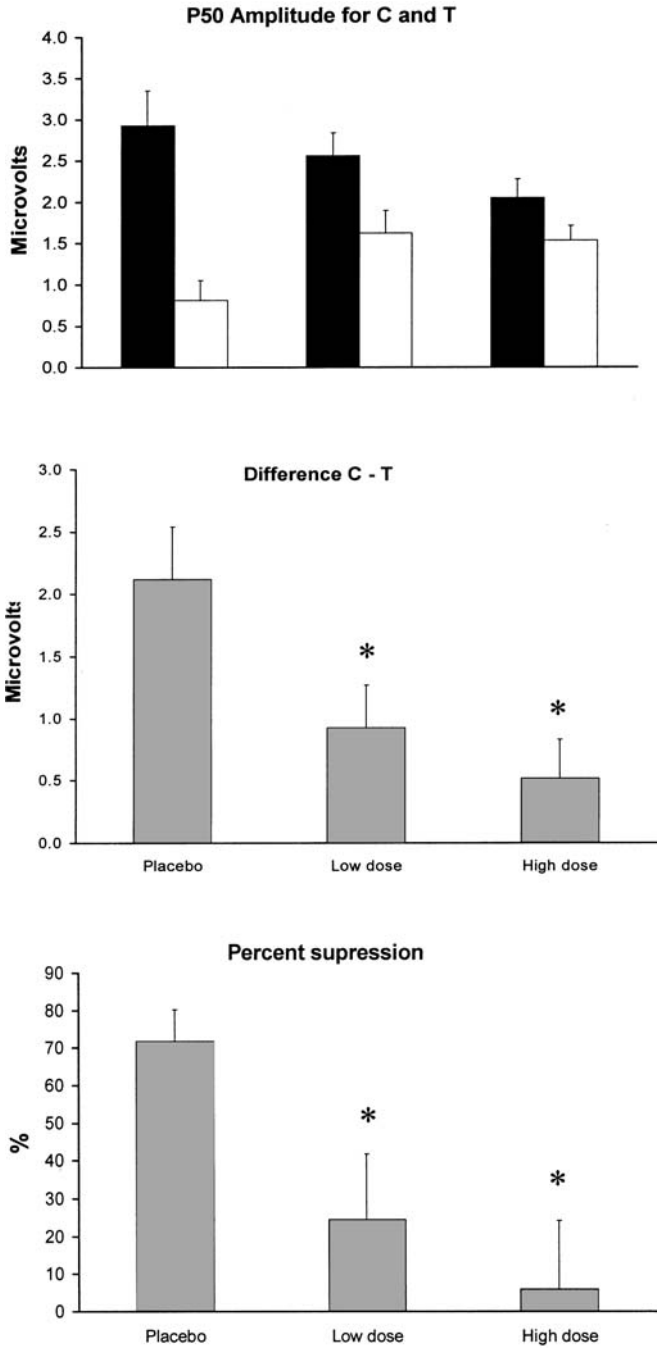

Fig. 2 *Upper panel* P50 amplitude to the conditioning (*closed square*) and testing (*open square*) stimuli under the three drug conditions. *Middle panel* Difference (conditioning–testing) of P50 amplitude values under the three drug conditions. *Lower panel* Percentage suppression values under the three drug conditions. In all three panels, *error bars* denote 1 SEM, and an *asterisk* indicates $P<0.05$ relative to placebo ($n=15$)

different prepulse-to-pulse intervals was: 74.03±13.79 μV pulse-alone, 21.14±3.80 μV PP60, 26.64±5.92 μV PP120, 48.65±11.45 μV PP240, and 80.79±16.96 μV PP2000). No significant effects of drug ($F_{2,28}=1.19$) or drug × prepulse condition ($F_{6,84}=0.65$) were observed.

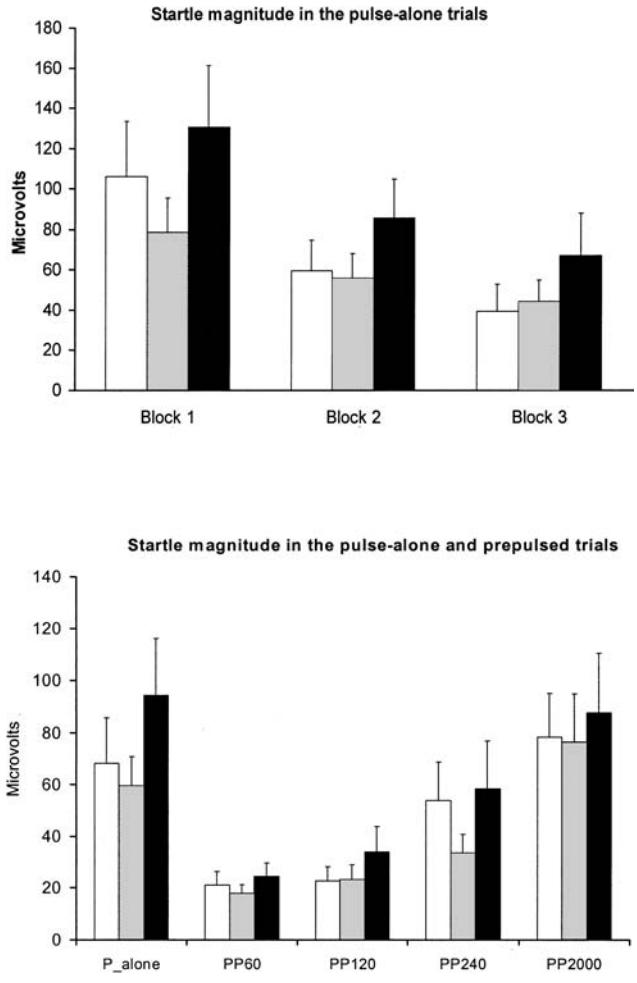

Fig. 3 *Upper panel* Mean startle magnitude values in the pulse-alone trials in each of the three blocks of trials comprising a recording session, after each of the three drug conditions. A main effect of block was found in the ANOVA ($F_{2,28}=12.91$, $P<0.01$), while no effects of drug or drug × block were observed. *Lower panel* Mean startle magnitude values after the pulse-alone and at each of the four prepulse-to-pulse intervals after each of the three drug conditions. In both panels (*open square*) placebo, (*shaded*) low dose, (*closed square*) high dose. *Error bars* denote 1 SEM ($n=15$). A main effect of prepulse condition was found in the ANOVA ($F_{3,42}=11.85$, $P<0.001$), while no effects of drug or drug × prepulse condition were observed

Figure 4 shows percentage inhibition (expressed as percentage facilitation for PP2000) values at the different prepulse-to-pulse intervals under the three drug conditions. A two-way ANOVA with drug and prepulse condition as factors revealed a main effect of prepulse condition ($F_{3,42}=11.85$, $P<0.001$, $\varepsilon=0.565$; linear contrast $F_{1,14}=36.35$, $P<0.001$; percentage inhibition in the four prepulse-to-pulse intervals ±SEM was: 59.16±5.93 PP60, 56.46±7.27 PP120, 21.13±20.87 PP240, and –19.89± 12.65 PP2000). No significant effect was seen for factor drug ($F_{2,28}=2.88$, $P<0.1$, $\varepsilon=0.938$; linear contrast $F_{1,14}=4.89$, $P<0.05$; percentage inhibition ±SEM across the four prepulse-to-pulse intervals for each drug condition was: 16.07±14.15 for placebo, 32.71±8.57 for the low dose,

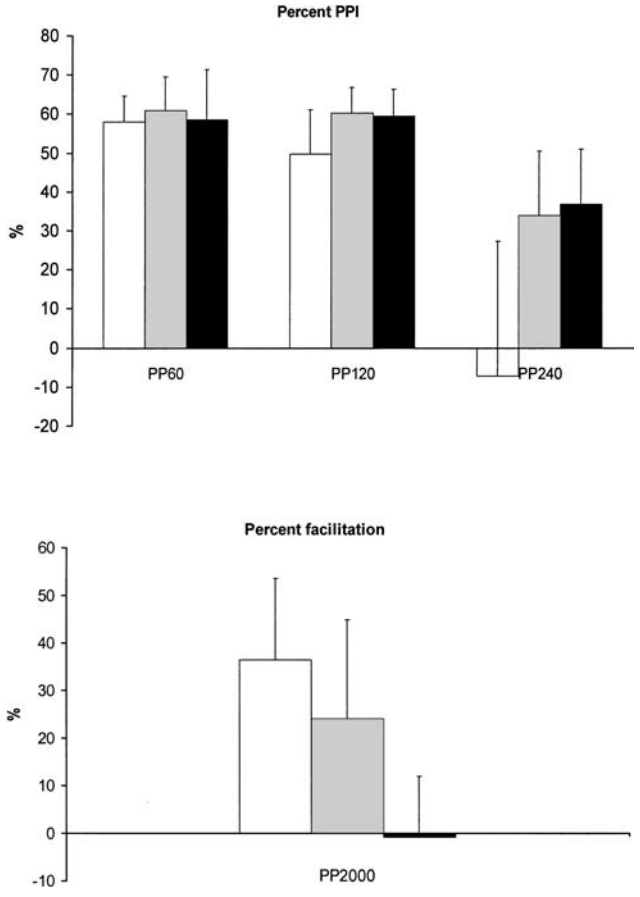

Fig. 4 *Upper panel* Mean values of percentage inhibition of the startle response at the 60, 120 and 240-ms prepulse-to-pulse intervals. *Lower panel* Mean values of percentage facilitation of the startle response at the 2000-ms prepulse-to-pulse interval. In both panels, (*open square*) placebo, (*shaded*) low dose, (*closed square*) high dose. *Error bars* denote 1 SEM (n=15). No effects of drug or drug × prepulse condition were observed

and 38.86±8.66 for the high dose). Finally, the interaction drug × prepulse condition was not found to be significant ($F_{6,84}$=1.42).

Subjective effects

The administration of the selected *ayahuasca* doses to a group of healthy volunteers with experience in the use of psychedelics induced a pattern of subjective effects that was reflected as increases in the scores of the HRS and APZ subscales, as shown in Table 1.

All HRS and APZ subscales showed statistically significant increases relative to placebo after *ayahuasca* administration, except for *volition*. The characteristic psychedelic pattern of effects reported by the volunteers had an overall duration of 4–6 h, reaching its maximum intensity between 90 min and 120 min. The most frequently reported perceptual effects were in the somatosensory and visual modalities. Somatosensory effects comprised altered bodily sensations, such as pins and needles, and increased skin sensitivity. Visual perception was characteristically modified, volunteers experiencing distortions of the visual field with eyes open, and more or less elaborate visions with eyes closed. Auditive phenomena were also present and consisted typically of alterations in external sounds, with true auditory hallucinations being less frequently reported. This modified state of awareness was also accompanied by changes in the cognitive sphere, with increased thought speed and associations, a reduction in the capacity to focus attention, and changes in mood, usually consisting of feelings of happiness and excitation. At the doses administered, *ayahuasca* did not induce full-blown psychotic symptoms and none of the participants lost insight into the

Table 1 Means (±SD) of the scores obtained for the Hallucinogen Rating Scale (HRS) and Spanish version of the Altered States of Consciousness (APZ) questionnaire subscales (n=15), and results of the statistical analysis performed. Student's t-tests were followed by Bonferroni correction. *ns* not significant

Variable	ANOVA		Student's t- test		
	P value	Placebo	vs Placebo		vs Low dose High dose
			Low dose	High dose	
HRS					
Somaesthesia	***	0.08±0.10	0.42±0.40*	0.93±0.36**	**
Perception	***	0.11±0.20	0.57±0.52**	1.11±0.68**	**
Cognition	***	0.07±0.18	0.44±0.48*	1.01±0.63**	**
Volition	(*)	0.93±0.81	1.23±0.68 ns	1.38±0.57 ns	ns
Affect	***	0.35±0.21	0.60±0.36*	1.02±0.38**	*
Intensity	***	0.22±0.44	1.27±0.79**	1.80±0.53**	**
APZ					
AIA	**	0.20±0.56	1.33±2.23 ns	3.40±2.77**	ns
OSE	***	0.20±0.41	2.53±2.90*	4.40±2.95**	ns
VUS	***	0.00±0.00	2.07±2.71*	4.07±3.33**	*

(*)P<0.1
*P<0.05
**P<0.01
***P<0.001

drug-induced nature of the psychological effects experienced.

Correlations

No significant correlations were found between drug-induced changes in P50 and PPI measures. Thus, the following results were obtained between drug-induced changes in (a) P50 difference values and drug-induced changes in PPI at the 60-ms (r=–0.253, P=0.362), 120-ms (r=0.212, P=0.449), 240-ms (r=0.151, P=0.590), and 2000-ms (r=0.412, P=0.127) intervals; and (b) P50 percentage suppression values and drug-induced changes in PPI at the 60-ms (r=–0.066, P=0.815), 120-ms (r=0.381, P=0.162), 240-ms (r=0.212, P=0.448), and 2000-ms (r=0.366, P=0.179) intervals.

Given that significant drug effects were found on P50 measures, these were correlated with subjective-effect scores. Again, no correlations were found between changes in (a) P50 difference values and drug-induced changes in HRS-somaesthesia (r=–0.244, P=0.382), HRS-perception (r=–0.313, P=0.255), HRS-cognition (r=–0.281, P=0.310), HRS-volition (r=–0.474, P=0.075), HRS-affect (r=–0.387, P=0.155), HRS-intensity (r=–0.225, P=0.421), APZ-AIA (r=–0.490, P=0.063), APZ-OSE (r=–0.319, P=0.246), and APZ-VUS (r=–0.393, P=0.147) scores; and (b) P50 percentage suppression values and drug-induced changes in HRS-somaesthesia (r=–0.207, P=0.458), HRS-perception (r=–0.321, P=0.243), HRS-cognition (r=–0.101, P=0.722), HRS-volition (r=–0.439, P=0.102), HRS-affect (r=–0.278, P=0.316), HRS-intensity (r=–0.235, P=0.400), APZ-AIA (r=–0.393, P=0.147), APZ-OSE (r=–0.247, P=0.374), and APZ-VUS (r=–0.186, P=0.507) scores.

Discussion

The results obtained in the present study indicate diverging effects for *ayahuasca* on P50 suppression and PPI. Whereas a statistically significant dose-dependent reduction of P50 suppression was observed following drug administration, no significant effects were seen on PPI values. Additionally, the rate of habituation of the startle reflex, another form of startle plasticity thought to reflect gating mechanisms, was not modified by *ayahuasca*. In addition, at the doses administered, *ayahuasca* induced a pattern of subjective effects, similar in nature to those reported in a previous study involving a smaller sample of volunteers (Riba et al. 2001a), as was evidenced by the self-report questionnaires administered.

The present results would argue for a disruptive effect of psychedelics on P50 suppression. Nevertheless, this conclusion should be regarded as preliminary and interpreted with caution, considering the presence of other pharmacologically active alkaloids in *ayahuasca*. The only studies that have evaluated the effects of pharmacological challenge on this measure in humans have concentrated mainly on cathecolaminergic drugs and NMDA antagonists. Thus, both D-amphetamine and the α_2-adrenoceptor antagonist yohimbine, a drug that increases noradrenaline release, have been shown to impair P50 suppression in healthy volunteers (Adler et al. 1994b; Light et al. 1999). Furthermore, while the dopamine agonist bromocriptine has also been found to disrupt P50 suppression (Adler et al. 1994a) in humans, a low dose of the NMDA antagonist ketamine failed to decrease P50 suppression (van Berckel et al. 1998).

Regarding data from animals, suppression of the N40 potential in rodents in a paired stimuli paradigm, homologous to that of the human P50, appears to be highly dependent on the integrity and functionality of cholinergic pathways (Adler et al. 1998). However, inhibition can be disrupted by amphetamine (Adler et al. 1986; Stevens et al. 1991) – analogously to data from humans – and by phencyclidine (Adler et al. 1986). This loss of N40 suppression has been found to depend on the noradrenergic and dopaminergic properties of these drugs, also in the case of phencyclidine (Stevens et al. 1991; Miller et al. 1992). The psychostimulant cocaine has also been found to cause a loss of N40 suppression (Boutros et al. 1994). Thus, increased catecholamine neurotransmission seems to exert the same disruptive effects on sensory gating in humans and lower animals. However, in the only study reported to date on the effects of 5-HT_2 modulation of N40 suppression, an unexpected disruptive effect was found for the 5-$HT_{2A/2C}$ antagonist ketanserin. Conversely, the 5-$HT_{2A/2C}$ agonist DOI increased filtering and was also capable of reverting the reductions in filtering caused by ketanserin and amphetamine (Johnson et al. 1998).

The effects of *ayahuasca* on PPI did not reach statistical significance at any of the prepulse-to-pulse intervals tested. In the only other human study performed to date involving serotonergic psychedelics, the administration of psilocybin provoked a mild though significant increase of PPI at a prepulse-to-pulse interval of 100 ms, with no significant effects on habituation (Gouzoulis-Mayfrank et al. 1998). Both in the present study and in that by Gouzoulis-Mayfrank and coworkers, the drug doses administered were moderate and, although causing modifications in thought processes and the sensorium, they did not induce a clear-cut psychotic syndrome. Vollenweider and coworkers (1999) administered the serotonin releaser MDMA to a group of healthy volunteers and found a significant increase in PPI at the prepulse-to-pulse interval of 120 ms, but no significant effects on habituation. Results in the present study replicate the absence of effects found for psychedelics and MDMA on the rate of habituation.

Recently, a mechanistic study has shown that pretreatment with the 5-$HT_{2A/2C}$ antagonist ketanserin has no effect on the PPI-enhancing activity of MDMA, even though the antagonist was able to attenuate some of the effects of the drug, fundamentally the MDMA-induced perceptual modifications (Liechti et al. 2001). Conversely, these authors reported a decrease in PPI after pretreatment with the serotonin re-uptake inhibitor citalo-

pram and concluded that the effects of MDMA on human PPI seem to be more dependent on serotonin release than on an interaction at the 5-$HT_{2A/2C}$ level. These results would question the role of the human 5-$HT_{2A/2C}$ site in the modulation of PPI, despite the fact that recent human data provide additional support to the role of these receptors in the genesis of the psychological effects of psychedelics (Vollenweider et al. 1998). Unfortunately, no studies to date have evaluated the effects of the blockade of this receptor on psychedelic-induced increases of PPI in humans. Interestingly, the pattern of effects shown by serotonergic drugs on the human PPI in the limited number of studies conducted to date is opposed to that by dopaminergic/noradrenergic agonists. Thus, D-amphetamine and bromocriptine have been shown to impair PPI in healthy volunteers (Abduljawad et al. 1998, 1999; Hutchinson and Swift 1999).

In contrast to the above data, a coincidental pattern of effects on startle habituation and PPI has been observed for dopaminergic and 5-$HT_{2A/2C}$ agonists in lower animals. Braff and Geyer (1980) demonstrated an impairment in habituation of tactile startle in rats after administration of the mixed serotonergic agonist LSD. PPI has also been found to be impaired in rats after the 5-$HT_{2A/2C}$ agonist DOI, an effect which can be prevented by mixed 5-$HT_{2A/2C}$ (Sipes and Geyer 1994) and selective 5-HT_{2A} antagonists (Sipes and Geyer 1995; Padich et al. 1996). In a recent article, LSD was found to disrupt PPI in rats, and this effect was prevented only by selective 5-HT_{2A} antagonists. Other antagonists with affinity for the 5-HT_{2C}, 5-$HT_{2B/2C}$, 5-HT_{1A}, and 5-HT_6 did not counteract LSD-induced disruptions (Ouagazzal et al. 2001). Similarly, in rats PPI is disrupted by systemic administration of dopamine agonists, such as apomorphine, amphetamine, or the D_2 agonist quinpirole, and reversed by antipsychotic agents showing anti-D_2 activity (Geyer et al. 2001). One aspect that may have been overlooked and that could be involved in the differences in PPI modulation found for indole psychedelics between species is the fact that these drugs interact with both the 5-$HT_{2A/2C}$ and 5-HT_{1A} sites. Activation of these receptors has been shown to mediate opposite behavioral effects (Krebs-Thomson and Geyer 1998) in animals, and 5-HT_{1A} activation has recently been found to increase PPI in mice (Dulawa et al. 2000). The degree to which either receptor is activated after indole psychedelics could vary between species, and, consequently, the overall drug-induced effects on PPI could also vary.

The diverging results obtained on PPI and P50 suppression after *ayahuasca* administration to humans seemingly indicate a differential drug action. In addition to differences in receptor-level interactions, P50 suppression and PPI may reflect different stages of information processing and involve different brain structures. While P50 suppression is essentially viewed as a hippocampal process (Freedman et al. 1996; Adler et al. 1998), based on data from animal studies, PPI is thought to be modulated by a complex circuit involving the limbic cortex, striatum, pallidum, and pontine tegumentum, (Swerdlow and Geyer 1999; Swerdlow et al. 2001), offering many targets for pharmacological modulation. Swerdlow et al. (2000) have postulated that P50 and PPI are interrelated to the extent that hippocampal circuitry participates in both processes. Thus, the sites of pharmacological action and the subsequent modulation of each gating measure by different neurotransmitter systems may consequently show considerable variation.

In conclusion, at the doses administered, *ayahuasca* induced a different pattern of effects on PPI and P50. The results obtained seemingly indicate no effect, or at best, a mild enhancing effect of the drug on PPI, a measure of sensorimotor gating. On the contrary, the observed significant dose-dependent decreases in P50 suppression after *ayahuasca* suggest a suppressing effect of the drug on normal sensory gating in humans. This differential modulation of sensorimotor and sensory gating by *ayahuasca* in humans could be due to differential drug effects on brain structures participating in each process. However, the fact that the subjective-effect profile induced by *ayahuasca*, which was typical of the psychedelics, did not resemble that of acute psychosis should also be taken into consideration. In addition, the pharmacological characteristics of the beverage, which combines MAO-inhibitors and DMT, precludes the generalization of the present findings to all 5-$HT_{2A/2C}$ agonists. Future studies with *ayahuasca* should examine wider dose ranges to better characterize the effects of this drug on gating mechanisms in the CNS.

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Psychometric assessment of the Hallucinogen Rating Scale☆

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Abstract

Reliability and convergent-discriminant validity of a Spanish version of the Hallucinogen Rating Scale (HRS) were assessed in two differentiated populations of hallucinogen users involving the retrospective assessment of drug effects. In Study 1 (immediate assessment), 75 European users of the South American hallucinogenic drink *ayahuasca* answered the HRS 4 h after drug intake in their habitual setting. In Study 2 (delayed assessment), 56 adult polydrug users answered the HRS and a short form of the Addiction Research Center Inventory (ARCI) recalling the effects they experienced when they last took a hallucinogen, in order to test the convergent-discriminant validity of HRS with the scales of the standard questionnaire used in most studies involving psychoactive drugs. The HRS scales showed increases after both the immediate and delayed retrospective assessment of drug effects. Reliability data indicated that four of the six scales show an acceptable level of internal consistency. Significant but limited correlations were found between the Perception and Somaesthesia scales and the ARCI LSD scale, pointing out the questionnaire's construct validity. Thus, the HRS was sensitive to hallucinogenic drug effects other than those elicited by intravenous *N*,*N*-dimethyltryptamine (DMT), for which it was originally designed, and showed reasonable reliability and convergent validity. Results suggest its usefulness in the evaluation of subjective effects elicited by psychoactive drugs with hallucinogenic properties, and constitute a preliminary approach to the effects of *ayahuasca* in European subjects.

Keywords: Hallucinogen Rating Scale (HRS); Immediate versus delayed retrospective assessment; Subjective effects; Reliability; Convergent analysis

1. Introduction

Experimental studies involving hallucinogenic drug administration to human subjects have awakened renewed interest since the research of Hermle et al. (1992) and Vollenweider et al. (1997a,b,c) with model psychoses in Europe and Strassman's studies involving the administration of *N*,*N*-dimethyltryptamine (DMT) to healthy volunteers in the US (Strassman et al., 1994, 1996; Strassman, 1996). Before the resumption of clinical studies, these intriguing drugs had received little attention in psychiatry and human experimental psychopharmacology in the last two decades, despite their popularity as recreational drugs. Hallucinogen consumption, especially of LSD, rather than decreasing, has remained stable or even increased throughout the years and has recently won new impetus in the form of synthetic amphetamine derivatives such as MDMA (Pope et al., 1990; Schuster et al., 1998). Additionally, in Europe, a new pattern of use is emerging involving the so-called 'natural drugs' or 'shamanic inebriants', such as *peyote* (a mescaline-containing cactus) or *ayahuasca* (a DMT containing-drink) rediscovered by religious cults or 'new age' groups. Regarding *ayahuasca*, several groups ingesting this South American hallucinogenic drink have settled in recent years in several European countries, particularly in Holland, Italy, Germany and Spain. This hallucinogenic beverage is obtained from infusing various plants native to the Amazon Basin, habitually *Banisteriopsis caapi* and

☆ The full text of Appendix A is available at the journal website at http://www.elsevier.com/locate/drugalcdep under 'Supplementary Materials'.

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Psychotria viridis (Rivier and Lindgren, 1972). The drink contains both the potent short-acting hallucinogen DMT, the same used parenterally in Strassman's study, and several alkaloids known generically as beta-carbolines, which render DMT orally active by preventing its peripheral metabolism due to their monoamine oxidase inhibiting properties (McKenna et al., 1984; Callaway et al., 1999). This preparation is becoming increasingly popular in the countries mentioned above probably because it is a 'plant' drug (i.e. not the product of chemical synthesis) and because both the tea and the several religious groups which consume it in a ritual setting enjoy legal protection in Brazil. All these aspects have made it possible for a growing number of people to come into contact with *ayahuasca* outside South America.

The maintained prevalence of use of the classical hallucinogens, and the emergence of new patterns of use, including plant drugs and the widespread consumption of the so-called 'entactogenic' amphetamine derivatives, with mixed psychostimulant and hallucinogenic properties, have prompted new research efforts directed to clarify the complex psychological effects elicited by these agents. In this context, new psychometric research tools have been designed to quantify their effects on the central nervous system. By measuring the mood-, perception- and cognition-altering effects that are characteristic of a given drug, these instruments can help characterize their activity profiles over time and establish dose-response data. Although neuroendocrine and neurophysiological measures have also been tested for this purpose, the psychometric approach is still widely accepted, as such biological variables are usually less sensitive and specific. To date, no reliable unique biological measures are available to study dose-response relationships after the administration of hallucinogens to human subjects.

In the present study one intended both to approach the new phenomenon of *ayahuasca* use in Spain and further explore the sensitivity and psychometric properties of a recently developed instrument used to evaluate the perceptual, somatic and psychological effects of hallucinogenic drugs: the Hallucinogen Rating Scale (HRS). This self-report questionnaire was first designed to quantify the subjective effects experienced after the administration of DMT, and thus facilitate neuropharmacological research with these drugs in human subjects (Strassman, 1994, 1995). The items included in the questionnaire were originally generated by analyzing the verbal reports obtained after interviewing a number of experienced American DMT users. The resulting instrument was subsequently modified during the course of a controlled dose-response study conducted with DMT in 12 volunteers (Strassman et al., 1994). Its utility in human ketamine research (Bowdle et al., 1998) has also been demonstrated, and a recent study incorporated the HRS in the evaluation of the subjective effects elicited by psilocybin, MDE and methamphetamine in a group of healthy volunteers (Gouzoulis-Mayfrank et al., 1999). The items are grouped in six empirically-derived scales which, in the original dose-response study conducted by Strassman et al., and in subsequent DMT (Strassman, 1996; Strassman et al., 1996) and ketamine (Bowdle et al., 1998) studies, provided a better resolution of effects among doses than other biological variables used. The aim of the present study was thus to test whether the HRS was sensitive to the immediate and delayed assessment of hallucinogenic drug effects, and to explore its reliability and convergent validity, in order to establish its suitability for human psychopharmacological research with hallucinogens other than intravenous DMT.

2. Methods

A Spanish version of the questionnaire (Appendix A) was evaluated by means of two independent studies involving different approaches. Both study protocols had been previously reviewed by the Research Institute's review board. Approval by the hospital's Ethics Committee was deemed unnecessary, since both studies were non-experimental designs, that is the independent variable (drug ingestion) was not manipulated by the researchers. These non-experimental designs are more specifically known as ex post facto designs (DePoy and Gitlin, 1993).

In Study 1 the questionnaire was administered to a group of local users (Barcelona area, Spain) of *ayahuasca* immediately after one of their drug sessions (immediate retrospective assessment). In Study 2, one wished to compare the HRS results with those from the Addiction Research Center Inventory (ARCI), by means of a delayed retrospective assessment. The ARCI is the standard questionnaire used in studies involving drugs of abuse and includes a scale theoretically sensitive to the effects of hallucinogens (Haertzen et al., 1963; Haertzen, 1966, 1974). The HRS and a short version of the ARCI already validated into Spanish were administered to a group of polydrug users with experience in the consumption of hallucinogens. This time, volunteers were requested to answer both questionnaires recalling the effects they had experienced the last time they were under the influence of a hallucinogen. In both studies, reliability was assessed for each scale using Cronbach's alpha coefficient, and in the second study a convergent/discriminant analysis between the HRS and the ARCI scales was performed. This methodology would allow one first to cross-evaluate the HRS in two different samples and determine whether or not the instrument was sensitive to hallucinogenic drug effects in two different conditions (im-

mediate vs. delayed retrospective assessment of drug effects) and secondly, establish the HRS' convergent/discriminant validity with the most frequently used instrument in studies involving psychoactive drugs. The delayed retrospective assessment approach had already been used by Lamas et al. (1994) in their validation procedure of the Spanish version of the ARCI used in the present study.

2.1. Questionnaires

The HRS (version 3.06) was adapted into Spanish using the back-translation method, a judgmental method for investigating the conceptual equivalence (i.e. meaning symmetry) of the original and translated versions of a scale, necessary for valid cross-cultural comparisons (Berry, 1980). The HRS was translated independently by two Spanish researchers working in the field of psychopharmacology and with a good knowledge of the English language. These two Spanish versions were then contrasted and a final Spanish version was adopted. This was translated back into English by two independently working translators. The differences between retranslations were discussed and a final retranslated version was agreed upon. Finally, the original source and the back-translated items were compared for non-equivalence of meaning, and any discrepancies were noted. The translation-retranslation process was repeated several times until no semantic differences were noticed between the two questionnaire forms (Brislin, 1980).

The version of the American scale used in the present translation and evaluation is shorter than that used originally by Strassman et al. in their initial DMT dose-response study. The questionnaire has undergone several modifications and it now includes 71 items distributed in six scales: *Somaesthesia*, reflecting somatic effects including interoceptive, visceral and tactile effects; *Affect*, sensitive to emotional and affective responses; *Volition*, indicating the subject's capacity to willfully interact with his/her 'self' and/or the environment; *Cognition*, describing alterations in thought processes or content; *Perception*, measuring visual, auditory, gustatory and olfactory experiences; and finally *Intensity*, which reflects the strength of the overall experience (Strassman et al., 1994). The 71 items, their Spanish translation and their scale location are shown in Appendix A. All items are scored 0–4 (0 = not at all, 1 = slightly, 2 = moderately, 3 = quite a bit, 4 = extremely). Scorings on items 19, 62, 63, 64, 65, 66, 67 and 68 are inverted, since lower values are expected with increasing doses. The scores for the different scales are obtained by summing the scores obtained for the scale's individual items, divided by the number of items included in a given scale. A detailed account of item selection and scale development can be found in Strassman et al. (1994).

The short version of the ARCI used in the present study was an already existing Spanish version of Martin et al.'s widely used 49-item short form (Martin et al., 1971). It was translated and evaluated by Lamas and colleagues and has shown adequate reliability and discriminant validity (Lamas et al., 1994). It contains five scales or groups: MBG, morphine–benzedrine group, measuring euphoria; PCAG, pentobarbital–chlorpromazine-alcohol group, measuring sedation; LSD, lysergic acid diethylamide scale, which measures dysphoria and psychotomimetic effects; BG, benzedrine group, a stimulant-sensitive scale; and the A scale, amphetamine, sensitive to the effects of *d*-amphetamine. Alpha coefficients for these scales range from 0.49 to 0.87.

2.2. Study 1

2.2.1. Subjects

Prior to the study, several groups taking *ayahuasca* for recreational purposes in the Barcelona area (Spain) were contacted. In this preliminary evaluation of the HRS' sensitivity to the subjective effects elicited by a hallucinogen other than intravenous DMT, emphasis was placed on obtaining as large a sample of users as possible, which inevitably militated against its homogeneity. It was also not feasible to obtain a psychological or psychiatric profile of the participants. Nevertheless, sex, age and number of previous ingestions were recorded. The nature and goals of the present study were explained to them, and a total of 75 adult *ayahuasca* users gave their written informed consent to participate. After pooling the questionnaires, four subjects were excluded because of unanswered items. The final study sample consisted of 71 subjects (38 men and 33 women) with a mean $\pm$ S.D. age of 36.59 ± 7.89 years (range: 18–50). Thirty-three subjects had ingested *ayahuasca* tea between 1 and 5 times (46.5%); 11 had between 6 and 10 times (15.5%) and 27 had taken it more than 10 times (38%).

2.2.2. Study procedure

The subjects were requested to complete the questionnaire on a single occasion 4 h after *ayahuasca* ingestion, once the psychoactive effects had resolved. Instructions were given by one of the researchers or by a trained collaborator in the habitual setting where the subjects ingested the tea. They were asked to answer according to the effects experienced on that particular occasion and to answer each item according to the maximum intensity with which each effect was felt, thus avoiding the possibility of doubt in case the same effect had been felt with different intensities on different occasions.

2.3. Study 2

2.3.1. Subjects

Fifty-six polydrug users (48 men and eight women), with a mean ($\pm$ S.D.) age of 26 ± 6.72 years and previous experience with hallucinogens, were recruited by word of mouth, in collaboration with the methadone maintenance program of the Hospital de Sant Pau, Barcelona. Eligibility criterion was hallucinogen use during the last 5 years. Of the 56 subjects included, four had used a hallucinogen the preceding week (7.1%); 16 during the preceding month (28.6%); another 16 during the preceding year (28.6%) and 20 during the last 5 years (35.7%). Regarding present use of other illicit drugs, 50 of the 56 subjects admitted they were presently taking the following: cannabis ($n = 47$), cocaine ($n = 27$), amphetamine ($n = 21$), MDMA ($n = 15$), opiates ($n = 10$) or others ($n = 10$). Prior to participation, the subjects signed a written informed consent.

2.3.2. Study procedure

In this second study, a delayed retrospective assessment (also termed 'simulation' by some authors, see Lamas et al., 1994) approach was used. That is, subjects were requested to complete the HRS and the ARCI according to their recollection of the effects they had experienced when they last took a hallucinogen. Again, they were instructed to indicate the maximum intensity with which a particular effect had been felt (HRS) and to answer 'true' or 'false' (ARCI) depending on whether a particular statement applied to their last experience with a hallucinogen. The order of administration of the two questionnaires was balanced to avoid an effect of order in the responses.

2.4. Data analysis

Two criteria were considered in the analysis of the Spanish version of the HRS scales: (1) reliability, measured using Cronbach's alpha coefficient (studies 1 and 2); and (2) convergence-discriminance between the HRS and the ARCI scales (study 2). This validity criterion was assessed by studying correlations between the HRS and ARCI scales. The occurrence of a significant correlation was hypothesized between at least the Somaesthesia scale included in the HRS and the ARCI LSD scale, together with an absence of significant correlations between the six HRS scales and the other four ARCI scales, as the ARCI LSD scale is known to reflect basically the somatic-dysphoric effects elicited by hallucinogens. Reliability and intercorrelations of the ARCI scales are also presented in Section 3.

Additionally, in order to cross-evaluate the pattern of responses in both samples, where different methodological approaches had been used, the following analysis was performed: First, Pearson's product-moment correlations among the six HRS scales were computed in both samples. Subsequently, a Principal Component Analysis was performed on the correlation matrix thus obtained.

The purpose of this analysis was merely to compare results obtained in the two different samples. Scale development has been reported previously (Strassman et al., 1994) and one desired to maintain the questionnaire's original structure, so no first order Principal Component Analysis was performed on the individual items. Moreover, the small sample size precluded such an approach.

Once the product-moment correlations were obtained, the decision about the appropriate number of factors was made considering those factors with an eigenvalue greater than 1. Oblique rotation of the principal components extracted was preferred to orthogonal rotation because of the intensity of the correlations obtained between some scales. In order to test the null hypothesis stating that the factor structure was similar in the two samples studied, both factor solutions were compared. The similarity of both oblique factorial solutions obtained in the two samples was evaluated using the following indices: (a) the factorial congruency index (Harman, 1976); (b) the discrepancy or distance index, which is the square root of the average squared difference between the corresponding elements of the respective columns of factor loadings (Mulaik, 1972); (c) the non-parametric salience index, s (Cattell and Baggaley 1960); and (d) Pearson's r between Fisher's Z-transformed factor loadings. Different indices were used because of controversy among authors over the most appropriate index to be employed. However, Cattell (1978) indicated that these four indices tend to yield similar results. Good agreement between factors in the different samples would yield a congruency index near 1 (acceptable values are those above 0.90), a discrepancy index near zero, and salience and Fisher's Z-transformed index values near 1.

3. Results

3.1. Mean scores and reliability of the HRS scales

Both the subjective effects elicited by *ayahuasca* (immediate assessment, Study 1) and the recollection of previous hallucinogen-induced subjective effects (delayed assessment, Study 2) produced increases in the six HRS scales. Mean scores, standard deviations and reliability estimates (Cronbach's alpha) obtained for the six HRS scales in studies 1 and 2 are shown in Table 1. In Study 1, alpha coefficients for Perception, Cognition, Somaesthesia and Affect were adequate and acceptable (range between 0.81 and 0.88), reflecting a good degree of internal consistency. However, alpha coefficients for

the Intensity and Volition scales failed to reach a reasonable value. The Intensity scale showed the lowest internal consistency of the six scales. In Study 2, alpha coefficient values for the HRS scales replicated those found in Study 1, showing similar figures, especially for Perception and Cognition and to a lower extent for Somaesthesia and Affect. Although the Intensity scale reached a higher alpha value in this sample than in the previous sample, it was still low for an acceptable internal consistency.

3.2. Intercorrelations and principal component analysis: cross-evaluation of the two samples

Pearson's product-moment correlations between HRS scales are also shown in Table 1.

The highest correlations were found between Perception and Somaesthesia (Study 1) and between Affect and Cognition (Studies 1 and 2). Volition was the only scale inversely related to the rest, showing the lowest correlations in the matrix. This pattern of correlations between Volition and the other scales suggested that Volition was measuring a different construct or mapping a different content compared to the other HRS scales, which were all positively related in the correlation matrix.

The pattern of correlations obtained in the two samples showed a high degree of similarity. To further test this behavior a principal component analysis of intercorrelations between the HRS scales was performed. This analysis rendered in Study 1 two factors explaining approximately 75% of the variance in the correlation matrix (first factor: 57.5%; second factor, 16.8%). After an oblique rotation of both factors, a clear pattern appeared, showing a negative association between the two components (see Table 2), the correlation between both being $r = -0.19$. The first component included Somaesthesia, Perception, Cognition, Affect and Intensity; and the second component included only the Volition scale. The same analysis was performed with the factor matrix obtained in Study 2. As shown in Table 2, the factorial matrix was practically equal in both samples. Taken together, the two factors that emerged explained approximately 68% of the variance in the

Table 1
Intercorrelations (Pearson's r) among Hallucinogen Rating Scale (HRS) scales in the two samples studied, plus means ± S.D. and reliability coefficients associated with each HRS scale in the two samples[a]

	Aff.	Cog.	Int.	Per.	Som.	Mean ± S.D.	Alpha
Aff.	1					1.82 ± 0.58	0.81
Cog.	0.69**	1				1.92 ± 0.77	0.87
Int.	0.39**	0.55**	1			2.56 ± 0.58	0.33
Per.	0.53**	0.65**	0.48**	1		1.87 ± 0.70	0.88
Som.	0.63**	0.66**	0.52**	0.74**	1	1.45 ± 0.64	0.82
Vol.	−0.30*	−0.23*	−0.24*	−0.06	−0.13	1.42 ± 0.44	0.51
Aff.	1					1.75 ± 0.47	0.72
Cog.	0.70**	1				1.85 ± 0.72	0.86
Int.	0.34**	0.45**	1			2.49 ± 0.62	0.50
Per.	0.62**	0.66*	0.49**	1		1.72 ± 0.73	0.91
Som.	0.49**	0.40**	0.27**	0.47**	1	1.76 ± 0.63	0.71
Vol.	0.12	−0.09	−0.09	0.05	0.02	1.58 ± 0.48	0.54

[a] Upper and lower panels show data for study 1 ($N = 71$) and study 2 ($N = 56$), respectively. Aff. = affect, Cog. = cognition, Int. = intensity, Per. = perception, Som. = somaesthesia.

* $P < 0.05$;

** $P < 0.01$ (two tailed).

Table 2
Oblique rotated factor pattern matrix for the Hallucinogen Rating Scale (HRS) scales in the two samples studied

	Study 1 ($N = 71$)			Study 2 ($N = 56$)	
	1st Factor	2nd Factor		1st Factor	2nd Factor
Somaesthesia	0.9074	0.1236	Somaesthesia	0.6690	0.1160
Perception	0.8949	0.2351	Perception	0.8589	0.0411
Cognition	0.8563	−0.0836	Cognition	0.8484	−0.1251
Affect	0.7458	−0.2183	Affect	0.8444	0.1985
Intensity	0.6684	−0.1536	Intensity	0.6277	−0.3084
Volition	−0.0344	0.9639	Volition	0.0480	0.9543

Table 3
Means, S.D., reliability coefficients and intercorrelations (Pearson's r) among Addiction Research Center Inventory (ARCI) scales[a]

	A	BG	LSD	MBG	PCAG
BG	0.66**				
LSD	0.05	−0.14			
MBG	0.62**	0.38**	−0.11		
PCAG	−0.19	−0.35**	0.29*	−0.14	
Mean	6.39	3.75	3.02	9.84	−0.80
S.D.	1.87	2.12	2.42	3.54	1.81
No. items	11	13	14	16	15
Alpha	0.42	0.48	0.57	0.70	0.30

[a] $N = 56$; PCAG, pentobarbital–chlorpromazine–alcohol group; LSD, lysergic acid diethylamide scale; BG, benzedrine group; A, amphetamine; MBG, morphine-benzedrine group.
* $P < 0.05$;
** $P < 0.01$ (two tailed).

second study (first factor: 50.2%; second factor: 17.9%), and, again, they showed a slight negative association ($r = -0.3$).

The similarity evaluation of the two oblique factorial solutions yielded the following indices for the first and second component, respectively: congruency index = 0.99 and 0.99; discrepancy index = 0.08 and 0.08; salience index = 1 and 1; and Fisher's Z-transform index = 0.97 and 0.99.

3.3. Convergent and discriminant validity of the HRS scales

The ARCI scales were used to assess convergent and discriminant validity of the HRS in Study 2. Mean scores, standard deviations, alpha coefficients and intercorrelations between the ARCI scales are shown in Table 3. As can be seen, the delayed assessment of hallucinogen effects produced increases in the MBG and A scales, and to a lower extent in the BG and LSD scales, in relation to the number of items. Scale A strongly correlated with both the BG and MBG scales. This correlation between the A and BG scales replicates that ($r = 0.70$) reported by Lamas et al. (1994) in their validation study. In addition, the BG scale was positively related to the MBG scale although with less intensity. As a group, these three scales could be considered to reflect stimulating effects. All three correlated negatively with the PCAG scale. In addition, the LSD scale was positively correlated with PCAG and tended to be inversely related with the three stimulant-sensitive scales, although in no case did correlations reach a significant value.

Correlations between the HRS (including a global score for the HRS obtained by summing the individual scores in the six scales), and ARCI scales are shown in Table 4. As hypothesized, a convergent correlation was obtained between the LSD scale and the HRS scales. The highest significant correlations were found with the Somaesthesia and Perception scales. The global HRS score was also significantly correlated with the LSD scale ($r = 0.32$, $P < 0.05$). In contrast, the stimulant-sensitive scales, A and BG did not correlate significantly with the different HRS or the global score, whereas MBG showed a significant correlation with the Intensity scale ($r = 0.32$, $P < 0.05$), but correlated negatively with the global HRS score. An exception to this behavior was the two significant negative correlations found between Volition and the A and BG scales. These correlations were consistent with the results mentioned above, and further support the hypothesis that the Volition scale was measuring an independent dimension not reflected by the other five scales.

A non-predicted correlation was obtained between PCAG and the HRS scales, mainly Cognition and Somaesthesia, also observed with the global HRS score ($r = 0.39$, $P < 0.01$). It should be noted, however, that PCAG and LSD share a number of items, and as the ARCI correlations already showed these two scales are partially related. In order to remove the influence of the LSD scale on PCAG scores, the partial correlation between the global HRS score and PCAG was computed ($r = 0.29$, $P < 0.05$). Although this correlation was lower than the previous one, it was still significant and indicated a positive relation between PCAG and some HRS scales. This unexpected correlation should

Table 4
Correlations (Pearson's r) among Hallucinogen Rating Scale (HRS) and Addiction Research Center Inventory (ARCI) scales in Study 2[a]

	Affect	Cognition	Intensity	Perception	Somaesth.	Volition	Global
A	−0.03	−0.02	−0.06	−0.05	0.13	−0.27*	−0.06
BG	−0.16	−0.06	−0.10	−0.09	−0.10	−0.27*	−0.18
LSD	0.14	0.23	0.23	0.28*	0.33*	0.03	0.32*
MBG	0.04	−0.01	0.32*	−0.01	0.10	−0.19	−0.07
PCAG	0.36	0.29*	0.21	0.19	0.38*	0.15	0.39**

[a] $N = 56$; Global, global HRS score; PCAG, pentobarbital–chlorpromazine–alcohol group; LSD, lysergic acid diethylamide scale; BG, benzedrine group; A, amphetamine; MBG, morphine–benzedrine group.
* $P < 0.05$;
** $P < 0.01$ (two tailed).

be interpreted cautiously, given the low alpha value obtained for PCAG in the present study, and considering the fact that Haertzen (1974) had already described a high correlation between the PCAG and LSD scales.

4. Discussion

The purpose of the present study was to test the sensitivity of the HRS to a hallucinogenic drug other than intravenous DMT and to evaluate the reliability and validity of the questionnaire in two different populations of hallucinogen users, implying the immediate and delayed retrospective assessment of hallucinogenic drug effects. Results showed increases in the six scales in both samples and acceptable reliability values in four of the six HRS scales. Significant correlations were also found between the Perception and Somaesthesia scales with the ARCI LSD scale.

One of the most interesting aspects of the data presented above is the notable similarity of the psychometric indices obtained in the two studies undertaken in the evaluation process.

Despite the limitations associated with the heterogeneous nature of the studied samples, similar alpha values were obtained in five of the six HRS scales using quite different approaches, one involving the immediately preceding consumption of a hallucinogen, and the other the recollection of drug effects more distant in time. Alpha values obtained for Perception, Cognition, Somaesthesia and Affect indicated a good internal consistency for these four scales in both studies. Only two scales, Volition and Intensity, yielded poorer levels, indicating a non-uniform covariation of items or, as was very likely the case for the Intensity scale, too low a number of items. The number of items included in this scale is indeed lower than in the other five (only four items), and as alpha values depend on the number of items (McDonald, 1998), this could explain the poor internal consistency found for this scale. To the authors' knowledge, no reliability analyses have been published regarding the original American questionnaire or any of the existing translations of the HRS. One is consequently unable to compare the alpha values with those from other studies, but they objectively reflect a good degree of internal consistency in at least four of the six scales.

The equivalence of the two different assessment approaches used in the present study, that is immediate versus delayed, was further confirmed by the principal component analyses performed. Similarity indices showed a high degree of convergence between the two factorial solutions extracted. Consequently, it can be inferred that the same underlying factors accounted for the relationships among variables in both cases. Furthermore, mean values obtained for the HRS scales in studies 1 and 2 were similar and higher than those reported by Grob et al. (1996) in a study in which a moderate-low dose of *ayahuasca* was administered, and fall between the scores obtained with dosage levels of 0.2–0.4 mg/kg IV DMT, in the study of Strassman et al. (1994), reflecting a full hallucinogenic effect. These data would support the value of delayed retrospective assessment of drug effects as a questionnaire-validation procedure, as previously suggested by Haertzen (1974) and Lamas et al. (1994) regarding the ARCI.

Before performing the convergent-discriminant analysis between the HRS and the ARCI, the internal consistency of the ARCI scales was studied. For some of them, Cronbach's alpha values tended to be lower in the sample than those reported in the original adaptation of the instrument (Lamas et al., 1994). Alpha coefficients were similar for MBG, LSD and A scales, but PCAG obtained a considerably lower value in the sample than in the original study. The BG scale also yielded a lower value in the present analysis. Showing a pattern described in previous studies, A, MBG and BG correlated strongly with each other. In this study, they showed negative correlations with PCAG and LSD, and these two scales showed a significant positive correlation.

Consistent with the hypothesis, the highest correlations between questionnaires were found between several HRS scales and the LSD scale included in the ARCI. LSD correlated significantly with Perception, Somaesthesia and the global HRS score. This pattern of convergent correlations is also an index of the construct validity of these scales (Cronbach and Meehl, 1955). The results obtained indicate that the ARCI LSD and the HRS (in a global sense) are measuring the same construct, though probably covering different aspects, given the moderate values of the correlations obtained. Regarding the already-mentioned correlation between PCAG and several HRS scales, no explanation was found other than the variance shared between PCAG and LSD scale (8%). Haertzen (1974) reported a 0.44 correlation between the two scales. Although high scores were obtained for the stimulant-sensitive ARCI scales (MBG, A, BG), no correlations were found between these and the HRS scales except between MBG and Intensity, a correlation that was not present when the global HRS score was considered. This can be interpreted as an ability of the Intensity scale to capture the euphoria and the stimulating aspects of hallucinogen-induced phenomena. This ability is not observed when the global HRS score is considered, presumably indicating a greater sensitivity of the HRS to hallucinogenic than stimulant effects. Although the second highest, the correlation between the global HRS score and the LSD scale was modest, explaining only 10% of the variance. This suggests that the dysphoric somatic effects measured by the LSD scale (Haertzen, 1974) are

part of the effects elicited by hallucinogens only to some extent, but are by no means central to the experience, as has long been argued.

Finally, a brief comment on the specificity of the HRS must be made. In Study 2, data on non-hallucinogen effects were not collected, so conclusions on this aspect of the questionnaire can not be drawn. Future studies should address this issue by asking drug abusers to score the HRS for their recollection of a wider variety of drug experiences.

To summarize, the similar results obtained in the two different settings indicate that the HRS was effectively sensitive to hallucinogenic drug effects in the samples, other than intravenous DMT, and also demonstrate the value of delayed retrospective assessment of drug effects in validation procedures. Four out of six scales showed an acceptable degree of internal consistency and therefore reasonable reliability. The HRS Intensity scale showed a positive correlation with an ARCI stimulant-sensitive scale (MBG). This pattern was not seen for the other five scales, probably indicating the questionnaire's greater sensitivity to hallucinogenic than stimulant effects. Finally, the HRS showed a significant but limited correlation with the LSD scale of the ARCI, which met the authors' expectations. In view of the results obtained in the present study, one believes the HRS will prove a valuable instrument in the assessment of subjective effects in those research trials involving the administration of drugs with hallucinogen-like properties. Nevertheless, future dose-response studies using both the HRS and the ARCI will help clarify the ability of the first to reflect and measure additional aspects of hallucinogen-induced phenomena, other than the somatic-dysphoric symptoms measured by the ARCI.

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Determination of N,N-dimethyltryptamine and β-carboline alkaloids in human plasma following oral administration of Ayahuasca.

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Determination of *N,N*-dimethyltryptamine and β-carboline alkaloids in human plasma following oral administration of *Ayahuasca*

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Abstract

Ayahuasca is a South American psychotropic beverage prepared from plants native to the Amazon River Basin. It combines the hallucinogenic agent and 5-$HT_{2A/2C}$ agonist *N,N*-dimethyltryptamine (DMT) with β-carboline alkaloids showing monoamine oxidase-inhibiting properties. In the present paper, an analytical methodology for the plasma quantification of the four main alkaloids present in *ayahuasca* plus two major metabolites is described. DMT was extracted by liquid–liquid extraction with *n*-pentane and quantified by gas chromatography with nitrogen–phosphorus detection. Recovery was 74%, and precision and accuracy were better than 9.9%. The limit of quantification (LOQ) was 1.6 ng/ml. Harmine, harmaline, and tetrahydroharmine (THH), the three main β-carbolines present in *ayahuasca*, and harmol and harmalol (*O*-demethylation metabolites of harmine and harmaline, respectively) were measured in plasma by means of high-performance liquid chromatography (HPLC) with fluorescence detection. Sample preparation was accomplished by solid-phase extraction, which facilitated the automation of the process. All five β-carbolines were measured using a single detector by switching wavelengths. Separation of harmol and harmalol required only slight changes in the chromatographic conditions. Method validation demonstrated good recoveries, above 87%, and accuracy and precision better than 13.4%. The LOQ was 0.5 ng/ml for harmine, 0.3 ng/ml for harmaline, 1.0 ng/ml for THH, and 0.3 ng/ml for harmol and harmalol. Good linearity was observed in the concentration ranges evaluated for DMT (2.5–50 ng/ml) and the β-carbolines (0.3–100 ng/ml). The gas chromatography and HPLC methods described allowed adequate characterization of the pharmacokinetics of the four main alkaloids present in *ayahuasca*, and also of two major β-carboline metabolites not previously described in the literature.

Keywords: *Ayahuasca*; *N,N*-Dimethyltryptamine; β-Carboline alkaloids

1. Introduction

Ayahuasca is a psychotropic plant tea which has traditionally played a central role in the magico-religious practices and folk medicine of indigenous

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peoples native to the Amazon and Orinoco river basins [1,2]. In recent years, *ayahuasca* has become increasingly popular in Europe and North America, where many individuals interested in shamanic practices reportedly use it as a means to facilitate self-knowledge and introspection or as a sacramental drug in the context of syncretic religions [3]. As the use of this tea has spread outside its original geographic area reaching Europe and North America, health issues regarding the safety of its use have been raised, and clinical data on its general pharmacology are warranted.

The psychotropic properties of *ayahuasca* are attributed mainly to the fact that it contains measurable amounts of the hallucinogenic indole DMT, a serotonergic 5-$HT_{2A/2C}$ agonist [4], such as LSD or psilocybin. DMT is known to elicit intense perceptual, cognitive and affective modifications when parenterally administered [5], but has been shown to be devoid of psychoactivity after oral ingestion [6], probably due to first-pass enzymatic degradation by monoamine oxidase (MAO) [6,7]. Interestingly, *ayahuasca* also contains levels of β-carboline alkaloids with MAO-inhibiting properties, which could explain the oral psychoactivity of the tea. The usual elaboration process of *ayahuasca* involves the combination in a single beverage of two different plants, one of which contributes the orally labile DMT, and the other, the MAO inhibitors. Thus, the pounded stems of *Banisteriopsis caapi* (Malphighiaceae) are infused together with the leaves of *Psychotria viridis* (Rubiaceae). While *B. caapi* contributes varying amounts of five different MAO-inhibiting β-carbolines, i.e. harmine, THH, harmaline and trace amounts of harmol and harmalol [8,9], *P. viridis* is the source of the DMT [8,10]. The chemical structures of DMT, harmine, harmaline, harmol and harmalol are shown in Fig. 1. In vitro, harmine and harmaline, and to a lesser extent THH, potently inhibit MAO [9], an effect which could prevent in vivo the oxidative deamination of the DMT present in the tea, allowing its access to systemic circulation and the central nervous system after oral ingestion.

Our research group has initiated a project directed at studying the pharmacology of *ayahuasca* in humans. It includes the assessment of the subjective, cardiovascular and neurophysiological effect profile of the beverage in healthy volunteers. In a clinical research setting, *ayahuasca* has demonstrated a

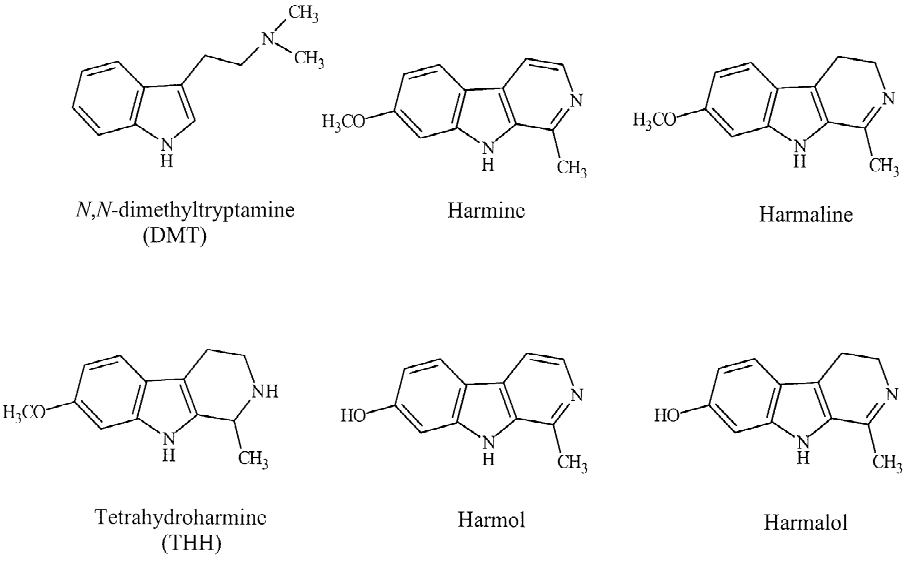

Fig. 1. Chemical structure of alkaloids from *P. viridis* (DMT) and *B. caapi* (harmine, harmaline and THH) typically found in *ayahuasca* brews. Harmol and harmalol are found in trace amounts in *B. caapi* and *ayahuasca* but appear in significant concentrations in human plasma samples following oral dosing with *ayahuasca*. These compounds are presumably formed in vivo by *O*-demethylation of harmine and harmaline, respectively.

combined stimulatory and hallucinogenic effect profile, as measured by subjective effect self-assessment instruments [11], and dose-dependent changes in spontaneous brain electrical activity, which parallel the time course of subjective effects and support the role of 5-$HT_{2A/2C}$ and D_2 agonism in mediating the effects of *ayahuasca* [12]. In the present paper, we describe an analytical methodology which was developed to characterize the pharmacokinetics of *ayahuasca* alkaloids in humans following oral administration of the tea.

2. Experimental

2.1. DMT

2.1.1. Chemicals and reagents

DMT was generously provided by the United Nations International Drug Control Programme, Technical Services Branch, Laboratory Operations. Diphenhydramine and blank plasma were supplied by Uriach Laboratories (Barcelona, Spain) and the blood bank of Hospital del Mar (Barcelona, Spain), respectively. Methanol, *n*-pentane, potassium hydroxide and sodium chloride were reagent-grade and purchased from Merck (Darmstadt, Germany).

2.1.2. Instrumentation

A gas chromatograph equipped with a nitrogen–phosphorus detection system (HP5890 series II, Hewlett-Packard, Palo Alto, CA, USA) was used. Samples were injected in splitless mode (30 s of purge off time) into 5% phenyl-methylsilicone capillary column, 12 m×0.2 mm I.D. and 0.33-μm film thickness (Ultra 2, Hewlett-Packard). Helium was used as carrier gas at a flow-rate of 0.7 ml/min (measured at 180 °C) and as make up gas at a flow-rate of 47 ml/min. Air and hydrogen detector flow were set at 80 and 5.5 ml/min, respectively. A temperature program for plasma DMT separation starting at 70 °C, was maintained for 1 min, and programmed to 120 °C at 30 °C/min, then to 280 °C at 20 °C/min; it was maintained for 1 min. Total run-time was 12 min. Injector and detector temperature were set at 280 °C.

2.1.3. Working standards

Working solutions of 1 μg/ml of diphenhydramine (internal standard) and DMT were prepared from a stock solution of 100 μg/ml by dilution with methanol. All solutions were checked for purity on a routine basis. Standard solutions were stored at −20 °C until analysis.

2.1.4. Preparation of calibration curve and quality control samples

A calibration curve was prepared for each analytical batch. Appropriate volumes of working solutions were added to test-tubes containing 1.0 ml of drug-free plasma and vortexed vigorously. Final concentrations were 2.5, 5.0, 10, 25 and 50 ng/ml. Control plasma samples containing DMT were prepared at three different concentrations, low control 2.5 ng/ml, medium control 25 ng/ml and high control 50 ng/ml. They were kept frozen at −20 °C in 1.0-ml aliquots. Control samples were included in each batch in duplicate.

2.1.5. Sample preparation

Test and control samples were allowed to thaw at room temperature. Aliquots of 1 ml of plasma were pipetted into 15-ml screw-capped tubes and processed together with a calibration curve after addition of 25 ng of internal standard. Samples were treated by adding 0.5 ml of 5 *M* potassium hydroxide and 1.0 ml of sodium chloride saturated solution. They were then extracted with 5 ml of *n*-pentane for 20 min. The organic phase was separated and evaporated to dryness under a nitrogen stream at 25 °C. The residue was redissolved in 50 μl methanol. Finally, 1.0 μl was injected into the chromatographic system.

2.2. β-Carbolines

2.2.1. Chemicals and reagents

Harmine, harmaline, harmol, harmalol, propranolol and yohimbine were purchased from Sigma (St Louis, MO, USA). THH was synthesized and generously provided by Dr James C. Callaway, University of Kuopio, Finland. Blank plasma was supplied by the blood bank of Hospital de Sant Pau (Barcelona, Spain). Acetonitrile, methanol, triethylamine, ammonium acetate and sodium hydroxide were HPLC grade and purchased from Scharlab (Barcelona, Spain). Ultra pure water was obtained using a Milli-

Q purification system (Millipore, Molsheim, France). Boric acid was purchased from Panreac (Barcelona, Spain), glacial acetic acid from Riedel-de-Haën (Seelze, Germany), ammonia from Merck (Darmstadt, Germany), and 0.9% v/v saline solution from B. Braun Medical (Barcelona, Spain). Bond-Elut C_{18} 100-mg solid-phase extraction cartridges and Vac-Elut SPE24 vacuum system were from Varian (Harbor City, CA, USA).

2.2.2. Instrumentation

Analysis by HPLC was carried out using an autosampler 717, pumps 515 and 510, a variable wavelength fluorescence 474 detector and Millenium[32] acquisition and processing software, all from Waters (Milford, MA, USA). The analytical column was a Kromasil 100 C_{18}, 5 μm, 150×4 mm I.D., and the guard column was a C_{18} 5 μm, 10×4 mm I.D., both purchased from Teknokroma (Barcelona, Spain).

The mobile phase for harmine, harmaline and THH consisted of solvent A which was a mixture (63:37 v/v) of ammonium acetate buffer 50 m*M* pH 8.0 and acetonitrile–methanol (20:30 v/v); and solvent B which was a mixture of acetonitrile–methanol (20:30 v/v). Gradient elution was initiated with 100% solvent A at a flow-rate of 0.8 ml/min for 6.5 min, then changed within 2 min to the following proportions: 68.3% solvent A and 31.7% solvent B and a flow-rate of 1.2 ml/min. These conditions were maintained for 7.5 min, to return to initial conditions thereafter. The fluorescence detector was operated at the following excitation/emission wavelengths: λ=260 nm/λ=370 nm to measure THH, harmine and yohimbine (internal standard), and λ=340 nm/λ=495 nm to measure harmaline.

The mobile phase for harmol and harmalol consisted of solvent A which was a mixture (73:27 v/v) of ammonium acetate buffer 50 m*M* pH 6.3 and acetonitrile–methanol (20:30 v/v); and solvent B which was a mixture of acetonitrile–methanol (20:30 v/v). Gradient elution was initiated with 100% solvent A at a flow-rate of 0.8 ml/min for 4.5 min, then changed within 1.5 min to the following proportions: 72% solvent A and 28% solvent B and a flow-rate of 1 ml/min. These conditions were maintained for 9 min, to return to initial values thereafter. The fluorescence detector was operated at excitation/emission wavelengths of λ=340 nm/λ=495 nm to measure harmol and harmalol, and λ=260 nm/λ=370 nm to measure propranolol (internal standard).

2.2.3. Working standards

Working solutions of 100, 10, 1 and 0.1 μg/ml of harmine and THH were prepared from stock solutions of 1 mg/ml of each alkaloid in methanol. The same working solutions plus 0.01 μg/ml were prepared for harmaline from a stock solution of 1 mg/ml in methanol. A stock solution of 1 mg/ml yohimbine in methanol was prepared. A 10 μg/ml solution in methanol was obtained by dilution and from this, a 100 ng/ml yohimbine in saline solution was prepared, which was later added to the samples.

Working solutions of 10, 1 and 0.1 μg/ml of harmol and harmalol were prepared from stock solutions of 1 mg/ml of each alkaloid in methanol. A stock solution of 10 mg/ml of propranolol in methanol was prepared. A working solution of 1 mg/ml was obtained by dilution of the latter, and this was used to obtain a 50 ng/ml propranolol solution in saline, which was later added to the samples. All standard solutions were stored at +4 °C during sample analysis.

2.2.4. Preparation of calibration curves and quality control samples

Calibration curves were constructed for each of the five β-carbolines (three original alkaloids plus two metabolites). Blank plasma was spiked with working solutions of each of the five compounds. Final concentrations were 0.5, 1, 2, 5, 10, 25 and 50 ng/ml for harmine; 0.3, 0.5, 1, 2, 5, 10 and 25 ng/ml for harmaline; 1, 1.5, 3, 10, 25, 50 and 100 ng/ml for THH; 0.3, 0.5, 1, 2, 5, 10, 25, and 50 ng/ml for harmol; and 0.3, 0.5, 1, 2, 5, 10, 25 and 50 ng/ml for harmalol. Analyte concentrations were calculated by comparison with calibration curves. THH/yohimbine, harmine/yohimbine, harmaline/yohimbine, harmol/propranolol and harmalol/propranolol area ratios were used. Quality control samples were prepared at the following concentrations: 2, 5 and 25 ng/ml for harmine; 1, 2 and 10 ng/ml for harmaline; 3, 10 and 50 ng/ml for THH; 2, 10 and 25 ng/ml for harmol and harmalol. Spiked plasma samples at the following concentrations: 2 and 10 ng/ml for harmine; 1 and 5 ng/ml for

harmaline; 3 and 25 ng/ml for THH; 2 and 25 ng/ml for harmol and harmalol were prepared in the same way for the stability study.

2.2.5. Sample preparation

Calibration, quality control, stability control and test samples were allowed to thaw at room temperature. Six hundred microliter aliquots of an internal standard solution were added to 600 μl of the samples. This was followed by gentle mixing and centrifugation for 10 min prior to solid-phase extraction. The cartridges were attached to the vacuum station and activated with 2 ml methanol and conditioned with 1 ml Milli-Q water and 2 ml borate buffer (50 m*M*) at pH 9.0. Next, 1 ml of a mixture containing 0.5 ml of plasma and 0.5 ml saline solution with 100 ng/ml of yohimbine (for harmine, harmaline and THH) or 50 ng/ml of propranolol (for harmol and harmalol), were transferred into the cartridges. After aspiration of the entire volume through the cartridges, they were washed with 1 ml Milli-Q water, followed by 1 ml acetonitrile–water (10:90 v/v). The cartridges were then dried under full vacuum and eluted with 0.5 ml of a 0.1% triethylamine solution in methanol. The eluate was allowed to dry for 1.5 h, approximately. The solid residue was redissolved in 100 μl mobile phase, vortexed for 10 s and placed in disposable microvials which were put in the autosampler. Sixty microliters were injected into the chromatographic system

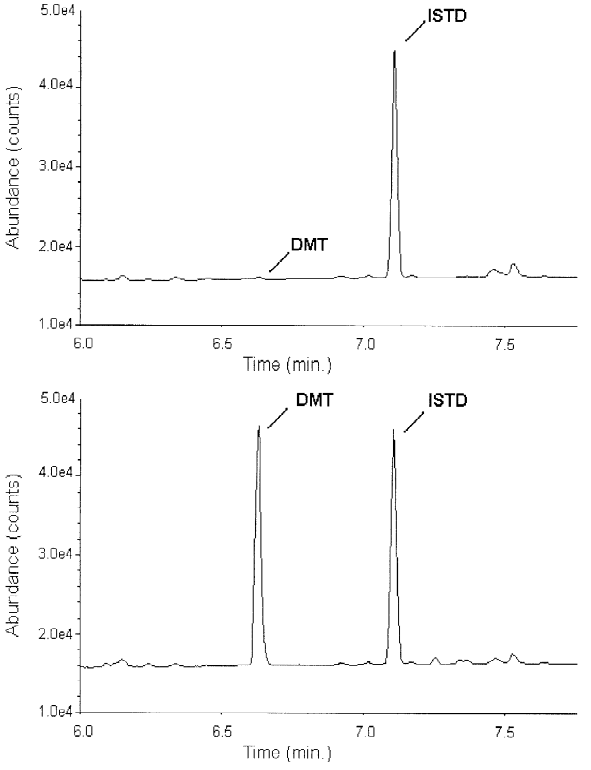

Fig. 2. Chromatogram obtained with gas chromatography with nitrogen–phosphorus detection of a plasma sample from a volunteer administered a 1 mg DMT/kg dose of *ayahuasca*. (A) Baseline plasma sample obtained just before drug administration. Expected retention time for DMT, and peak corresponding to the internal standard (ISTD). (B) Plasma obtained at 2.5 h after drug administration. The calculated concentration for DMT was 10.97 μg/l.

3. Results

3.1. DMT

Fig. 2 shows a representative plasma chromatogram obtained after method application. No interfering peaks were observed at the elution time of analyte and the internal standard. The DMT retention time was 6.61 min.

3.1.1. Recovery

Extraction efficiencies for DMT and diphenhydramine were calculated by comparing the areas of the chromatographic peaks of equal concentrations of drug extracted and non-extracted. The experiment was carried out with concentrations of analytes (in duplicate) identical to those used for calibration. Recoveries for DMT and the internal standard in the concentration range studied were 74±8.2 and 81±7.4%, respectively.

3.1.2. Linearity

Five concentrations ranging from 2.5 to 50 ng/ml were used to assess the linearity of the method. Results obtained from regression analysis of the theoretic concentrations versus ratio are shown in Table 1.

3.1.3. Precision, accuracy and robustness

Intra-assay precision and accuracy were determined by testing five replicates of blank plasma

Table 1
Linearity parameters of plasma determinations of DMT (gas chromatography) and β-carbolines (HPLC) obtained in the routine phase

	n	Intercept $x \pm SD$	Slope $x \pm SD$	Determination coef. r^2 $x \pm SD$
DMT	36	−0.0416±0.0484	0.0754±0.0152	0.9946±0.0032
Harmine	10	0.0051±0.0040	0.0189±0.0016	0.9929±0.0028
Harmaline	10	0.0036±0.0050	0.1115±0.0150	0.9916±0.0020
THH	10	0.0077±0.0069	0.0094±0.0013	0.9948±0.0018
Harmol	10	−0.0006±0.0011	0.0284±0.0021	0.9981±0.0005
Harmalol	10	−0.0005±0.0027	0.0526±0.0042	0.9964±0.0015

n, no. of days.

spiked with 5, 20, and 40 ng/ml (low, medium and high control samples). Each control sample was analyzed in 36 consecutive analytical batches over a 3-month period. Analytical batches were carried out by different scientists, thus emphasizing the method's robustness. Precision was expressed as the %CV of the calculated concentration. Accuracy was expressed as the relative error (R.E.) of the calculated concentration. Results obtained are shown in Table 2. The intra-assay precision was lower than 7.5% for the three concentrations tested.

3.1.4. Estimation of limits of detection and quantification

Five replicates of the low concentration value of the calibration curve of DMT (2.5 ng/ml) were processed for their calculation. An estimate of the limits of detection and quantification was calculated as three and 10 times the standard deviation of the estimated concentration, respectively. The detection and quantification limits obtained following this method were 0.5 and 1.6 ng/ml, respectively.

Table 2
Precision and accuracy of plasma determinations of DMT (gas chromatography) and β-carbolines (HPLC)

	Intra-day		Inter-day	
	Precision (CV, %)	Accuracy (R.E., %)	Precision (CV, %)	Accuracy (R.E., %)
DMT	≤7.5	≤8.7	≤9.9	≤−7.4
Harmine	≤6.2	≤10.1	≤7.8	≤11.2
Harmaline	≤6.9	≤10.4	≤13.4	≤12.5
THH	≤10.9	≤9.5	≤9.8	≤8.8
Harmol	≤6.2	≤13.2	≤7.6	≤11.3
Harmalol	≤6.3	≤5.4	≤8.2	≤7.6

3.2. β-Carbolines

Fig. 3 shows typical sample plasma chromatograms of the β-carbolines. The mean retention times in minutes found in the validation study were 5.42 for THH, 6.95 for harmaline, 11.77 for harmine, and 13.00 for yohimbine (internal standard). Retention times for the *O*-demethylated metabolites were 3.87 for harmalol, 6.02 for harmol, and 14.07 for propranolol (internal standard). Variability in retention times for all compounds tested was less than 5% (CV).

3.2.1. Recovery

Peak areas of the β-carbolines and internal standards were measured after injection of the same amounts of the respective alkaloids and internal standard in mobile phase. These were compared with those obtained with blank plasma spiked with known amounts of the three β-carbolines. Recoveries, expressed in %, near the LOQ were 102.1±6.8 for 2 ng/ml harmine, 91.5±1.6 for 1 ng/ml harmaline, 87.4±2.9 for 3 ng/ml THH, and 90.6±3.7 for yohimbine. Recoveries for the *O*-demethylated metabolites were 89.8±4.4 for 0.5 ng/ml harmol and 91.9±6.3 for 0.5 ng/ml harmalol and 91.2±5.7 for propranolol.

3.2.2. Linearity

Linearity was evaluated for concentrations ranging from 0.5 to 50 ng/ml for harmine, from 0.3 to 25 ng/ml for harmaline, from 1 to 100 ng/ml for THH, and from 0.3 to 50 ng/ml for harmol and harmalol. The response ratio of the peak alkaloid area/internal standard area was fitted versus effective concentration by means of least-squares linear regression. The inverse of the square concentrations

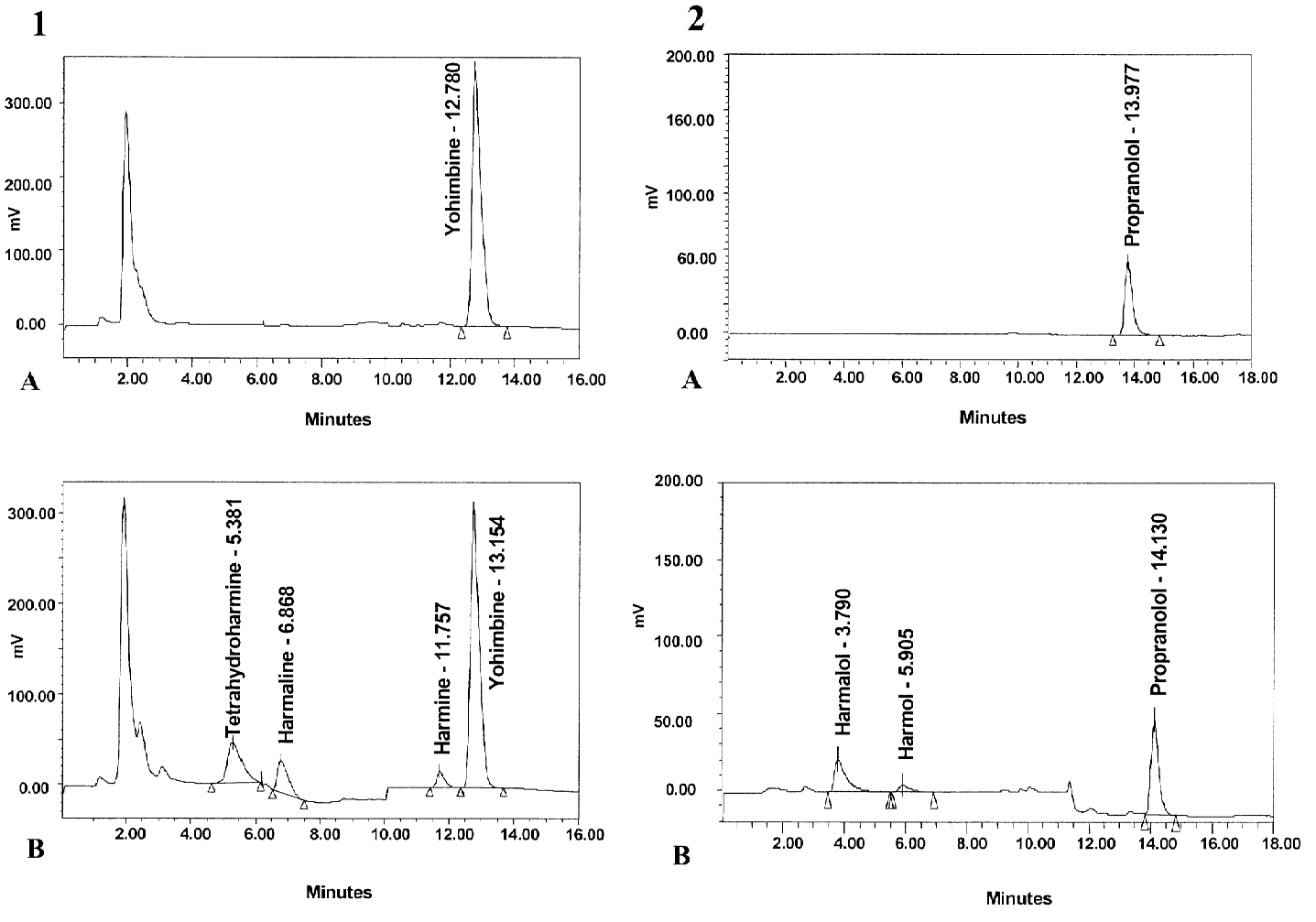

Fig. 3. Representative chromatograms of solid-phase extracted plasma samples obtained with HPLC. (1) Method for the quantification of harmine, harmaline and THH: (a) typical chromatogram of a blank plasma sample with internal standard (yohimbine), free of interfering peaks; (b) typical plasma sample from a volunteer after oral dosing with *ayahuasca*. (2) Method for the quantification of harmol and harmalol, the *O*-demethylated metabolites of harmine and harmaline: (a) typical chromatogram of a blank plasma sample with internal standard (propranolol), free of interfering peaks; (b) typical plasma sample from a volunteer after oral dosing with *ayahuasca*.

$(1/C^2)$ was used as a weighting factor. Regression analysis yielded the following results in the validation phase ($n=4$ days): $y=0.1620x+0.0018$ ($r^2=0.9912$) for harmine, $y=0.1071x+0.0019$ ($r^2=0.9915$) for harmaline, $y=0.0091x-0.0019$ ($r^2=0.9943$) for THH, $y=0.0265x-0.0017$ ($r^2=0.9962$) for harmol, and $y=0.0519x-0.0011$ ($r^2=0.9962$) for harmalol. Results in the routine phase are shown in Table 1.

3.2.3. Precision, accuracy and robustness

Intra-assay and inter-assay precision was determined by testing six replicates (during method validation) and 10 replicates (during routine analysis) of blank plasma samples spiked with the control samples of the respective alkaloids. Precision (expressed as %CV) and accuracy (expressed as R.E.) results obtained in the method validation phase are shown in Table 2.

In the routine phase, precision values (%CV) between assays for quality controls were below 12.4 for harmine, 11.1 for harmaline, 9.1 for THH, 3.5 for harmol and 5.9 for harmalol; and accuracy values (%R.E.) between 10 assays were below 6.9 for harmine, 9.0 for harmaline, 5.0 for THH, 10.4 for harmol and 5.4 for harmalol.

3.2.4. Estimation of limits of detection and quantification

The LOQ was established as the lowest concentration values in the calibration curve with acceptable precision and accuracy, i.e. 0.5 ng/ml for harmine, 0.3 ng/ml for harmaline, 1.0 ng/ml for THH, and 0.3 ng/ml for harmol and harmalol. The limit of detection was 0.1 ng/ml for harmine, harmaline, harmol and harmalol, and 0.3 ng/ml for THH.

3.2.5. Stability study

Freeze and thaw stability was assessed with two analyte concentrations for each alkaloid (one high and one low) in spiked plasma samples stored at −80 °C. The samples were subjected to three freeze and thaw cycles in three consecutive days. Concentrations determined in each cycle were within the method's accuracy (<10%) except for harmaline, which showed a 16% variation in the third cycle. To test the stability of samples in the autosampler, three sets of spiked samples where analyzed at $t=0$, $t=24$ h, $t=48$ h with a fresh calibration curve. Concentrations determined at each time tested were within the method's accuracy (<10%). Additional stability studies were carried out, i.e. stability in solution kept in the refrigerator and stability in dried residue. All compounds were stable except for harmaline and harmalol. Harmaline showed a 30% concentration decrease and harmalol a 21% concentration decrease at 24 h in dried residue. For this reason, dried residues were redissolved immediately in mobile phase during routine analysis. The stability study showed that sample degradation was low, being inferior to 10% for all the β-carbolines evaluated with the exception of the two cases mentioned. No significant variations were observed in the quality controls during the entire routine process, as indicated in Section 3.2.3.

3.3. Pharmacokinetic results

Blood samples from 18 healthy volunteers with previous experience with hallucinogen drug use were obtained at 0, 30, 60, 90, 120, 150 min, and 3, 4, 6, 8 and 24 h following oral administration of two doses of encapsulated freeze-dried *ayahuasca* in a double-blind placebo-controlled clinical trial. The study was approved by the local ethics committee and the Spanish Ministry of Health. Signed informed consent was obtained from all participants. The freeze-dried material administered in the study was obtained from a 9.6 l batch of *ayahuasca* and contained 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline and 11.36 mg THH per g. The alkaloid concentrations in the original tea were 0.53 mg/ml DMT, 0.90 mg/ml harmine, 0.06 mg/ml harmaline and 0.72 mg/ml THH. The two doses administered to the volunteers were equivalent to 0.6 and 0.85 mg DMT/kg body weight, and were chosen based on tolerability data obtained in a previous study [11]. The blood samples were collected in tubes containing EDTA, centrifuged, and the plasma frozen at −20 °C and stored at −80 °C until analysis.

Fig. 4 shows mean concentration versus time curves for DMT, harmine, harmaline, THH, harmol and harmalol obtained after analysis of plasma samples from four male volunteers who received two

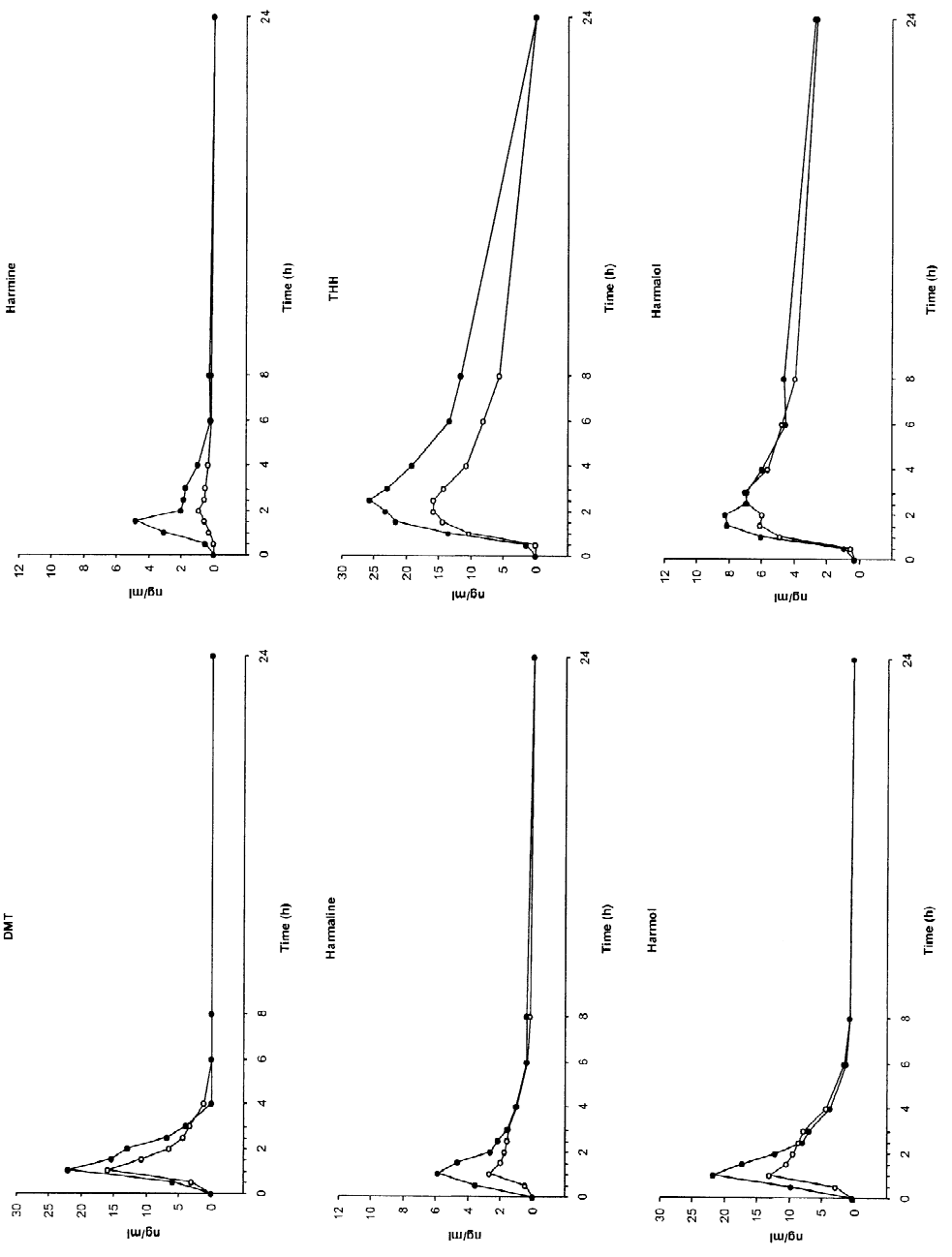

Fig. 4. Plasma concentration–time curves (n=4) for the four main alkaloids present in *ayahuasca* (DMT, harmine, harmaline, and THH) and the *O*-demethylated metabolites of harmine (harmol) and harmaline (harmalol); ○, low 0.6 mg DMT/kg dose of *ayahuasca*; ●, high 0.85 mg DMT/kg dose of *ayahuasca*.

oral doses of *ayahuasca* corresponding to 0.6 and 0.85 mg DMT/kg body weight.

4. Discussion and conclusions

There is a high variability in the bioavailability of *ayahuasca* alkaloids in humans, as observed in the present study. Some of the clues to these variations have already been provided in the Introduction. From an analytical perspective, for a given dose, one should be prepared to cover differences of peak plasma concentrations of one order of magnitude. The present work has proven sufficiently specific, sensitive and robust to perform this task. The GC and HPLC methods described in the present paper allowed for the adequate characterization of the pharmacokinetics of the six compounds evaluated after oral ingestion of *ayahuasca*.

Regarding the DMT quantification method, the use as salting-out effect of sodium chloride to saturate the aqueous phase and *n*-pentane as organic solvent, instead of *n*-butyl chloride as described in a previously reported method [13], provides an adequate recovery and very clean extracts. There is an overall improvement of three times the LOQ (from 5 to 1.6 ng/ml) which facilitates the pharmacokinetic study, taking into account the variability in the absorption of DMT when orally administered. The method described for the determination of the β-carbolines also introduces several improvements from previously described procedures [13]. Sample preparation is thus facilitated by solid-phase extraction, and quantification of the three alkaloids plus two metabolites, harmol and harmalol, is accomplished using a single fluorescence detector, the procedure requiring only slight modifications in the chromatographic conditions, and the use of two internal standards. The LOQ is also lower than previously reported values. Results demonstrate the present HPLC methodology is a rapid, simple and sensitive procedure for the determination of β-carboline compounds at low concentrations in human plasma, enabling the automation of the process.

In conclusion, a previously reported method was modified [13] in order to facilitate analyte extraction, improve sensitivity and allow the quantification of two metabolites not previously studied. DMT was determined by gas chromatography with selective nitrogen–phosphorus detection following liquid–liquid extraction. The three main β-carbolines present in *ayahuasca*, i.e. harmine, harmaline and THH, were determined by means of HPLC with fluorescence detection following solid-phase extraction. Additionally, harmol and harmalol, two alkaloids present in trace amounts in *ayahuasca* but showing significant levels in plasma following ingestion of the tea, were also determined. Harmol and harmalol had not been assessed previously in plasma following oral dosing with *ayahuasca*, and are presumably formed in vivo by the *O*-demethylation of harmine and harmaline, respectively. The quantification of these two metabolites was accomplished with slight changes in the chromatographic conditions necessary to determine the parent compounds.

Acknowledgements

We are grateful to Dr James C. Callaway of the Department of Pharmaceutical Chemistry of the University of Kuopio, Finland, for supplying the THH standard, and to the United Nations International Drug Control Programme, Technical Services Branch, Laboratory Operations for generously providing the DMT standard.

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Appendix I

Effects of the South American psychoactive beverage Ayahuasca on regional brain electrical activity in humans: a functional neuroimaging study using low resolution electromagnetic tomography (LORETA)

[In preparation]

Effects of the South American psychoactive beverage Ayahuasca on regional brain electrical activity in humans: a functional neuroimaging study using low resolution electromagnetic tomography (LORETA)

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Abstract

Ayahuasca, a South American psychotropic plant tea obtained from *Banisteriopsis caapi* and *Psychotria viridis*, combines monoamine-oxidase-inhibiting β-carboline alkaloids with *N*,*N*-dimethyltryptamine (DMT), a psychedelic agent showing 5-HT_{2A} agonist activity. The spatial distribution of *ayahuasca*-induced changes in brain electrical activity was investigated by means of low resolution electromagnetic tomography (LORETA). Electroencephalography (EEG) recordings were obtained from eighteen volunteers with prior experience in the use of psychedelics after the administration of a 0.85 mg DMT/kg body weight dose of encapsulated freeze-dried *ayahuasca* and placebo. The intracerebral power density distribution was computed with LORETA from spectrally analyzed data. Statistically significant differences with placebo were observed at 60 and 90 minutes after dosing. *Ayahuasca* decreased power density in the alpha-2, delta, theta and beta-1 frequency bands. This pattern of effects is analogous to that of the classical psychedelics and point out the involvement of 5-HT_{2A} receptor agonism in the neurochemical effects of *ayahuasca*. Power decreases in the delta, alpha-2 and beta-1 bands were found predominantly over the temporo-parieto-occipital junction, whereas theta power was reduced in the temporomedial cortex and in frontomedial regions. The present results suggest the involvement of unimodal and heteromodal association cortex and limbic structures in the psychological effects elicited by *ayahuasca*.

Key words: Ayahuasca, DMT, EEG, Psychedelics, Brain electrical sources, LORETA, Human.

1. Introduction

The psychoactive plant tea known as *ayahuasca*, a Quechuan name meaning vine of the souls or vine of the dead, is a traditional shamanic inebriant used in the Upper Amazon since pre-Columbian times for religious and medicinal purposes (Schultes and Hofmann, 1982). In the second half of the last century, the use of *ayahuasca* reached the urban areas of Amazonian countries, where it is used by *ayahuasqueros* for healing and divination. However, modern non-indigenous use of *ayahuasca* mainly takes place within the context of syncretic religious groups, particularly the Brazilian *Santo Daime* and *União do Vegetal*, that have combined Old World religious beliefs with the sacramental use of the beverage (Dobkin de Rios, 1996). In recent years groups of followers of these Brazilian religions have become established in the United States and in several European countries (Anonymous, 2000).

Botanical research into the plant sources of *ayahuasca* has shown that the main ingredient of the tea is the woody vine *Banisteriopsis caapi* (malpighiaceae). *Ayahuasca* is obtained by infusing the pounded stems of the vine either alone or more frequently in combination with the leaves of *Psychotria viridis* (rubiaceae) or *Diplopterys cabrerana* (malpighiaceae). *B. caapi* contains notable amounts of β-carboline alkaloids, mainly harmine and tetrahydroharmine (THH), and to a lesser extent harmaline and traces of harmol and harmalol (Rivier and Lindgren, 1972; McKenna et al., 1984). *P. viridis* and *D. cabrerana* also contain indole alkaloids, mainly the potent short-acting psychedelic agent DMT (River and Lindgren, 1972). DMT is structurally related to the neurotransmitter serotonin, and like better-characterized psychedelics such as LSD and mescaline (a phenethylamine), binds to the 5-HT_{2A} receptor sites in the central nervous system (CNS), where it acts as an agonist (Smith et al., 1998).

The combination of DMT from *P. viridis* with the β-carboline alkaloids from *B. caapi* in a single oral preparation is most remarkable from the pharmacological point of view. It takes advantage of the pharmacodynamic properties of the β-carbolines, which allow access to the system of the otherwise orally inactive tryptamine component. Indeed, DMT is known for its lack of psychoactivity when orally ingested (Ott, 1999), probably due to metabolism by the enzyme monoamine oxidase (MAO) (Suzuki et al., 1981). On the other hand, the β-carbolines present in *ayahuasca*, particularly harmine and harmaline, are potent natural MAO inhibitors (McKenna et al., 1984), apparently preventing the extensive gut and liver first-pass effect on DMT, which is subsequently able to reach unaltered systemic circulation and the CNS.

In a clinical research setting, *ayahuasca*, has been found to induce transient modifications in perception, thought processes and mood, that fit a combined stimulatory and psychedelic effect profile, as measured by subjective effect self-assessment instruments (Riba et al., 2001a).

The aim of the present study was to assess the differential involvement of cortical brain regions in the acute central effects of *ayahuasca* by means of a recently developed neuroimaging technique: low resolution electromagnetic tomography (LORETA). Based on the scalp electrical potential distribution obtained by means of classical EEG measures, LORETA provides three-dimensional information regarding the cortical neural generators of brain electrical activity (Pascual-Marqui et al., 1994). Furthermore, LORETA computes a unique three-dimensional intracerebral power density distribution for the different EEG frequency bands, allowing their separate analysis. Unlike dipole modeling, LORETA makes no a-priori assumptions about the number of sources involved. The only constraint implemented is that of maximal smoothness of the solution, based on the assumption that neighboring neuronal sources are likely to be similarly active (i.e., have similar orientations and strengths). The distribution obtained is thus the smoothest of all possible inverse solutions, as it is considered the most plausible. In a new implementation of LORETA, an additional neuroanatomical constraint restricts the solution space to cortical gray matter volume (Pascual-Marqui et al., 1999). The technique has previously been used in the evaluation of acute effects of psychoactive drugs (Anderer et al., 2000; Frei et al., 2001).

To our knowledge, regional brain electrical activity has not been evaluated previously by means of LORETA following the administration of *ayahuasca* or other psychedelics with 5-HT_{2A} agonist activity. It is consequently difficult to establish a-priori hypothesis regarding the brain areas involved in the effects of *ayahuasca* on the EEG. However, PET and SPECT investigations on blood flow and glucose metabolism after acute psilocybin (an indoleamine structurally similar to DMT) and mescaline administration have evidenced increased activation in prefrontal regions (Hermle et al., 1992; Vollenweider et al., 1997a; Gouzoulis-Mayfrank et al., 1999). Consequently, we postulated that changes in electrical activity would be identified at least in the prefrontal cortex.

2. Materials and methods

2.1. Volunteers

Eighteen healthy volunteers (fifteen males and three females) participated in the study. Eligibility criteria in-

cluded prior experience with psychedelic drugs at least on five occasions without sequelae derived thereof, no current or previous history of neurological or psychiatric disorder, and no family history of Axis-I psychiatric disorder in first degree relatives. Volunteers were given a structured psychiatric interview (DSM-III-R) and completed the trait-anxiety scale from the State-Trait Anxiety Inventory (STAI). Exclusion criteria included alcohol or other substance dependence, and high scores on trait anxiety (over 1 standard deviation above normative mean). Each participant underwent a complete physical examination that included a medical history, laboratory tests, ECG and urinalysis. Their mean age was 25.7 years (range: 19-38), mean weight 66.47 kg (range: 50.7-79.5) and mean height 175.11 cm (range 158-188). In addition to their prior intake of psychedelics, all volunteers had previous experience with *Cannabis* and cocaine. Although prior exposure specifically to *ayahuasca* was not required for participation, two of the volunteers had ingested the drug before inclusion in this study. 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. Written informed consent was obtained from all participants.

2.2. Drug

The *ayahuasca* employed in the present study was not administered in its original liquid form, but as a lyophilizate. The freeze-dried homogenized material had been obtained from a 9.6 liter batch of *ayahuasca*. The DMT content in the lyophilizate had been determined by HPLC, as described by Callaway and coworkers (1996), and the β-carboline constituents following a modification of the method described therein. The 9.6 liter batch yielded 611 grams of freeze-dried powder, containing 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline and 11.36 mg THH per gram. Based on tolerability and subjective effects assessed previously (Riba et al., 2001a), an *ayahuasca* dose containing 0.85 mg DMT/kg body weight was administered to the volunteers. The calculated individual dose for each volunteer was administered by combining 00 gelatin capsules containing 0.5 g, 0.25 g or 0.125 g of freeze-dried *ayahuasca.* Placebo capsules were 00 gelatin capsules containing 0.75 g lactose. These were administered on placebo day, and were also combined with active *ayahuasca* capsules when necessary, so that all volunteers took the same number of capsules on each experimental day.

2.3. Study design and experimental procedure

EEG recordings were obtained in a double-blind placebo-controlled randomized crossover clinical trial. Two weeks prior to the beginning of the experimental procedure, volunteers were requested to abstain from any medication or illicit drug use until the completion of the study. Volunteers also abstained from alcohol, tobacco and caffeinated drinks 24 hours prior to each experimental day. Urine was screened for illicit drug use on each experimental day. Experimental days were at least one week apart.

On each experimental day, volunteers remained in the clinical research unit for a period of approximately ten hours. Following arrival in the morning under fasting conditions, EEG electrodes were placed on the scalp, and drug/placebo capsules were administered at approximately 10:00 am with 250 ml tap water. EEG recordings were obtained at baseline and at regular intervals after treatment administration. For the first four hours, volunteers remained seated in a reclining chair in a quiet and dimly-lit room. The experimenter remained outside the room during the EEG recordings The last recording was performed at eight hours and volunteers were discharged approximately nine hours after administration

3. Measurements

3.1. EEG acquisition and processing and LORETA analysis

Nineteen-lead EEG recordings were obtained by means of scalp electrodes placed according to the international 10/20 system: Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1 and O2, referenced to averaged mastoids. Additionally, vertical and horizontal electrooculograms were recorded. The signal was acquired through a Neuroscan SYNAMPS amplifier. Three-minute vigilance-controlled EEG (V-EEG) with eyes closed was recorded at baseline, prior to drug administration, and at different time points after dosing. During the V-EEG recordings, the experimenter tried to keep the volunteers alert; as soon as drowsiness patterns appeared in the EEG they were aroused by acoustic stimulation. The EEG signal was band-pass filtered at 0.3-30 Hz, and digitized online with a sampling frequency of 100 Hz. EEG recordings were obtained prior to drug administration (-15 min and baseline), and at 30, 60, 90, 120, 180, 360 and 480 minutes after dosing.

A two-step artifact processing procedure was used

(Anderer et al., 1992). It included ocular artifact minimization based on regression analysis in the time domain, as described by Semlitsch et al. (1986), and automatic artifact rejection based on a time and frequency domain approach as described by Anderer et al. (1987). Validity of the artifact processing procedure was visually assessed. After recomputation to average reference, spectral analysis was performed for artifact-free 5-s epochs. For each recording, spectral power of six 5-s epochs of artifact-free, vigilance-controlled EEG were averaged. Data were digitally filtered into seven frequency bands according to Kubicki et al. (1979): delta [1.5-6 Hz], theta [6-8Hz], alpha-1 [8-10 Hz], alpha-2 [10-12 Hz], beta-1 [12-18 Hz], beta-2 [18-21 Hz] and beta-3 [21-30 Hz].

Subsequently, LORETA was used to estimate the three-dimensional intracerebral current density distribution from the voltage values recorded at the scalp electrodes. The LORETA version employed implements a three-shell spherical head model (Ary et al., 1981) registered to the Talairach human brain atlas (Talairach and Tournoux, 1988) available as a digitized MRI from the Brain Imaging Centre, Montréal Neurological Institute. The EEG electrode coordinates reported by Towle et al. (1993) were used for registration between spherical and realistic head geometry. The LORETA solution space was restricted to cortical gray matter and hippocampus, based on the Digitized Probability Atlas corresponding to the Talairach and also available from the Brain Imaging Centre, Montréal Neurological Institute. A voxel was included in the solution space if its probability of being gray matter was higher than 33%, and higher than its probability of being either white matter or cerebrospinal fluid. The final solution space consisted of 2394 voxels with a spatial resolution of 0.343 cm^3 (Pascual-Marqui et al., 1999). The EEG lead field was computed numerically with the boundary element method (Pascual-Marqui, 1999). LORETA images represent the power (i.e., squared magnitude of computed intracerebral current density) in each of the 2394 voxels. "LORETA power", synonymous to "EEG power", refers to the spectral power of current density as estimated by LORETA. Thus, in a first step, current density values were estimated based on the EEG cross-spectral matrix and then squared for each voxel and frequency band (for mathematical details see Frei et al., 2001).

3.2. Subjective effects

Volunteers were requested to answer the Hallucinogen Rating Scale (HRS), a self-report questionnaire measuring psychedelic-induced subjective effects (Strassman et al., 1994). The HRS includes six subscales: *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 version of the questionnaire was used (Riba et al., 2001b). The HRS was administered at 240 minutes post-administration.

4. Statistical analysis

4.1. LORETA data

In a first step, in order to explore the time course of *ayahuasca* effects, paired-samples *t*-tests were computed for log-transformed LORETA power at each voxel and frequency band for the different time points. To correct for multiple comparisons, a non-parametric single-threshold test was applied on the basis of the theory for randomization and permutation tests developed by Holmes et al. (1996). The omnibus null hypothesis of no activation anywhere in the brain was rejected if at least one t-value (i.e. voxel, t_{MAX}) was above the critical threshold t_{CRIT} for P= 0.05 determined by 5000 randomizations. The total number of suprathreshold voxels was plotted versus time in order to select the time points with the largest effects. Subsequently, LORETA images were computed for log-transformed normalized data for each separate frequency band at the selected time points and following the same statistical approach. On the basis of the Structure-Probability Maps Atlas (Lancaster et al., 1997), the number of significant voxels in each lobe (frontal, parietal, occipital, temporal, limbic and sub-lobar), gyrus, and Brodmann area (BA) of the left and the right hemisphere was computed separately for each suprathreshold region.

4.2. Subjective effects

Scores on the HRS questionnaire after *ayahuasca* were compared with placebo by means of paired-samples Student's *t*-tests.

5. Results

5.1. LORETA data

Fig. 1 shows the results for the omnibus significance test performed for all voxels and frequency bands at the different time points in order to explore the time course of

effects.

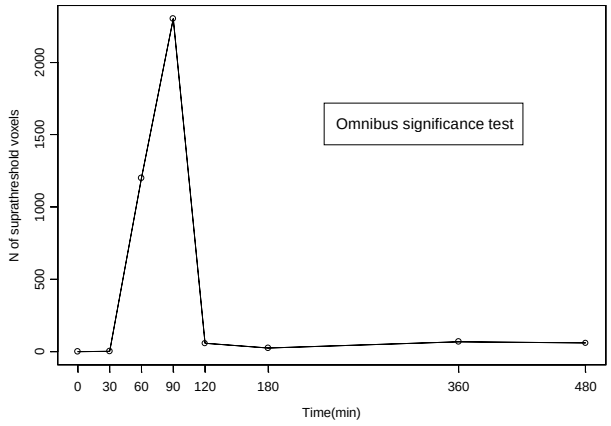

Fig. 1. Omnibus significance test. The total number of suprathreshold voxels at different time points after ayahuasca (0.85 mg DMT/kg body weight) administration are shown.

A steep rise in the number of suprathreshold voxels was observed at 60 min following drug administration, and the pharmacodynamic peak at 90 min after dosing. LORETA images were thus computed for the *ayahuasca*- vs. placebo-induced changes at these two time points.

The voxel by voxel statistical comparison of *ayahuasca*-induced vs. placebo-induced effects at 60 min after drug administration (0.85 mg DMT/kg body weight dose), followed by Holmes correction, showed statistically significant decreases mainly in the alpha-2 frequency band (459 suprathreshold voxels). As listed in Table 1, power density decreases were found in the parietal (135), occipital (79), temporal (170) and limbic (69) lobes in both hemispheres, and at the left frontal (3) and sublobar level (3). Suprathreshold voxels were thus found over extensive cortical areas around the temporo-parieto-occipital junction predominantly in the angular gyrus, supramarginal gyrus, precuneus, superior and middle temporal gyri and fusiform gyrus. In the limbic lobe, suprathreshold voxels covered mainly the cingulate and the parahippocampal gyrus. The BAs showing the highest percentage of suprathreshold voxels were BA 7 in the parietal lobe, BA 19 in the occipital lobe, BA 39 and BA 37 in the temporal lobe and BA 36 and BA 35 in the limbic lobe.

Table 1
Ayahuasca- vs. placebo-induced decreases in Alpha-2 power (10-12 Hz) 60 min post-administration. The number of significant voxels (N_{sig}), the total number of voxels (N_{total}) and the percentage of significant voxels (%) for each gyrus and hemisphere are given (*n*=18).

Alpha-2 (10-12 Hz)	**Suprathreshold voxels**					
	Left hemisphere			**Right hemisphere**		
Gyrus	**N_{sig}**	**N_{total}**	**%**	**N_{sig}**	**N_{total}**	**%**
Frontal lobe						
Subcallosal gyrus	2	7	29	0	7	0
Precentral gyrus	1	38	3	0	37	0
Parietal lobe						
Postcentral gyrus	6	39	15	6	44	14
Supramarginal gyrus	2	10	20	11	11	100
Superior parietal lobule	16	24	67	4	17	24
Precuneus	48	73	66	18	65	28
Inferior parietal lobule	1	56	2	13	50	26
Angular gyrus	3	4	75	7	7	100
Occipital lobe						
Cuneus	9	35	26	2	30	7
Lingual gyrus	4	38	11	13	31	42
Superior occipital gyrus	3	4	75	5	5	100
Middle occipital gyrus	16	26	62	20	24	83
Inferior occipital gyrus	5	10	50	2	9	22
Temporal lobe						
Superior temporal gyrus	10	85	12	20	95	21
Middle temporal gyrus	30	89	34	30	88	34
Inferior temporal gyrus	10	51	20	4	52	8
Fusiform gyrus	43	58	74	18	53	34
Sub-gyral	4	12	33	1	9	11
Limbic lobe						
Cingulate	8	8	100	1	8	13
Posterior cingulum	1	15	7	1	20	5
Parahippocampal gyrus	32	33	97	12	31	39
Uncus	14	24	58	0	24	0
Sub-lobar						
Insula	3	38	8	0	32	0

Fig. 2 shows LORETA axial brain slices as statistical non-parametric maps corresponding to the suprathreshold regions found for the alpha-2 frequency band, at 60 min after drug administration.

voxels, i.e., the pharmacodynamic peak, was observed at 90 min post-administration. At this time point statistically significant decreases were found for the delta, theta and beta-1 frequency bands. Alpha-2 power density was also

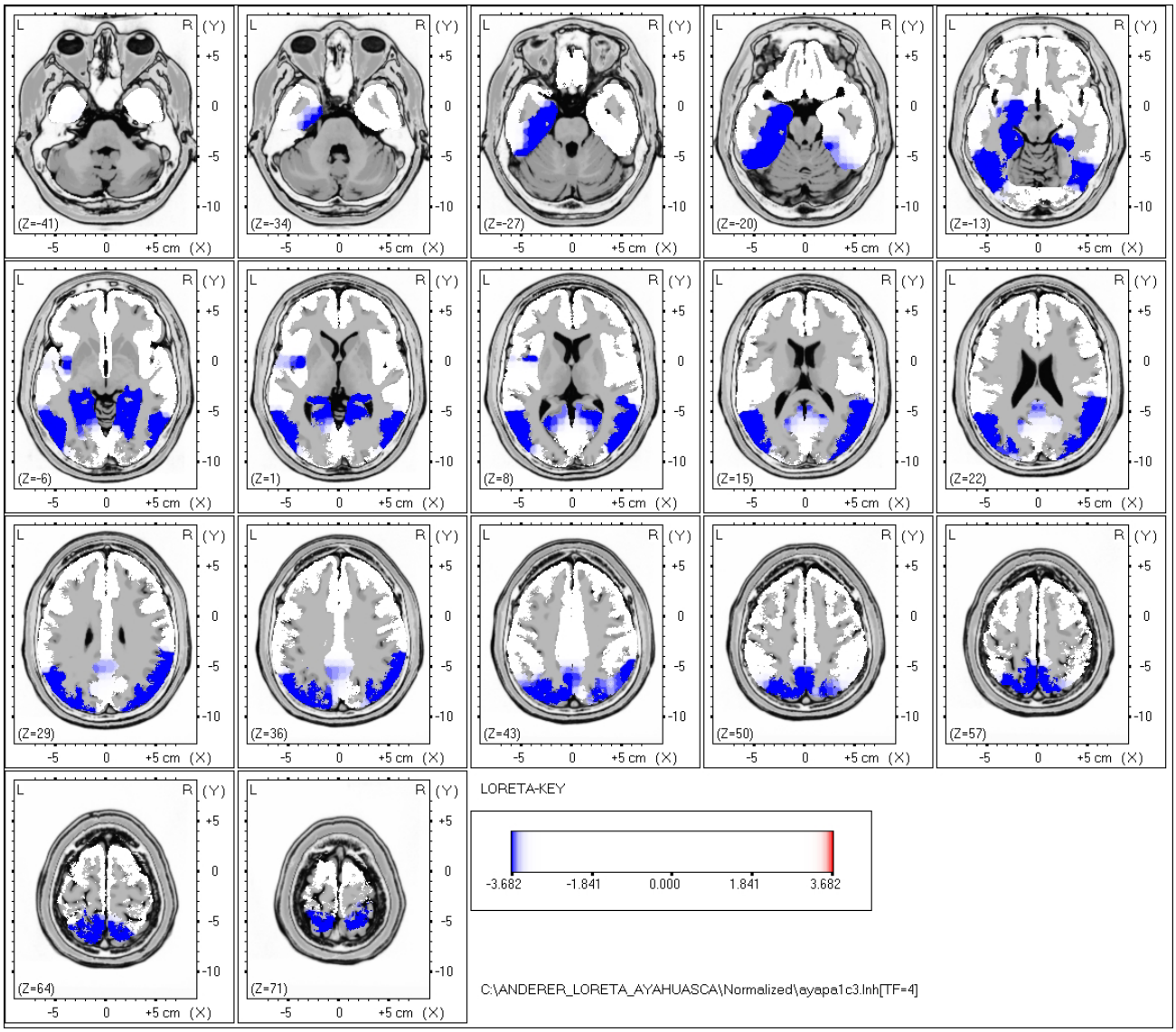

Fig. 2. Effects of ayahuasca (0.85 mg DMT/kg body weight) on regional cortical electrical activity at 60 min after administration (n=18): Statistical non-parametric maps based on t-values of differences between ayahuasca-induced and placebo-induced changes in the alpha-2 (10-12 Hz) frequency band. Blue indicates significant decreases after Holmes correction ($P < 0.05$) as compared to placebo. Axial slices (head seen from above, nose up, L = left, R = right) in steps of 7 mm from most inferior (Z = -41) to the most superior (Z = 71).

In addition to the above results, power decreases were observed for the delta frequency band in a small area covering 15 suprathreshold voxels in the border between the left occipital and temporal lobes in BA 19, BA 37 and BA 21. Finally, decreases in the theta band fell short of statistical significance with the lowest *t*-value equal to –3.58 (*P*=0.0522; cuttoff *t*-value =3.61). This local minimum was located in BA 24 in the medial frontal cortex.

As shown in Fig. 1, the largest number of suprathreshold

reduced relative to placebo, but contrary to what was observed at 60 min after administration these decreases were not statistically significant.

Table 2 lists the anatomical distribution of the power decreases observed for the delta frequency band. Thus, 471 suprathreshold voxels were located in the parietal (135), occipital (70), temporal (263) and limbic (3) lobes in both hemispheres. Suprathreshold voxels were found over extensive cortical areas around the temporo-parieto-occipi-

tal junction predominantly in the angular gyrus, superior occipital gyrus, middle temporal gyrus and fusiform gyrus. The BAs showing the highest percentage of suprathreshold voxels were BA 37, BA 19 and BA 39.

Fig. 3 shows LORETA axial brain slices as statistical non-parametric maps corresponding to the suprathreshold regions found for the delta frequency band. Note the marked overlap between these brain areas and those found at 60 min for the alpha-2 frequency band.

Areas of power density decrease in the theta frequency band are indicated in Table 3. One hundred and twenty-eight voxels showed *t*-values below the statistical threshold. These suprathreshold voxels were found in the frontal lobe (13), in the temporal lobe (58) and in the limbic lobe (57). Suprathreshold voxels were found distributed in three non-confluent areas: i.e., in the medial and superior frontal gyri (BA 6, 8), in the anterior cingulate (BA 24, 32), and in the left temporomedial cortex comprising mainly the fusiform and parahippocampal gyri and the uncus (BA 36, 35, 28).

Fig. 4 shows LORETA orthogonal slices (axial, sagittal and coronal views) as statistical non-parametric maps corresponding to the three non-confluent suprathreshold regions found for the theta frequency band, through the voxel of the extreme *t*-value.

Areas of beta-1 power density decrease are shown in Table 4. These comprised 139 suprathreshold voxels, located in the parietal lobe (122), occipital lobe (3), temporal lobe (5), and limbic lobe (9). Suprathreshold voxels were found on two non-confluent areas. The first area comprised voxels in the parietal lobe, extending medially and bilaterally from the posterior cingulate gyrus (BA 30, 31) to the precuneus and superior parietal lobule (BA 7, 19). The second area comprised voxels in the right supramarginal and angular gyri (BA 39).

Fig. 5 shows LORETA orthogonal slices (axial, sagittal and coronal views) as statistical non-parametric maps corresponding to the two non-confluent suprathreshold regions found for the beta-1 frequency band, through the voxel of the extreme *t*-value.

Table 2
Ayahuasca- vs. placebo-induced decreases in Delta power (1.5-6 Hz) 90 min post-administration. The number of significant voxels (N_{sig}), the total number of voxels (N_{total}) and the percentage of significant voxels (%) for each gyrus and hemisphere are given (n=18).

Delta (1.5-6 Hz)	**Suprathreshold voxels**					
	Left hemisphere			**Right hemisphere**		
Gyrus	**N_{sig}**	**N_{total}**	**%**	**N_{sig}**	**N_{total}**	**%**
Parietal lobe						
Postcentral gyrus	1	39	3	9	44	20
Supramarginal gyrus	5	10	50	8	11	73
Superior parietal lobule	9	24	38	13	17	76
Precuneus	37	73	51	17	65	26
Inferior parietal lobule	4	56	7	21	50	42
Angular gyrus	4	4	100	7	7	100
Occipital lobe						
Cuneus	10	35	29	1	30	3
Superior occipital gyrus	4	4	100	5	5	100
Middle occipital gyrus	20	26	77	19	24	79
Inferior occipital gyrus	8	10	80	3	9	33
Temporal lobe						
Superior temporal gyrus	19	85	22	15	95	16
Middle temporal gyrus	62	89	70	42	88	48
Inferior temporal gyrus	20	51	39	24	52	46
Fusiform gyrus	41	58	71	40	53	75
Limbic lobe						
Parahippocampal gyrus	0	33	0	3	31	10

5.2. Subjective effects

Ayahuasca induced a series of perceptual, mood and cognitive modifications with a characteristic psychedelic pattern, as measured by the HRS questionnaire. Table 5 shows the mean values obtained after placebo and *ayahuasca* for the HRS scores, and the results of the statistical analyses performed.

Drug-induced subjective effects were first noted as early as 15 minutes, became more marked between 30 and 45, showed a steep rise at 60 minutes, reached a maximum

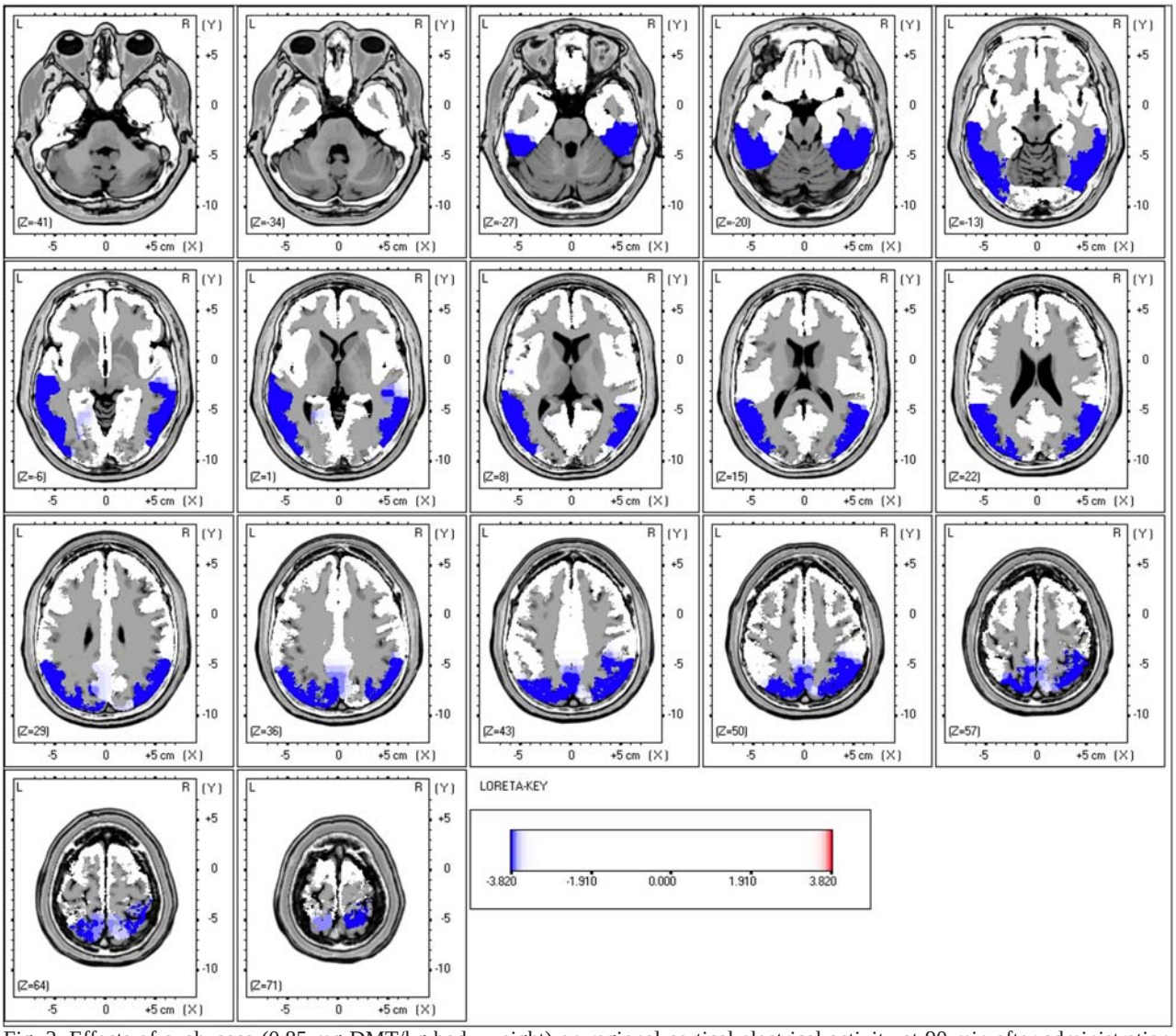

Fig. 3. Effects of ayahuasca (0.85 mg DMT/kg body weight) on regional cortical electrical activity at 90 min after administration (n=18): Statistical non-parametric maps based on t-values of differences between ayahuasca-induced and placebo-induced changes in the delta (1.5-6 Hz) frequency band. Blue indicates significant decreases after Holmes correction ($P < 0.05$) as compared to placebo. Axial slices (head seen from above, nose up, L = left, R = right) in steps of 7 mm from most inferior (Z = -41) to the most superior (Z = 71).

Table 3
Ayahuasca- vs. placebo-induced decreases in Theta power (6-8 Hz) 90 min post-administration. The number of significant voxels (N_{sig}), the total number of voxels (N_{total}) and the percentage of significant voxels (%) for each gyrus and hemisphere are given (n=18).

Theta (6-8 Hz)	**Suprathreshold voxels**					
	Left hemisphere			**Right hemisphere**		
Gyrus	**N_{sig}**	**N_{total}**	**%**	**N_{sig}**	**N_{total}**	**%**
Frontal lobe						
Medial Frontal gyrus	2	61	3	2	59	3
Superior Frontal gyrus	4	100	4	5	98	5
Temporal lobe						
Middle Temporal gyrus	1	89	1	0	88	0
Inferior Temporal gyrus	13	51	25	0	52	0
Fusiform gyrus	43	58	74	0	53	0
Sub-gyral	1	12	8	0	9	0
Limbic lobe						
Anterior cingulum	2	25	8	2	25	8
Cingulate gyrus	8	42	19	7	41	17
Cingulate	6	8	75	0	8	0
Parahippocampal gyrus	18	33	55	0	31	0
Uncus	14	24	58	0	24	0

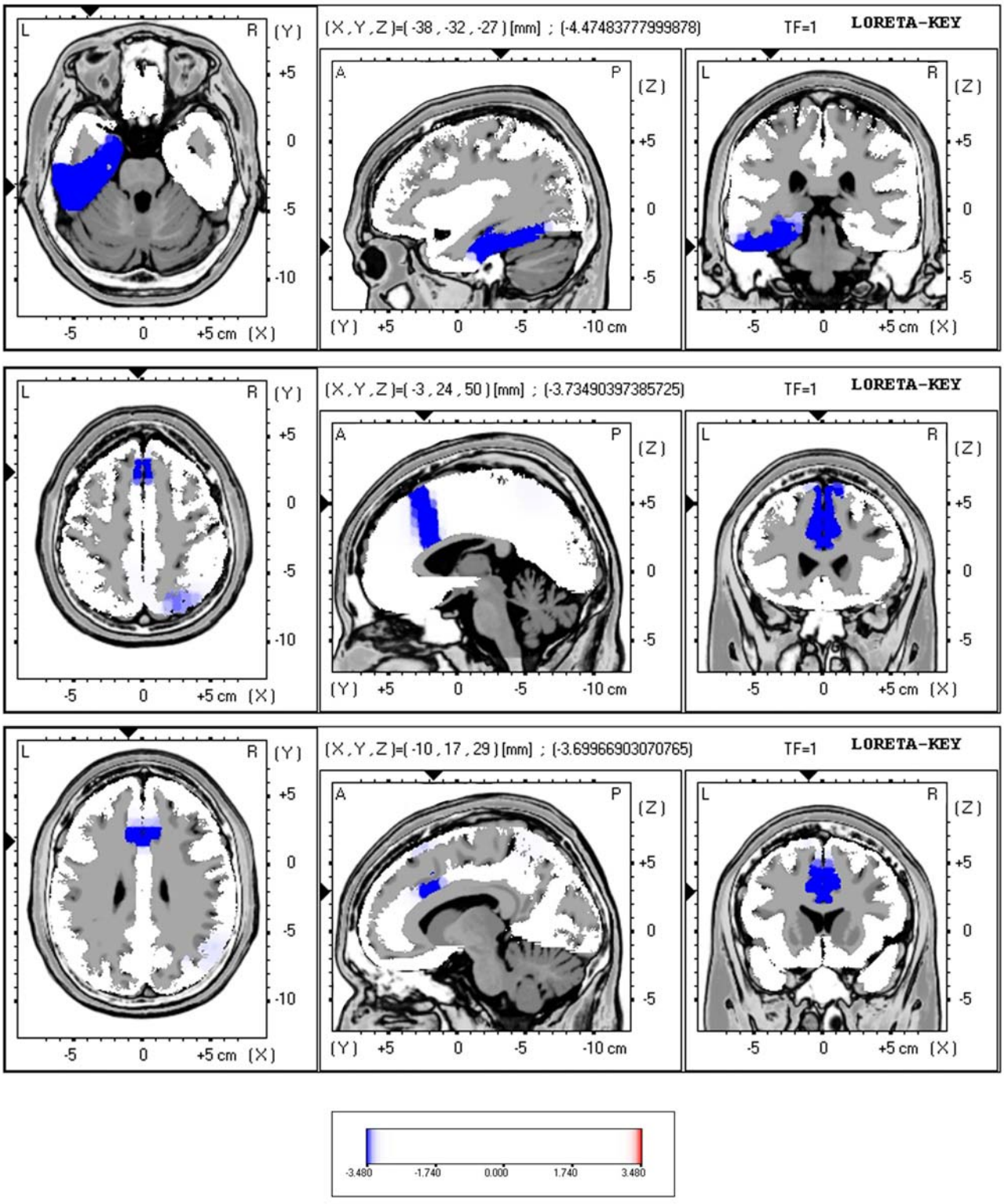

Fig. 4. Effects of ayahuasca (0.85 mg DMT/kg body weight) on regional cortical electrical activity at 90 min after administration (n=18): Statistical non-parametric maps based on t-values of differences between ayahuasca-induced and placebo-induced changes in the theta (6-8 Hz) frequency band. Blue indicates significant decreases after Holmes correction ($P < 0.05$) as compared to placebo. Orthogonal (axial, sagittal, coronal) slices for each of the three non-confluent suprathreshold regions, i.e., temporomedial (upper row), frontomedial (middle row), and cingulate (lower row), through the voxel of the extreme t-value. The Talairach coordinates (x, y, z) of the displayed extreme t-value are indicated by black triangles on the coordinate axes. L, left; R, right, A, anterior; P, posterior.

Table 4
Ayahuasca- vs. placebo-induced decreases in Beta-1 power (12-18 Hz) 90 min post-administration. The number of significant voxels (N_{sig}), the total number of voxels (N_{total}) and the percentage of significant voxels (%) for each gyrus and hemisphere are given (*n*=18).

Beta-1 (12-18 Hz)	**Suprathreshold voxels**					
	Left hemisphere			**Right hemisphere**		
Gyrus	**N_{sig}**	**N_{total}**	**%**	**N_{sig}**	**N_{total}**	**%**
Parietal lobe						
Postcentral gyrus	6	39	15	6	44	14
Supramarginal gyrus	0	10	0	3	11	27
Superior parietal lobule	9	24	38	9	17	53
Precuneus	38	73	52	45	65	69
Inferior parietal lobule	0	56	0	2	50	4
Angular gyrus	0	4	0	4	7	57
Occipital lobe						
Cuneus	0	35	0	3	30	10
Temporal lobe						
Superior temporal gyrus	0	85	0	3	95	3
Middle temporal gyrus	0	89	0	2	88	2
Limbic lobe						
Cingulate gyrus	4	42	10	3	41	7
Posterior cingulum	1	15	7	1	20	5

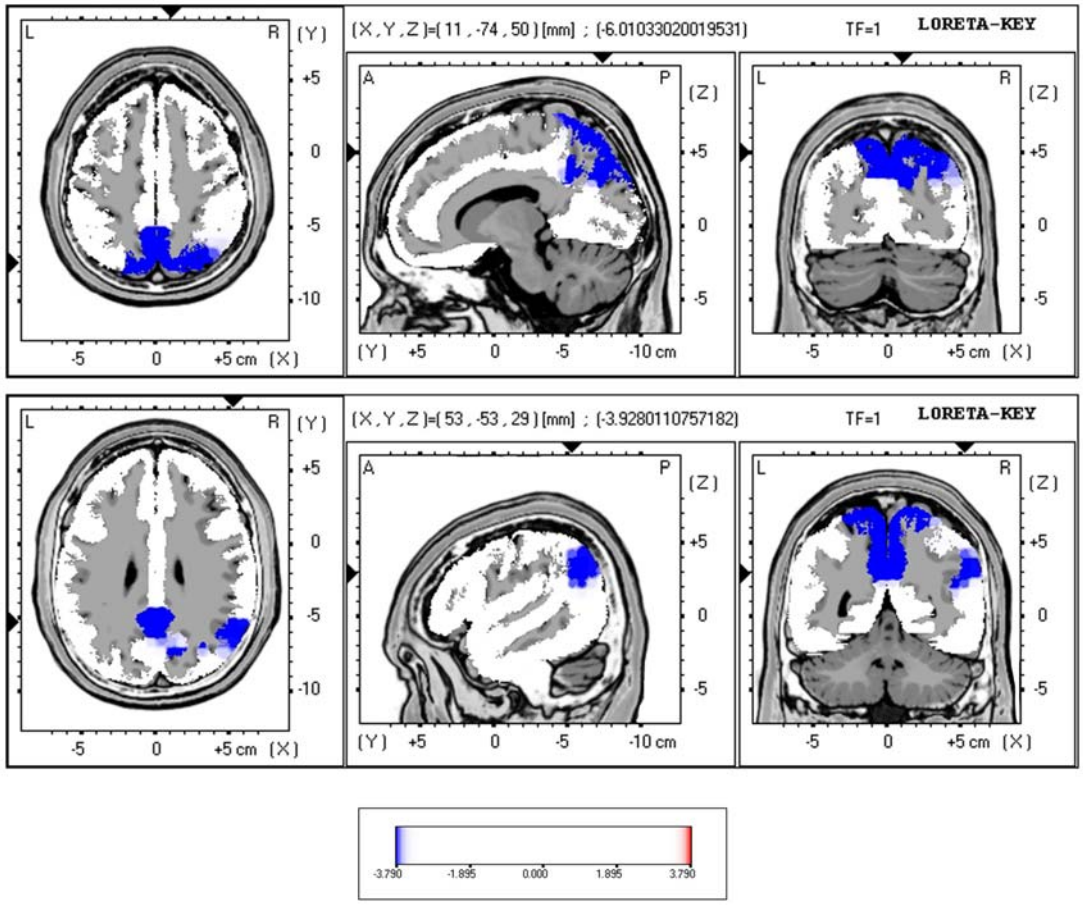

Fig. 5. Effects of ayahuasca (0.85 mg DMT/kg body weight) on regional cortical electrical activity at 90 min after administration (n=18): Statistical non-parametric maps based on t-values of differences between ayahuasca-induced and placebo-induced changes in the beta-1 (12-18 Hz) frequency band. Blue indicates significant decreases after Holmes correction ($P < 0.05$) as compared to placebo. Orthogonal (axial, sagittal, coronal) slices for each of the two non-confluent suprathreshold regions, i.e., parietomedial (upper row), and parietolateral (lower row), through the voxel of the extreme t-value. The Talairach coordinates (x, y, z) of the displayed extreme t-value are indicated by black triangles on the coordinate axes. L, left; R, right, A, anterior; P, posterior.

Table 5
Means (SD) of the scores obtained for the HRS questionnaire subscales, and results of the statistical comparisons performed by means of paired-samples Student's t-tests (n=18).

	HRS scores	
	Placebo	High dose
Somaesthesia	0.07 (0.10)	0.97 (0.40) **
Perception	0.09 (0.19)	1.10 (0.67) **
Cognition	0.06 (0.16)	0.96 (0.59) **
Volition	0.81 (0.79)	1.35 (0.61) **
Affect	0.32 (0.21)	1.02 (0.38) **
Intensity	0.24 (0.45)	1.85 (0.51) **

** = $P < 0.01$

between 90 and 120 minutes and decreased thereafter, disappearing entirely at 360 minutes. All volunteers experienced somatic modifications, which included altered bodily sensations, pins and needles, increased skin sensitivity and physical comfort. Visual and auditory phenomena were also frequently reported but greatly varied in quality and intensity between volunteers, ranging from distortions of objects and sounds, to elaborate visions with eyes closed and perception of non-existing noises. Five subjects reported experiencing auditory and visual synesthesia. In the cognitive sphere, the sense of time was altered, thought speed increased, and the ability to focus attention was subjectively perceived to be reduced. Mood modifications were also present, the experience being globally regarded as satisfactory, with most volunteers reporting having experienced happiness, excitation and a "high". 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.

6. Discussion

The analysis of brain electrical activity by means of LORETA identified significant drug-induced changes in the intracerebral power density distribution after 60 and 90 min following *ayahuasca* administration. At the peak of pharmacodynamic effects, the slow delta rhythm was decreased in posterior brain regions. Additionally, decreases in theta were observed in the medial frontal and medial temporal cortices. Similar effects, although less intense (delta) or bordering statistical significance (theta), were also observed in analogous brain regions at 60 min after dosing. At this time point, however, a widespread power reduction in the alpha-2 band was observed in posterior areas showing considerable overlap with those demonstrating significant decreases in delta power at 90 min post-administration. These modifications of the EEG power spectrum were accompanied by a constellation of perceptual, cognitive and mood modifications typical of the psychedelics, as evidenced by significant increases in all subscales of the HRS. The pattern of subjectively-reported effects corresponded qualitatively and in time course with previous results obtained in a smaller sample of volunteers (Riba et al., 2001a). It is of particular interest that these effects involving perceptive, and cognitive modifications were regarded by the majority of volunteers as positive and desirable, in contrast with the more psychosis-like profile, including paranoid thoughts and fear of control loss, reported in studies in which other psychedelics, such as psilocybin, have been administered to drug-naive subjects (Vollenweider et al., 1997a). The fact that volunteers who enrolled in the present study had prior experience with psychedelics may account for these differences.

The effects of *ayahuasca* on the EEG power spectrum are compatible with its proposed neurochemical mechanism of action. Decreases in slow activity, i.e., delta and theta power, are a general feature of psychostimulants, such as amphetamine and methylphenidate, serotonin releasers such as fenfluramine, and psychedelics displaying 5-HT_2 agonist activity (Itil et al., 1966; Herrmann et al., 1986; Saletu et al., 1993). Early pharmaco-EEG research on LSD, a 5-HT_{2A} agonist like DMT -the main psychotropic principle found in *ayahuasca*- had also shown decreases in slow activity after acute drug administration (Itil et al., 1966). Delta activity has traditionally been thought to reflect inhibitory activity, and increases in theta have been observed in relaxed and meditative states. Thus, the present results would rather suggest an excitatory or arousing effect for *ayahuasca*. This assumption is further supported by the fact that major tranquilizers with D_2 or mixed D_2/5-HT_2 antagonist activity, such as chlorpromazine and risperidone, are characterized by their delta and theta-promoting effects (Saletu et al., 1993; Lee et al., 1999). An important difference between *ayahuasca* and psychostimulants is the alpha-2 decreasing properties found in the present study. In addition to slow wave dampening effects, amphetamine is known to enhance alpha, a feature not shared by *ayahuasca*. As mentioned above, Itil and Fink (1966) found LSD had inhibitory effects on slow waves, but also reduced alpha. Interestingly, other drugs, such as scopolamine or ketamine, with various mechanisms of action and different overall EEG profiles but able to elicit hallucinatory states, also display an alpha suppressing effect. Thus, *ayahuasca* would combine alpha-dampening effects, a feature shown by other perception-

altering drugs, with slow wave reduction properties, in an overall profile which would bring it closer to drugs such as LSD rather than to pure psychostimulants.

Given the exploratory nature of the present study, it is the authors' view that some hypotheses should be postulated regarding the brain networks and processes targeted by *ayahuasca*, based on the spatial information provided by the LORETA analysis. The changes in intracerebral electrical source distribution showed considerable overlapping between frequency bands, mainly between alpha-2 and delta, although these effects were observed at different time points. Delta and alpha-2 power decreases were located bilaterally over somatosensory, auditory and visual association cortices and over temporo-parietal heteromodal association cortex (Mesulam, 2000). Power decreases in the beta-1 frequency band were predominantly found in somatosensory and visual association cortex, and also in heteromodal association cortex. Areas showing a theta power decrease, however, did not predominate in association cortex, but in paralimbic structures with relevant roles in emotion and memory processes (Mesulam, 2000). Thus, it can be hypothesized that drug-induced bioelectrical changes on unimodal sensory association cortex may have played a role in the modality-specific modifications in the visual, somatic and auditory perception reported. Additionally, it appears reasonable to assume that effects on transmodal brain areas could account for more complex cognitive modifications which also characterize the subjective experience elicited by *ayahuasca* (Riba et al., 2001a). In this respect, the temporo-parietal and frontomedial heteromodal association cortex, the cingulate and the temporomedial cortices play relevant roles in the neurobiology of attention, emotion, and memory (Squire and Zola-Morgan, 1991; Devinsky et al., 1995; Nyberg et al., 1996).

The results obtained in the present study show interesting similarities, but also differences, with previous SPECT and PET studies involving psychedelics. A recent PET investigation revealed that the most important metabolic increases after psilocybin administration to humans occur predominantly in the temporomedial, frontomedial, frontolateral and anterior cingulate cortices (Vollenweider et al., 1997a). These areas largely coincide with those showing theta decreases after *ayahuasca*. Nevertheless, PET and SPECT studies following psilocybin and mescaline have emphasized a hyperfrontality pattern, i.e., increased blood perfusion or glucose metabolism in frontal regions (Hermle et al., 1992; Vollenweider et al., 1997a). Metabolic increases in frontomedial regions, and more specifically in the anterior cingulate cortex appear to be a common feature of psychedelic drug effects, as these have been observed after psilocybin (Vollenweider et al., 1997a; Gouzoulis-Mayfrank et al., 1999) and after subanesthesic doses of ketamine (Vollenweider et al., 1997b). In the present study, however, only small areas within the frontal lobes were found to show drug-induced changes in power density distribution. Besides of the obvious differences in the drugs being administered, a possible explanation for this divergence could be the different nature of the variables under study (regional brain electrical activity vs. glucose metabolism or blood perfusion). Also, the aforementioned differences in the reported pattern of subjective effects should be taken into consideration.

In conclusion, *ayahuasca* effects on the EEG power spectrum involved mainly reductions in the slow delta and theta activity together with decreases in beta-1 and in the alpha-2 frequency band. The assessment of the spatial distribution of intracerebral power density changes singled out the temporo-parieto-occipital junction, and temporomedial and frontomedial areas as target regions of *ayahuasca* in the CNS. These areas comprise unimodal association cortex in the somatosensory, auditory and visual perception modalities, heteromodal association cortex, and also key regions within the limbic neural network involved in the integration of multimodal sensory information, and in emotion and memory processes. Future studies specifically addressing drug effects on these aspects of human cognition are needed in order to further our understanding the complex psychological modifications elicited by *ayahuasca*.

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Appendix II

Estimation of the bioavailability of DMT in Ayahuasca

Estimation of the bioavailability of DMT in *ayahuasca*

Introduction

It is a widely accepted hypothesis that the psychotropic effects of *ayahuasca* are mainly caused by the DMT present in the tea. It can be postulated that in order to obtain psychoactivity, a sufficient amount of DMT must survive first-pass metabolism in the gastrointestinal tract and liver, and reach unaltered systemic circulation and the CNS. In vitro and animal studies have shown that DMT can be degraded by MAO and that the β-carbolines in the tea are potent MAO inhibitors. Oral psychoactivity of DMT has also been demonstrated when MAO inhibitors other than the harmala alkaloids are ingested concomitantly. Additional support has thus been given to the DMT-β-carboline interaction hypothesis in *ayahuasca*. However, no data are available regarding the efficacy of this mechanism in terms of how much of the DMT found in the tea is successfully absorbed undegraded when *ayahuasca* is ingested.

Aim

The calculations presented in this appendix of the present dissertation aimed to provide an estimate of the bioavailability of DMT, i.e., the absorbed fraction or percentage of the total administered amount of DMT in *ayahuasca* reaching systemic circulation.

Methods

Bioavailability of DMT in *ayahuasca* was estimated by combining pharmacokinetic data from the final study presented in the present dissertation, with pharmacokinetic data obtained in a previous study conducted by other researchers following the i.v. administration of DMT to a group of healthy volunteers (Strassman and Qualls, 1994).

Design of the i.v. DMT study

Twelve volunteers received doses of 0.05, 0.1, 0.2 and 0.4 mg of DMT hemifumarate per kg body weight in a 30 second i.v. bolus followed by a 15 second flush with saline. Volunteers received the DMT injections on different experimental days and according to a double-blind, randomized placebo-controlled design. In the course of the experiment, various pharmacodynamic variables were assessed and blood samples were

drawn at 2, 5, 10, 15, 30 and 60 min after completion of the bolus. Time "0" coincided with the end of the 45 second bolus (Strassman and Qualls, 1994).

Design of the *ayahuasca* study

The design of the final *ayahuasca* study has been presented in detail in a preceding chapter of the present dissertation. In summary, in a double-blind, placebo-controlled, randomized, crossover clinical trial, 18 volunteers received two different doses of *ayahuasca* corresponding to 0.6 and 0.85 mg DMT/kg body weight. The low and the high dose contained the following amounts of β-carbolines per kg body weight: 1.0/1.4 mg harmine, 0.07/0.09 mg harmaline and 0.82/1.16 mg THH. DMT plasma levels were quantified from blood samples drawn at baseline, 30, 60, 90, 120, and 150 min, and 3, 4, 6, 8 and 24 h after *ayahuasca* administration (Riba et al., 2003).

Pharmacokinetic parameters

Pharmacokinetic parameters for i.v. DMT were calculated based on plasma concentrations at the following time points: 2, 5, 10, 15 and 30 min measured after administration of the 0.1, 0.2 and 0.4 mg DMT/kg doses. Both the lower 0.05 mg DMT/kg dose, and the 60 min time point after each of the other three doses were not analyzed due to values being too close to the limit of detection of the analytical method. Parameters were calculated for the 8 volunteers for whom DMT plasma levels were successfully measured after the three selected doses. The following pharmacokinetic parameters were calculated using a non-compartmental approach by means of WinNonlin software (version 3.0, Pharsight Corporation, California, USA): maximum observed concentration (C_{max}, at t=2 min); area under the concentration-time curve from 0 to infinity ($AUC_{0-\infty}$); $AUC_{0-\infty}$ normalized by dose ($AUC_{0-\infty}/D$); terminal half-life ($t_{1/2\lambda z} = \ln 2/\lambda_z$), obtained by linear regression analysis of the terminal log-linear portion of the plasma-concentration curve; clearance (Cl) determined as dose/$AUC_{0-\infty}$; and finally volume of distribution (V_z) calculated as dose/($\lambda_z \bullet AUC_{0-\infty}$). All data presented in the results section are expressed as mean (SD). In order to examine possible differences in pharmacokinetic parameters between doses, comparisons between doses were performed by means of Student's t-test for $AUC_{0-\infty}/D$, $t_{1/2\lambda z}$, Cl and V_z.

Pharmacokinetic parameters for DMT in *ayahuasca* were calculated for 15 of the 18 volunteers as described in a previous section of the present dissertation. In the results section of this appendix only V_z values are presented.

Estimation of bioavailability

Bioavailability (*F*) of DMT in *ayahuasca* was calculated by dividing the V_z value obtained from the i.v. administration study by the apparent volume of distribution V_z/F obtained for DMT in *ayahuasca*: $F=V_z/(V_z/F)$. *F* was calculated for each of the two *ayahuasca* doses administered. The method employed for the calculation of *F* is based on the assumption that *V*z does not change for DMT whether the drug is administered alone (i.v. study) or in combination with the other alkaloids present in *ayahuasca*. We assumed that while the *β*-carbolines, whose main pharmacological action is the inhibition of MAO, would affect DMT absorption and elimination, they would no affect the distribution of the drug in the organism. On the other hand, the inhibition of MAO by the *β*-carbolines would most likely alter the clearance value of DMT in *ayahuasca*. Consequently, the expression $F=Cl/(Cl/F)$ would not be adequate to calculate bioavailability in this case; nor would $F=(AUC_{oral}/AUC_{iv})$x(i.v. dose/oral dose) since the AUC value is mainly affected by the elimination phase of the drug.

Results

The calculated pharmacokinetic parameters for i.v. DMT are shown in table 1. Values indicate mean (SD).

Table 1

i.v. DMT

Dose	C_{max} (ng/ml)	$AUC_{0-\infty}$ (ng/ml . min^{-1})	$AUC_{0-\infty}/D$	$t_{1/2\lambda z}$ (min)	Cl (l/min)	V_z (l)
0.1 mg/kg	16.78 (9.77)	215.51 (105.41)	0.042 (0.021)	9.03 (3.77)	31.12 (18.51)	363.89 (173.80)
0.2 mg/kg	58.70 (56.77)	577.83 (471.02)	0.054 (0.04)	11.01 (10.02)	26.57 (16.43)	398.32 (438.78)
0.4 mg/kg	81.38 (57.68)	952.53 (512.06)	0.045 (0.021)	8.39 (2.09)	27.61 (14.53)	325.72 (183.25)

No differences were found between doses for $AUC_{0-\infty}/D$, $t_{1/2\lambda z}$, *Cl* and V_z calculated for i.v. DMT.

Bioavailability values for DMT in *ayahuasca* are presented in Table 2. Values were calculated for each of the two administered *ayahuasca* doses and employing each of the three V_z values calculated for i.v. DMT (one V_z per dose).

Table 2

i.v. DMT	***Ayahuasca***			
	Low dose		High dose	
V_z *(l)*	V_z/F *(l)*	*F (%)*	V_z/F *(l)*	*F (%)*
363.89	3509.86	10.37	2505.97	14.52
398.32	3509.86	11.35	2505.97	15.89
325.72	3509.86	9.28	2505.97	13.00
Mean *F*		**10.33**		**14.47**

As shown in the table, mean *F* values were around 10% for the low *ayahuasca* dose and around 15% for the high *ayahuasca* dose.

Comment

The obtained *F* values indicate that a relatively small fraction of the ingested DMT in *ayahuasca* is actually absorbed. The approach used to calculate *F* is not ideal, since i.v. and oral data were obtained in different groups of volunteers. Calculations had to be performed with grand-mean values rather than on an individual basis. Results should consequently be considered an approximation to the real *F* value. In their study, Callaway and coworkers (1999) provide a V_{ss}/F value of 54.8 l/kg, which would result in 4,066 l in a 74.2 kg individual (reported as the mean weight of the volunteers in their study). Thus, applying the calculation method used in the present work, *F* values in the Callaway et al. study (1999) ranged from 8% to 10%, which is in line with our own findings. A 10-15% bioavailability appears to be sufficient to elicit psychotropic effects, as demonstrated in the previous sections of the present dissertation. However, these

values pose questions regarding the metabolism of DMT in vivo. First, not all DMT in *ayahuasca* reaches systemic circulation, and second, the fraction which does pass is eventually cleared from the bloodstream. Thus, MAO activity has either only partially been inhibited by the *β*-carbolines, or DMT is redirected to other metabolic routes. In actual fact, metabolites other than 3-indoleacetic acid have been detected when DMT is incubated in several biological matrices (see the introduction section of the present dissertation). Future studies should identify the metabolism products of oral DMT administered alone in order to better understand the fate of this compound in the organism.

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Summary of results

Main Results

1. Results on subjective effect measures in the pilot and final study demonstrated statistically significant psychotropic effects for *ayahuasca,* with dose-dependent increases in five of the six subscales of the HRS and in the LSD, MBG and A scales of the ARCI. Subjective effects included feelings of increased activation (ARCI-A, VAS-stimulated), euphoria and well-being (ARCI-MBG, VAS-high, VAS-liking, VAS-good effects) and somatic modifications (ARCI-LSD), in addition to perceptual modifications (HRS-Perception, VAS-visions), changes in thought content (HRS-Cognition) and increased emotional lability (HRS-Affect). Psychological effects were first noted after 30-60 min, peaked between 60-120 min and were resolved by 4-6 h.

2. *Ayahuasca* induced dose-dependent modifications of brain electrical activity as compared with placebo. These effects consisted of an overall decrease in absolute power for all the frequency bands evaluated, and an acceleration of the center-of-gravity frequency. Absolute power decreases were most prominent in theta, delta and beta-1 bands at 90-120 min, while the alpha and fast beta rhythms were less intensely affected. However, the alpha-2 band showed a highly significant decrease at all leads at 60 min. Relative power was most prominently increased in the beta-3 and beta-4 bands. Additionally, the alpha/delta-theta ratio, an index of activation, was found to be increased after *ayahuasca*. Non-parametric analysis of all variables and leads over time showed the first changes relative to placebo between 15-30 min. These were followed by a steep rise at 45 min, reaching the maximum effects between 45-90 min. EEG measures gradually declined thereafter to reach baseline values around 4-6 h.

3. Intracerebral power density showed the maximum differences with placebo at 60 and 90 min. *Ayahuasca* decreased power density in the alpha-2, delta, theta and beta-1 bands. At 60 min, a widespread power reduction in the alpha-2 band was observed in extensive areas around the temporo-parieto-occipital junction in both hemispheres. At 90 min, the slow delta rhythm was decreased also in posterior brain regions around the temporo-parieto-occipital junction. Theta was found to be decreased in the medial frontal and medial temporal cortices. Finally, beta-1 decreases were found mainly in the parietal lobe.

4. *Ayahuasca* produced dose-dependent reductions of P50 suppression measured both as amplitude difference between the response to the conditioning and testing stimuli and as percent inhibition of the testing response. On the contrary, no statistically significant effects were found on the magnitude of startle response, its habituation rate or on the percentage prepulse inhibition at any of the prepulse-to-pulse intervals studied.

5. The major *ayahuasca* alkaloids, DMT, harmaline and THH, could be measured in plasma following *ayahuasca* administration, while levels of harmine were negligible and only observed in a small subset of volunteers. Additionally, levels of harmol and harmalol, the *O*-demethylated analogues of harmine and harmaline, were detected in all volunteers. C_{max} and AUC values increased with dose for all measured compounds. 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 and coincided with the peak of subjective effects. The AUC normalized by dose increased with dose for DMT. Conversely, V_z/F and *Cl/F* values calculated for DMT decreased with dose. These decreases were statistically significant for V_z/F and showed a tendency for Cl/F. The calculation of the bioavailability of DMT based on i.v. DMT data from another study by other researchers yielded a value around 10-15 %.

6. *Ayahuasca* induced a statistically significant increase in 24 h normetanephrine excretion. However, instead of the expected decreases in deaminated monoamine metabolites (VMA, HVA, 5-HIAA), drug administration did not significantly modify excretion of these compounds.

7. In both the pilot and the final study, mean SBP, DBP and HR values were found to be increased at specific time points following *ayahuasca* administration as compared with placebo. However, statistically significant results were only obtained for DBP, these being in the final study. This variable showed a moderate 9 mm Hg increase at 75 min after the 0.85 mg DMT/kg body weight dose. Somatic-dysphoric effects, particularly nausea, were frequently associated to drug intake. However, vomiting was only observed in 4 of 53 occassions in which *ayahuasca* was administered (pilot and final studies combined). In the course of the pilot study one volunteer experienced an intensely dysphoric reaction with

transient disorientation and anxiety which led to his voluntary withdrawal from the study. No clinically relevant alterations were observed in the hematological or biochemical parameters tested in any volunteer.

Secondary Results

1. The HRS questionnaire was translated into Spanish and evaluated in two groups of volunteers. Scores above zero (no effect) were observed both in the immediate and delayed retrospective assessment of drug effects. Reliability data indicated that four of the six scales, i.e., Affect, Cognition, Perception and Somaesthesia, show an acceptable level of internal consistency. Significant but limited correlations were found between the Perception and Somaesthesia scales and the ARCI LSD scale, indicating the construct validity of the questionnaire. The HRS was sensitive to psychedelic drug effects other than those elicited by intravenous DMT, for which it was originally designed, and showed reasonable reliability and convergent validity.

2. DMT was quantified by gas chromatography with nitrogen-phosphorus detection, following liquid–liquid extraction with *n*-pentane. Recovery was 74%, and precision and accuracy were better than 9.9%. The limit of quantification (LOQ) was 1.6 ng/ml. The three main *β*-carbolines present in *ayahuasca*, i.e., harmine, harmaline and THH, plus harmol and harmalol (*O*-demethylation metabolites of harmine and harmaline, respectively) were quantified by means of high performance liquid chromatography (HPLC) with fluorescence detection. Sample preparation was accomplished by solid-phase extraction. All five *β*-carbolines were measured using a single detector by switching wavelengths. Method validation demonstrated good recoveries, above 87%, and accuracy and precision better than 13.4%. The LOQ was 0.5 ng/ml for harmine, 0.3 ng/ml for harmaline, 1.0 ng/ml for THH, and 0.3 ng/ml for harmol and harmalol. Good linearity was observed in the concentration ranges evaluated for DMT (2.5-50 ng/ml) and the *β*-carbolines (0.3-100 ng/ml).

DISCUSSION

The aim of the present study was to study the human pharmacology of *ayahuasca* in a controlled clinical trial. Working with the lyophilizate of the tea proved a convenient method for this purpose. However, the difficulties involved in the obtention, handling and storing of the freeze-dried material in order to administer accurate dosings in a standardized and stable pharmaceutical form, made this an achievement in itself. At the beginning of the research project, the option of studying combinations of pure DMT plus pure β-carbolines was considered. Although this approach would have simplified the matter enormously, it is our view that results from these preparations could not have been considered to fully reflect *ayahuasca* pharmacology. While some findings might not have changed much from one approach to the other, other aspects of the clinical picture certainly would have. A natural extract such as *ayahuasca* contains a myriad of substances. Some are pharmacologically active, but inert plant material is likely at least to influence the gastrointestinal tolerability of the tea. Although not ideal for the precise study of drug-drug interactions, ingestion of the natural tea may lead to a different experience in terms of unpleasant somatic effects, nausea or vomiting, compared with the simple combination of pure compounds in a capsule. The use of the lyophilizate thus appeared to be a reasonable compromise.

The constellation of subjective effects elicited by *ayahuasca* could be effectively quantified by means of the battery of self-administered instruments used for this purpose, i.e., the HRS, the ARCI and the VAS. The HRS was sensitive to *ayahuasca* effects, as it has previously been shown to be sensitive to other psychedelics such as i.v. DMT and oral psilocybin (Gouzoulis-Mayfrank et al., 1999b; Strassman et al., 1994). VAS data indicated that *ayahuasca* shows a distinct duration of effects, longer than those of i.v. DMT, but shorter than those of mescaline or LSD (Strassman, 1994). Finally, ARCI results revealed that *ayahuasca* effects are not merely perceptual, but share common features with psychostimulants (Martin et al., 1971) and elicit marked somatic-dysphoric symptoms. The coexistence of stimulation with modifications in the sensorium portrays *ayahuasca* as a psychedelic, and its subjective effects are dose-dependent and can be evidenced by the instruments used. This information being established for *ayahuasca*, blockade studies can be proposed in order to better characterize the neurochemical mechanisms involved in its effects. Additionally, we may now be able to address the long neglected study of the individual β-carbolines in humans and examine which of these and at what doses they can elicit subjective effects

analogous to those of *ayahuasca*, if at all. If the *β*-carbolines prove devoid of psychedelic activity, the use of subjective-effect measures combined with the administration of pure compounds may alternatively help tease apart the contribution of each of the *β*-carbolines in the facilitation of DMT absorption per os.

The EEG variables provided a quantitative and dose-dependent measure of *ayahuasca* effects on the CNS, which is totally objective and not easily influenced by the subject's expectations or will. Although complex, *ayahuasca* effects on the EEG power spectrum are compatible with its proposed neurochemical mechanism of action. Decreases in slow activity, i.e., delta and theta power, are a general feature of psychedelics displaying 5-HT_2 agonist activity, but also of psychostimulants such as amphetamine and methylphenidate, and serotonin releasers such as fenfluramine, (Herrmann and Schaerer, 1986; Itil and Fink, 1966; Saletu et al., 1993). Delta activity has traditionally been thought to reflect inhibitory activity, and increases in theta have been observed in relaxed and meditative states. Results thus suggest an excitatory or arousing effect for *ayahuasca*. This assumption is further supported by the fact that major tranquilizers with D_2 or mixed D_2/5-HT_2 antagonist activity, such as chlorpromazine and risperidone, are characterized by their delta and theta-promoting activity (Lee et al., 1999; Saletu et al., 1993). An important difference between *ayahuasca* and psychostimulants is the alpha-2 decreasing properties found in the present study. Early pharmaco-EEG research on LSD had also shown decreases in alpha activity after acute drug administration (Itil and Fink, 1966). Amphetamine, on the other hand, is known to enhance alpha in addition to its slow wave dampening effects, a feature not shared by *ayahuasca*. Future studies could benefit from comparing *ayahuasca* effects with those of more prototypical psychostimulants such as *d*-amphetamine. Besides, pretreatment with selective 5-HT_{2A} antagonists would allow for the identification of individual EEG variables specifically modified by 5-HT_{2A} activation.

The LORETA results obtained in the present study should be regarded as exploratory. The technique is relatively recent and this is the first time it has been applied to study *ayahuasca* or any other psychedelic. Based on the cortical areas targeted, it could be hypothesized that drug-induced bioelectrical changes on unimodal sensory association cortex may have played a role in the modality-specific modifications in the visual, somatic and auditory perception reported by the volunteers. Additionally, it appears

reasonable to assume that effects on transmodal brain areas could account for more complex cognitive modifications which also characterize the subjective experience elicited by *ayahuasca*. In this respect, the temporo-parietal and frontomedial heteromodal association cortex, the cingulate and the temporomedial cortices play relevant roles in the neurobiology of attention, emotion, and memory (Devinsky et al., 1995; Nyberg et al., 1996; Squire and Zola-Morgan, 1991). Clearly, additional studies of *ayahuasca* and other psychoactive drugs by means of LORETA are warranted. Comparison of the LORETA results with data from nuclear medicine techniques are also indicated. In contrast with the widespread power density decreases we observed for *ayahuasca* in posterior regions with LORETA, PET and SPECT studies of acute mescaline and psilocybin administration have shown blood flow and metabolic increases in the frontal cortex (Gouzoulis-Mayfrank et al., 1999a; Hermle et al., 1992; Vollenweider et al., 1997).

At the doses administered, *ayahuasca* induced a different pattern of effects on PPI and P50. The results obtained seemingly indicate no effect, or at best, a mild enhancing effect of the drug on PPI, a measure of sensorimotor gating. In the only other human study performed to date involving serotonergic psychedelics, the administration of psilocybin provoked a significant increase of PPI at a prepulse-to-pulse interval of 100 ms, with no significant effects on habituation (Gouzoulis et al., 1998). Interestingly, the pattern of effects exerted by serotonergic drugs on the human PPI in the limited number of studies conducted is opposed to that by dopaminergic/noradrenergic agonists. Thus, *d*-amphetamine and bromocriptine have been shown to impair PPI in healthy volunteers (Abduljawad et al., 1998; Abduljawad et al., 1999; Hutchison and Swift, 1999). On the contrary, the significant dose-dependent decreases in P50 suppression after *ayahuasca* suggest a suppressing effect of the drug on normal sensory gating in humans. To our knowledge, P50 suppression has not been assessed previously in humans following the administration of a 5-$HT_{2A/2C}$ agonist. The only studies that have evaluated the effects of pharmacological challenge on this measure in humans have concentrated mainly on cathecolaminergic drugs. Both *d*-amphetamine and the α_2-adrenoceptor antagonist, yohimbine have thus been shown to impair P50 suppression in healthy volunteers (Adler et al., 1994a; Light et al., 1999). Furthermore, the dopamine agonist bromocriptine has also been found to disrupt P50 suppression (Adler et al., 1994b) in humans. The pharmacological characteristics of the beverage, which combines MAO-

inhibitors and DMT, precludes the generalization of the present findings to all 5-$HT_{2A/2C}$ agonists. Results in the present study should therefore be regarded with caution and should be replicated using wider dose ranges.

The time course of DMT plasma concentrations closely paralleled that of subjective effects, with peak DMT concentrations and peak effects obtained between 1.5 and 2 h. It is worth noting the small percentage of DMT which apparently reaches systemic circulation after *ayahuasca* administration. Based on Strassman's i.v. data, our calculations yield a bioavailability of only 10-15% for DMT in *ayahuasca*. An analogous value is obtained performing the calculations with the apparent volume of distribution reported by Callaway and coworkers (1999). In fact, the above bioavailavility figure may be overestimated. In their study, Strassman et al. (1994) draw the first blood sample two min after the end of the DMT bolus. Thus, the maximum plasma concentration –which is obtained immediately after completion of the i.v. bolus- was missed. Greater DMT plasma levels after i.v. administration would have led to a smaller volume of distribution value and this in turn to a smaller bioavailability for DMT in *ayahuasca*. The role of the *β*-carbolines on DMT clearance deserves further analysis. The normalized AUC calculated for DMT in the present study showed a statistically significant increase between the low and the high *ayahuasca* doses. This is suggestive of a non-linear increment of DMT levels following the administration of increasing doses of *ayahuasca* and could be due to the action of the higher amounts of *β*-carbolines administered. In line with this possibility, while Callaway and coworkers found an apparent volume of distribution value for DMT similar to that in the present study, they reported higher half-life and lower clearance values. The role of the *β*-carbolines in the possible non-linearity of DMT levels between *ayahuasca* doses could be addressed in a repeated administration study.

The absence of measurable levels of plasma harmine suggests efficacious metabolism of this alkaloid. The rapid turnover of harmine to harmol has been observed in vitro (Yu et al., 2003). Phenotyping or genotyping volunteers for the CYPs involved in harmine *O*-demethylation could shed some light on the differences observed between our sample and that of Callaway and coworkers (1999), where measurable levels of harmine were found in plasma.

In the present investigation we could not demonstrate a clear-cut MAO inhibition by measuring urinary monoamine metabolite levels. This may have been related to the β-carboline amounts administered with the *ayahuasca* doses. These were established in terms of administered DMT per kg body weight. At the 0.85 mg DMT/kg dose which was used in the final study as the high dose, volunteers received 1.45 mg/kg harmine. This value is slightly below the 1.5 mg/kg found by Ott (1999) to be the threshold dose necessary to render DMT orally active in self-experiments. Although 1.45 mg/kg may have been sufficient to allow sufficient DMT to reach undegraded systemic circulation and to elicit psychotropic effects, it may not have been enough to modify the profile of endogenous monoamine metabolites in urine. The assessment of the in vivo MAO inhibition effect with this methodology may render positive results employing *ayahuasca* batches with higher β-carboline amounts. Alternatively, a repeated dose administration study could possibly yield a greater degree of MAO inhibition.

Increases in SBP, DBP and HR did not appear to be a robust effect of *ayahuasca*. While a tendency was found for SBP increases in the pilot study, this variable was not found to be affected in the final study. Instead, increments in DBP reached statistical significance, although the magnitude of the effect was only moderate. The small sample of the pilot study (only 5 volunteers) may have been insufficient to demonstrate the cardiovascular effects of *ayahuasca*. Both in the pilot and final studies, increases in SBP, DBP and HR were milder than those reported for other more prototypical sympathomimetics, such as amphetamine or MDMA, at doses showing psychotropic properties (de la Torre et al., 2000; Mas et al., 1999). The use of positive controls in future studies on the cardiovascular effects of *ayahuasca* is also indicated.

CONCLUSIONS

1. The psychotropic effects of *ayahuasca* vs. placebo were demonstrated by means of structured self-assessment instruments both in naive and in experienced *ayahuasca* users. The tea induces dose-dependent changes in the perceptual, affective, cognitive and somatic spheres, with a combination of stimulatory and psychedelic effects. The overall experience is of longer duration and milder intensity than that previously reported for intravenously administered DMT.

2. The central effects of *ayahuasca* were objectively measured by means of q-EEG, showing a time pattern which closely paralleled that of subjective effects. Results in the individual q-EEG variables are in line with those previously described for other serotonergic psychedelics and share some features with the profile of effects shown by pro-serotonergic and pro-dopaminergic drugs.

3. The use of the LORETA source location technique identified the EEG power decreases over somatosensory, auditory and visual association cortices, over temporo-parietal heteromodal association cortex and in paralimbic structures with relevant roles in emotion and memory processes. These areas may be involved in the psychological effects elicited by *ayahuasca*.

4. Diverging effects are observed on each of the two gating measures evaluated. While a decremental effect of *ayahuasca* on sensory gating was found, no distinct effects were observed on sensorimotor gating.

5. Following the oral administration of *ayahuasca*, measurable plasma levels were observed for DMT, harmaline and THH. Based on the calculated bioavailability, only a small percentage of the total DMT in *ayahuasca* appears to reach systemic circulation. The time course of DMT plasma concentrations parallels the evolution of subjective effects, with peak plasma concentrations and peak subjective effects attained at 1.5 h. While harmine was not found in plasma except for a few time points in four of eighteen volunteers, all volunteers showed measurable levels of harmol and harmalol, the *O*-demethylated analogues of harmine and harmaline. Results suggest an intense first-pass metabolism for harmine.

6. In vivo MAO inhibition following *ayahuasca* administration could not be firmly and definitely established measuring the excretion of urinary monoamine metabolites. While *ayahuasca* increased normetanefrine excretion in line with the hypothesis, the levels of the MAO-dependent metabolites did not show the decreases expected for a MAO inhibitor.

7. *Ayahuasca*-induced increases in cardiovascular measures were moderate and statistical significance could only be demonstrated for DBP. The drug exerts a series of unpleasant somatic-dysphoric effects, the most common of which are altered physical sensations and nausea. However, in contrast with reports from field observations, *ayahuasca*-induced vomiting was infrequent. Transient disorientation and anxiety experienced by one volunteer was the most disturbing adverse event observed.

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