Ayahuasca's 'afterglow'

Improved mindfulness and cognitive flexibility in naïve and experienced ayahuasca drinkers

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Background
There is a growing body of evidence demonstrating the therapeutic potential of ayahuasca for treating depression and anxiety. However, the mechanisms of action involved in ayahuasca's therapeutic effects are unclear. Mindfulness and cognitive flexibility may be two possible psychological mechanisms. Like other classic psychedelics, ayahuasca also leads to an 'afterglow' effect of improved subjective wellbeing that persists after the acute effects have subsided. This period may also offer a window of increased therapeutic potential.

Objectives/Method
This research explored the effect of ayahuasca on mindfulness and cognitive flexibility in the afterglow period by comparing three self report measures of mindfulness (Five Facets Mindfuless Questionnaire, FFMQ), decentring (Experiences Questionnaire, EQ) and cognitive flexibility (Cognitive Flexibility Scale, CFS), and neuropsychological task performance on the Stroop and Wisconsin Picture Card Sorting Task (WPCST). Participants were measured before drinking ayahuasca and again approx. 24 hours after ingestion, in a sample of 48 ayahuasca drinkers, whilst controlling for prior ayahuasca use.

Results
We found mindfulness (as measured by the FFMQ total scores) and decentring (measured by the EQ) significantly increased in the 24 hours after use. Four of the five distinct mindfulness facets were also significantly increased (Observe, Describe, Act with Awareness and Non-reactivity). Cognitive flexibility (as measured by the CFS and the WPCST) was also significantly improved in the 24 hours after ayahuasca use. Changes in mindfulness and cognitive flexibility were not influenced by prior ayahuasca use.

Conclusions. The present study provides further evidence of ayahuasca's ability to enhance mindfulness and highlights it as a potential psychological mechanism of the psychotherapeutic effects of ayahuasca. This was the first known study to measure cognitive flexibility in the 'afterglow' period and suggests it is worthy of further exploration as another possible psychological mechanism. Given psychological gains to mindfulness and cognitive felxibility occurred regardless of prior ayahuasca use suggests ayahuasca offers potentially therapeutic effects for both psychedelic naïve and experienced ayahuasca drinkers.

Renewed interest in the effects of classic psychedelics is providing evidence for their potential efficacy in treating a range of psychiatric illnesses, and specifically, in improving outcomes for treatment resistant patients (Bouso & Riba, 2014; Griffiths et al., 2016; Grob et al., 2011; Moreno, Wiegand, Taitano, and Delgado, 2006). Given the high failure rates in current treatments the search for alternative treatment strategies is crucial (Vollenweider & Kometer,

2010). Ayahuasca is one such classic psychedelic with potential therapeutic effects; shown to be beneficial in treating depression (Griffiths et al., 2016), anxiety (Grob et al., 2011) and substance abuse disorders (Bouso & Riba, 2014). Ayahuasca is a dimethyltryptamine (DMT) containing psychoactive plant tea most commonly combining two plants with water; the vine of Banisteriopsis caapi (B. caapi) and the leaves of Psychotria Viridis (P.Viridis) (Domínguez-Clavé et. al, 2016). The combination of these plants renders the DMT in P. Viridis orally active by inhibiting its degradation in the body and allowing uptake into the central nervous system, producing an altered state of consciousness that lasts between four to six hours, with peak effects between one and two hours after ingestion (Prickett & Liester, 2002).

Like other classical psychedelics, ayahuasca's primary neural mechanism of action is as a 5HT agonist (Sampedro et al., 2016). Secondary effects as a 5HT2a agonist are thought to underlie the increased positive mood experienced in ayahuasca users for up to two months after use, commonly referred to as the afterglow period (Sampedro et al., 2016). However, given the complexity and high co-morbidity of the mental health problems that ayahuasca treats, there are likely to also be psychological mechanisms that contribute to the increased psychological wellbeing observed in ayahuasca users in this afterglow period and longer term (Soler et al., 2016). Mindfulness and cognitive flexibility have recently been suggested as two such possible mechanisms which may be associated with ayahuasca's therapeutic effects (Kuypers et al., 2016; Carhart-Harris et al., 2014).

Mindfulness is a multi-faceted construct which is broadly defined as "paying attention in a particular way: on purpose, in the present moment, and non-judgmentally." (Kabat-Zinn, 1994, p. 4). It is considered to have five distinct facets which can be measured using the Five Facets Mindfulness Questionnaire (FFMQ; Baer et., al 2006) including: 1) observing, 2) describing, 3) acting with awareness, 4) non-judging of inner experience and 5) non-reactivity to inner experience (Baer et al., 2006). Mindfulness-based interventions have proven clinical efficacy in treating numerous psychiatric disorders, including depression, anxiety and addictions (Hoffman et al., 2010). Decentering is a closely related psychological construct, which is the ability to take a detached view of one's own thoughts and emotions, considering them as temporary events of the mind (Fresco et al., 2007). It is considered to mediate positive behavioural and psychological changes following mindfulness training (Hayes-Skelton & Graham, 2012). Both decentering and mindfulness have been shown to increase following a single dose of ayahuasca. Soler et. al, (2016) found levels of mindfulness (as measured by the FFMQ) and

decentering (as measured by the Experiences Questionnaire, Fresco et al., 2007) significantly increased in the 24 hours after ingestion in 25 ayahuasca users (23 had prior experience with ayahuasca, using on average 79 times (range 1–500). They found that ayahuasca affected the mindfulness facets differently, with non-judging and non-reactivity to inner experience significantly increasing following use.

Sampedro et al., (2017) subsequently replicated Soler et. al's (2016) findings in 16 experienced ayahuasca users (previous use of an average of 62 ± 99 times), and further assessed the neural correlates 24 hours after use and again two months later. They found that increased connectivity between the anterior cingulate cortex (ACC), a structure involved in emotional processing and cognitive control, and the posterior cingulate cortex (PCC), a key hub of the default mode network (DMN) involved in the sense of "self," predicted increases in mindfulness 24 hours after using ayahuasca, and sustained elevations in the non-judge facet of mindfulness two months after use. The neural correlates of ayahuasca users also matched those of meditators in the 24 hours after using ayahuasca, specifically decreased DMN-TPN (Task-positive network) anti-correlation, which may point to a decrease in the 'maladaptive ruminations' present in depression, which are associated with greater DMN 'dominance' over TPN activity (Sampedro et al., 2017) Ayahuasca may therefore also lead to mid-term increases in mindfulness, which offers enhanced therapeutic potential and supports the assertion by Dominguez-Clave et. al, (2016), who suggested that the mindfulness enhancing properties of ayahuasca could be used therapeutically to promote emotional reprocessing in patients with depression, addiction and personality disorders. This is further supported by a recent study which found that two ayahuasca sessions led to increases in the non-judging subscale of the FFMQ which are comparable to gains made in an 8-week Mindfulness-based Stress Reduction course (Soler et. al., 2018). Improving this capacity is therapeutically significant as it promotes a person's ability to tolerate potentially distressing thoughts, emotions and life situations by taking a less judgmental and more impersonal stance towards them (Soler et. al., 2018).

A second possible psychological mechanism that could account for the psychotherapeutic benefits seen with ayahuasca is cognitive flexibility. Broadly defined, cognitive flexibility is the ability to shift perspective or approach in order to adapt to change in the environment (Johnco, Wuthrich, & Rapee, 2014) and involves executive function abilities of problem solving and response inhibition (Johnco, Wuthrich, & Rapee, 2014). Neuro-anatomically, cognitive flexibility is associated with the ACC and medial pre-frontal cortex (Bissonette, Powell & Roesch, 2013;

Kim, Johnson, Cilles & Gold, 2011). More recently its definition has expanded to a broader concept of 'mental flexibility' which also involves psychological and behavioural processes. Higher cognitive flexibility has been associated with increased psychological well-being (Hayes, Luoma, Bond, Masuda & Lillis, 2006), whereas cognitive inflexibility or rigidity is associated with psychopathology (Hayes, Luoma, Bond, Masuda & Lillis, 2006). Low cognitive flexibility can also be a barrier to achieving therapeutic outcomes in treatment and therapeutic approaches which help to increase cognitive flexibility have shown greater efficacy (Johnco et al., 2014). To date no known studies have explored cognitive flexibility, specifically as a potential psychological mechanism of ayahuasca's therapeutic effects in the afterglow period. A growing body of neuropsychological evidence points to its potential. Divergent thinking was enhanced acutely following ayahuasca administration in 25 experienced (used 104-153 times) ayahuasca users (Kuypers et. al, 2016). Divergent thinking is considered to strengthen psychological and cognitive flexibility (Kuypers et. al, 2016), and has been shown to be an important aspect of cognitive therapy (Forgeard & Elstein, 2014).

Research has also shown ayahuasca acutely affects three key brain networks which are implicated in cognitive flexibility; the default mode network (DMN) (in which the PCC is a key hub), central executive network (CEN) and the Salience Network (SN) (in which the ACC is involved). These studies have suggested that ayahuasca temporarily disrupts the neural hierarchies of these networks by exciting posterior regions while loosening the "cognitive grip" exerted by frontal regions responsible for executive control (Alonso, Romero, Mañanas & Riba, 2015). Palhano-Fontes et. al, (2015) also found decreased functional connectivity in parts of the DMN acutely under the effects of ayahuasca, consistent with other classical psychedelics (Hermle et al. 1992; Vollenweider et al, 1997; Gouzoulis-Mayfrank et. al, 1999; Riba et. al, 2006). Carhart Harris et al., (2014) suggested that this decreased functional connectivity could lead to increased cognitive flexibility. Kuypers et al., (2016) suggest that the SN in particular might have an effect on cognitive flexibility due to ayahuasca's disruption of neural hierarchies on the DMN, CEN and SN which, "leads to an increase in information fed into the salience network," (Kuypers et. al, 2016). This is also supported by Riba et al., (2006) who reported ayahuasca lead to an increase in blood profusion in the SN acutely. Ayahuasca's effect on the SN is particularly interesting in terms of cognitive flexibility, as it is responsible for monitoring events occurring outside of the body as well as internal consciousness, and is able to direct attention to whatever is more important at a certain moment (Riba et al., 2006) SN abnormalities are also prominent in mood and anxiety disorders. (Yuen et al., 2014). The major nodes of the

SN were found to be affected in patients with major depression suggesting that a negativity bias in attention and repetitive thinking may underlie SN response (Hamilton, Chen, & Gotlib, 2013).

Ayahuasca has also been associated with better neuropsychological performance in experienced users relative to controls and less regular users in areas of cognitive flexibility. Bouso et al., (2012) found experienced ayahuasca users (n=127) performed better than closely matched controls both at baseline and one year later on two measures of cognitive flexibility; Stroop and the Wisconsin Picture Card Sorting Task (WPCST). This suggests repeated ayahuasca use may be associated with sustained improvements in cognitive flexibility. Neuroanatomically, the Stroop is associated to activation in the medial prefrontal cortex and ACC, an area of the brain shown to be thicker in experienced ayahuasca users than controls (Bouso et al., 2015). The WPCST is associated with activation in the anterior cingulate and medial prefrontal cortex. Given evidence of long term structural brain changes to the PCC and ACC (Bouso et al., 2015), it is possible that ayahuasca use leads to structural brain changes that account for positive changes in cognitive flexibility (Bouso et al., 2012) and this could also account for different responses seen in the response to ayahuasca between experienced and less experienced users in mindfulness (e.g. Soler et al, 2016), as well as neuro-psychologically (Bouso, Fàbregas, Antonijoan, Rodríguez-Fornells & Riba, 2013).

However, not all studies have shown acute or long-term differences in cognitive flexibility between experienced regular ayahuasca users and psychedelic naïve controls or inexperienced sers (Barbosa et al., 2016; Doering-Silveira et al., 2005). For example, another study comparing inexperienced and experienced users found both groups reaction times on the Stroop colour and word task to significantly decrease and accuracy to be maintained acutely under the effects of ayahuasca. (Bouso, Fàbregas, Antonijoan, Rodríguez-Fornells & Riba, 2013). To date there are no known studies that have investigated changes to cognitive flexibility in the afterglow period of ayahuasca use.

In summary, previous research has found increases in mindfulness in the afterglow period (24 hours) following ayahuasca use, as well as acute and long-term changes to cognitive flexibility, with prior ayahuasca use potentially affecting these changes. To further explore whether these are potential psychological mechanisms which could exert ayahuasca's therapeutic effect the current study aimed to assess changes in mindfulness and cognitive flexibility over two time points; baseline (pre-ayahuasca) and within 24 hours following ayahuasca use (post-

ayahuasca) in less experienced users relative to previous studies, and at the same time controlling for prior ayahuasca experience. Given the previous literature demonstrating an association between ayahuasca use and mindfulness gains in this 24-hour afterglow period, we expect to see a significant increase in participants' total mindfulness and decentering scores in the 24 hours following ayahuasca use relative to baseline and to determine whether there are any changes in cognitive flexibility in this same period.

Method

Participants

Participants were recruited through advertisements emailed to the mailing lists of public groups with an interest in psychedelics. Participants were eligible to take part in the study if they were over 18, had used ayahuasca in the past or had considered using it in the future. Participants were excluded if they self-reported current or prior drug dependency (including alcohol), current psychiatric or medical diagnoses, use of prescribed psychiatric medication, were receiving any form of psychological treatment and had a history of brain damage, head injury or epilepsy. The study was granted ethical approval by the University of East London Ethics Committee.

48 participants completed both assessment points. Twenty-six (54%) were female. Ages ranged from 26 to 58 (mean=38.48, SD=7.21). Participants were predominantly white (67% ,n=32), and highly educated (83%,(n=40) to degree level or above and spoke English as their first language (75%, n=36).

Measures

Personal history questionnaire: This captured basic demographic demographic information including: gender, age, ethnicity, first language and highest level of education.

Drug use history questionnaire: designed specifically for this study, capturing participants' of self-reported drug use history for ayahuasca, other classic psychedelics and recreational drugs (including alcohol and tobacco), including details on date first taken, date last taken, estimated lifetime number of uses and number of uses in the past 12 months.

Severity of Dependence Scale (SDS; Gossop, 1995)
The SDS is a brief 5-item questionnaire used to assess participants' levels of psychological dependence on ayahuasca. Items are scored on a 4-point scale ranging from 0-3. Higher total scores indicate higher levels of dependence. The scale has demonstrated excellent psychometric properties and validity for use with a diverse range of drug users (Gossop, 1995).

Five Facets Mindfulness Questionnaire-Short Form (FFMQ; Baer et al., 2006)
The FFMQ-Short Form is a 24 item validated self-report questionnaire which measures five mindfulness facets; 1) observe: noticing internal and external experiences e.g. body sensations, thoughts, emotions, sounds, 2) describe: putting words to or labelling the internal experience, 3) act with awareness: focusing on activities in the present moment rather than responding habitually, 4) non-judge: taking a non-evaluative view of internal and external experiences in the present moment, and 5) non-react: maintaining equanimity with the flow of thoughts and feelings as they arise without getting caught up in or carried away by them (Bohlmeijer, Ten Klooster, Fledderus, Veehof & Baer, 2011). Items are scored on a 5 point Likert scale; from 1 (never or very rarely true) to 5 (very often or always true). Higher scores indicate higher levels of mindfulness, with a total score and five subscale scores. The FFMQ has demonstrated good internal reliability and adequate psychometric properties in both clinical and non-clinical samples (Baer et al., 2008).

Experiences Questionnaire (EQ; Fresco et. al, 2007)
The EQ is a validated 11 item measure of decentering. EQ items are scored on a 5 point Likert scale ranging from never (1) to all the time (5). Higher total and mean scores represent higher levels of decentering. The EQ has demonstrated good construct validity and internal consistency, for use in meditating and non-meditating samples (Soler et al., 2014).

Cognitive Flexibility Scale (CFS; REF)
The CFS measures the ability to switch between thoughts and actions, based on the willingness to be flexible, a belief in self efficacy and awareness of alternative ways of responding to situations and experiences (Martin & Rubin, 1995) The CFS is a validated 12 item measure of cognitive flexibility. CFS items are scored on a 6 point Likert Scale ranging from (1) strongly disagree to (6) strongly agree. Higher total scores indicate higher cognitive flexibility. The CFS has proved effective in predicting symptoms in depression, anxiety and substance abuse disorders as well psychological wellbeing in non-clinical samples (Lee & Orsillo, 2014).

Stroop colour and word task (Stroop; Stroop,1935)

A computerised version of the Stroop test was used, measuring cognitive flexibility, as well as selective attention, conflict monitoring and resistance to interference. Thirty-eight colour-words were displayed in turn, on screen (“red”, “green”, “blue”) in congruent and incongruent colours. Participants had to use a key on the keyboard that corresponded to a colour word (Z=red, X=blue, N=green, M=yellow) to select the written colour word whilst ignoring the colour it is written in. Performance was measured by number of errors and reaction time in the congruent and incongruent conditions. Better performance was reflected as lower mean reaction times and lower number of mean errors.

Wisconsin Picture Card Sorting Task (WPCST; (Berg, 1948)

The WPCST is a neuropsychological test of "set-shifting," measuring cognitive flexibility by requiring participants to creatively problem solve to work out the picture card sorting rule, and adapting to changing rules whilst inhibiting impulsive responding (Berg, 1948). It is the most recognised task-based test of cognitive flexibility and is widely used (Heaton, 1981). A simplified, computerised version of the WPCST was used where by participants had to match 36 test cards to stimulus cards presented on screen, without knowing the rule by which the test card matched the stimulus cards. cards were displayed in 3 separate trial blocks, one for colour, shape and number. The sorting rule changes for each trial block, but with no warning provided. In each trial, 4 stimulus cards appear at the top of the computer screen. These cards vary in colour, type of shape, and number of shapes on the cards. A test card is then displayed at the bottom of the screen. Participants were instructed to match the test card with one of the 4 stimulus cards by clicking on the stimulus card they thought it matched. Feedback is displayed on screen after a selection indicating whether the card has been matched correctly or not. Better performance was indicated by higher mean number of correct answers, indicating a participant had worked the rule out more quickly and continued to guess correctly after this throughout the trial bock. Mean reaction times of correct answers was also calculated; faster reaction times indicating better performance.

Procedure

Agreed testing times were made with all individuals who expressed an interest in participating in the study and met the inclusion criteria. Participants were instructed to abstain from using any

drugs or alcohol (except nicotine) for at least 24 hours prior to the testing session at time-point 1. Following informed consent participants completed the self-report questionnaires; personal history, drug use history, FFMQ, EQ, CFS, SDS, followed by the Stroop and WPCST at time point 1 (baseline). These were then repeated again 24 hours after using ayahuasca (time-point 2). The order of the trials presented in the WPCST differed at each time-point. Each assessment session lasted approximately 30 minutes.

Data Analysis

Total and mean scores were then calculated for questionnaire measures including each of the five FFMQ subscales. For the Stroop task, mean scores were calculated for reaction time and number of errors in the congruent and incongruent conditions. For the WPCST, mean scores were calculated for reaction time and number of correct answers. Analyses was conducted using SPSS 23. An ANCOVA was conducted on all outcome measures; the FFMQ, EQ, CFS and WPSCT between the two-time points (baseline pre-ayahuasca and 24-hours post-ayahuasca), with previous ayahuasca lifetime use as a covariate. Additionally, a 2x2 repeated measures ANCOVA was conducted on the Stroop reaction times based on congruency (congruent and incongruent) and time (baseline and post ayahuasca) to establish the Stroop interference effect over time, with previous ayahuasca use as the covariate. A significance cut off of of $p= 0.05$ was used for all statistical analyses.

Results

Ayahuasca Experience

Table 1 provides details on participants' patterns of previous ayahuasca use. There were 6 psychedelic naive participants. Total years of use for the sample ranged from 0-9 years. One participant in the sample (2.1%) had last used ayahuasca two weeks before testing, and the rest of the sample (97.9%) had not used ayahuasca for one month or more.

Table 1: Patterns of previous ayahuasca use

	N	Min	Max	Mean (SD)
Lifetime use	48	0	130	22.1 (33.1)
Use in the last 12 months	44	0	26	5.59 (6.82)
Months since last used	42	0.5	24	4.94 (4.72)
SDS (total)	48	0	1	0.02 (0.14

SDS=Severity of Dependence Scale

Questionnaire Measures

Means (and SD) for all questionnaires at both time-points are shown in Table 2. Mean scores showed increases on all questionnaire measures of mindfulness and cognitive flexibility and all five subscales of the FFMQ between the two time points. Significant increases were found for the FFMQ total ($F(1,46)=-8.21$, $p=.003$, $\eta^2=.15$) and observed power was.80, CFS $F(1,46)= 8.25$, $p=0.006$, $\eta^2= .15$) and observed power was .80, and EQ ($F(1,46)= 4.15$, $p=.05$, $\eta^2= .08$). Of the five FFMQ subscales, significant differences were found for observe ($F(1,46) =13.38$, $p<.001$, $\eta^2= .24$) and observed power was .95, describe $F(1,46)= 3.76$, $p=.0.03$, $\eta^2=.08$ and observed power was .48, act with awareness $F(1,46)=7.08$, $p=0.01$, $\eta^2=.13$) and observed power was .74, and non-react $F(1,46)=0.02$, $p=0.03$, $\eta^2= .10$) and observed power was .61. In all cases there were no significant covariate interaction between time and previous ayahuasca use (all p's>0.05).

Table 2. Mean (SD) and *P* value scores for all measures of mindfulness and cognitive flexibility at time-point 1 (baseline) and time-point 2 (post-ayahuasca)

	Baseline	Post Ayahuasca	*P*
FFMQ (total)	3.58 (0.46)	3.75 (0.62)	.003*
FFMQ Subscales:			
1. Observe	3.99 (0.76)	4.23 (0.79)	<.001**
2. Describe	4.76 (0.89)	5.00 (0.95)	.03*
3. Act with awareness	3.55 (0.67)	3.74 (0.79)	.005*
4. Non-judge	3.23 (0.78)	3.33 (0.92)	.06
5. Non-react	3.36 (0.76)	3.55 (0.81)	.015*
EQ	40.60 (5.48)	41.75 (7.29)	.025*
CFS	57.38 (7.11)	59.35 (7.87)	.006*
WPCST (ms)			
Reaction Time	3572.38 (2615.94)	4195.61(4617.67)	0.14
Correct responses	.63 (0.15)	.71 (0.14)	.005*

FFMQ=Five Facets Mindfulness Questionnaire, EQ = Experiences Questionnaire, CFS=Cognitive Flexibility Scale, WPCST=Wisconsin Picture Card Sorting Task RT=reaction time
*= p<.05, **= p ≤ .001

Neuropsychological Task Performance

Stroop: Figures 1 and 2 show the mean reaction times (in ms) and number of errros for the Stroop by congruency and over the two-time points. There was a significant main effect of condition on reaction time ($F(1, 46) = 58.28$, p=<.001, $\eta^2 = .56$) and number of errors ($F(1, 46) = 20.42$, p=<.001, $\eta^2 = .31$). Observed power =1.00 and .99 respectively. There was no significant main effect of time on reaction time ($F(1, 46) = 1.65$, p=.20, $\eta^2 = .04$) or number of errors ($F(1, 46) = 1.64$, p=.21, $\eta^2 = .03$). Observed power = 0.24 in both cases. There was a significant interaction between time and condition on number of errors $F_{[KS3]}(1, 46 = 4.11$, $p<0.05$, (see figure 2) , but not for reaction time ($F(1, 46) = .02$, p=.90, $\eta^2 = .001$) (See figure 1). Observed power was .30 and ….. [KS4] In all cases there were no significant covariate interaction between time and previous ayahuasca use (all p's>0.05).

Figure 1: Mean Stroop reaction times (ms) by congruency and time

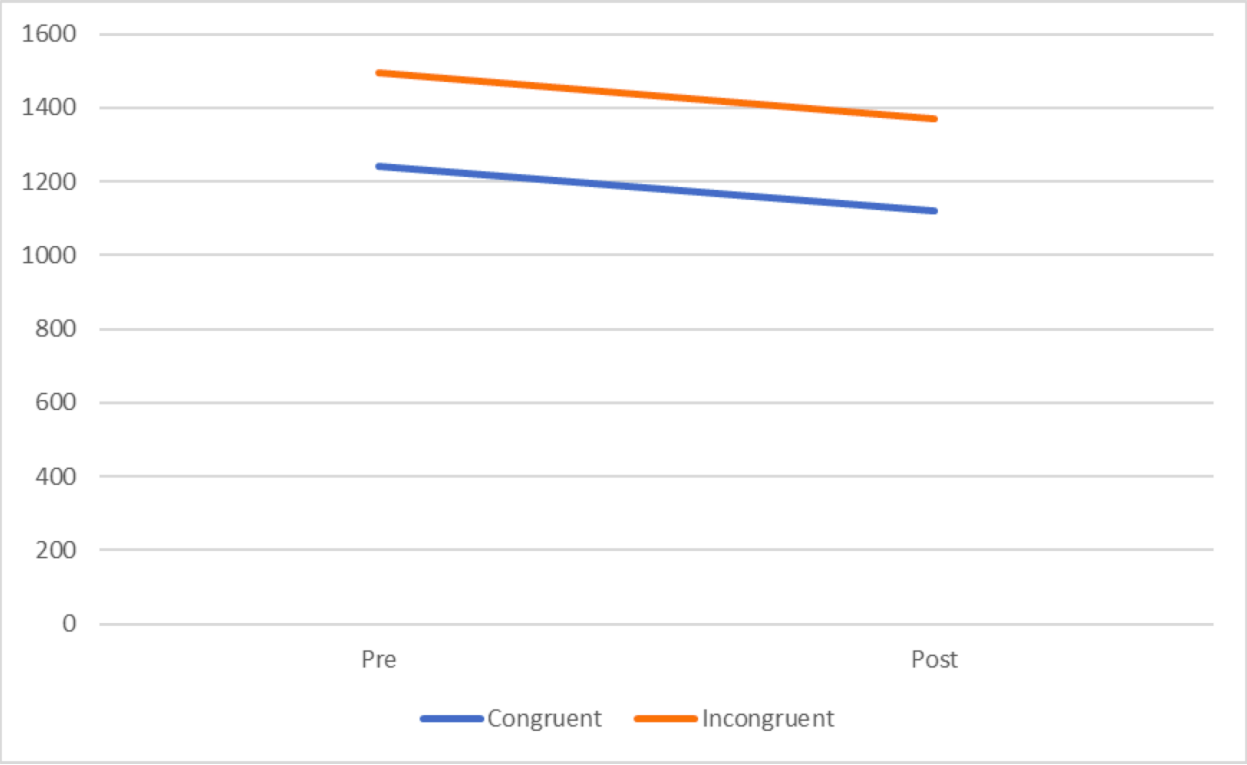
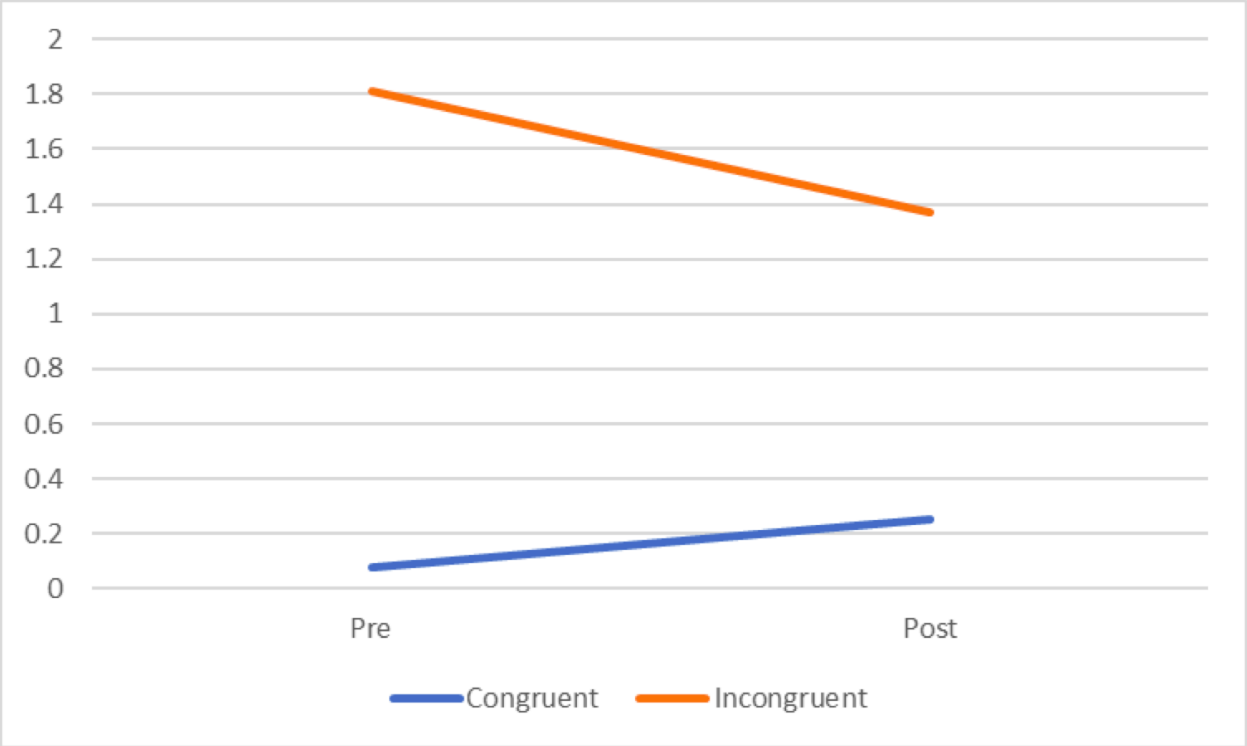


Figure 2: Mean Stroop errors (n) by congruency and time



WPCST: Means (and SD) for the WPCST outcome measures at both time-points are reported in Table 2. There was no significant effect of time on reaction times on the WPSCT ($F(1, 46)=2.30$, p=.14, $\eta^2= .05$). Observed power = 0.32. Participants made significantly more correct responses 24 hours after ayahuasca relative to before ($F(1, 46)=2.30$, p=.14, $\eta^2= .05$). Observed power = 0.82. In all cases there were no significant covariate interactions between time and previous ayahuasca use (all p's>0.05).

Discussion

The current study aimed to assess changes in mindfulness and cognitive flexibility over two time points; baseline (pre-ayahuasca) and within 24 hours following ayahuasca use (post-ayahuasca). We found mindfulness (as measured by the FFMQ) and decentering (measured by the EQ) significantly increased in the 24 hours after use. Cognitive flexibility (as measured by the CFS and the WPCST) was also significantly improved in the 24 hours after ayahuasca use

compared to baseline. Changes in mindfulness and cognitive flexibility were not influenced by prior ayahuasca use.

Consistent with the existing literature this study found the sample's self-reported levels of mindfulness and decentering to be increased in the 24 hours after drinking ayahuasca, evidenced by higher scores on the FFMQ and EQ at time-point two (post-ayahuasca) relative to time-point one (baseline) respectively. Mindfulness increases were seen in four of the five subscales of the FFMQ; Observing, Describing, Acting with Awareness and Non-reactivity to inner experience, indicating that both attentional and emotional acceptance facets of mindfulness were affected by ayahuasca use.

These findings are consistent with those of Soler et al., (2016) and Sampedro et al., (2017) insofar as they demonstrate overall mindfulness (assessed by total scores on the FFMQ) and decentering is increased in the 24 hours after drinking ayahuasca. However, differences reside in the findings on individual subscales. They found effects on only two of the five subscales (non-reactivity and non-judging of inner experience); both of which represent facets of emotional acceptance, whereas we have also demonstrated effects of ayahuasca on the observing and acting with awareness subscales, suggesting that both attentional and emotional acceptance facets are affected by ayahuasca use. Such inconsistencies could be explained by the differences in measures and samples (e.g. sample size and range of ayahuasca use). For example, previous studies (REF) have used the full length 39-item version of the FFMQ rather than the shortened version in the current study. However, given that an improvement in decentering abilities (measured by the EQ) was found in addition to one of the emotional acceptance facets in the current study after ayahuasca use, further supports the notion that ayahuasca has the capacity to affect both attentional and emotional acceptance components of mindfulness in the afterglow period. This is because decentering to some extent involves a non-evaluative (or non-judgmental) perception of present moment experience in order for a person to take a more detached stance from their experiences as they arise into awareness.

Given that changes in mindfulness and cognitive flexibility following ayahuasca use remained significant regardless of previous ayahuasca use in the current study, suggests that ayahuasca

can affect mindfulness abilities in even experienced ayahuasca users as well as less experienced users; even psychedelic naïve users. This is also supported by Soler et al (2017). Whilst they found some evidence that previous use may have some effect on mindfulness gains in the afterglow period, after further controlling for prior use they found this did not significantly alter the overall pattern of their results. Clinically this is particularly beneficial since it suggests even patients who have never previously used ayahuasca may benefit from improved mindfulness following a single ayahuasca session and that this effect is still possible after multiple uses. It thus warrants further investigation as a possible psychological mechanism of the benefits of ayahuasca. Further research exploring how mindfulness is affected beyond the 24 hour afterglow period in the days and weeks after, would offer insight into the therapeutic value of mindfulness gains made.

The current study has also reported significant differences in cognitive flexibility 24 hours after ayahuasca ingestion relative to baseline, with changes in performance unaffected by prior ayahuasca use. Evidence for changes in cognitive flexibility come from both self-report questionnaire measures (e.g. significantly higher mean scores on the CFS 24 hours after ayahuasca use) and more objective measures of cognitive flexibility, the WPCST. Mean number of correct answers on the WPSCT was significantly increased after ayahuasca use, with reaction times maintained, suggesting accuracy was improved without a significant slowing of response time.

Whilst the Stroop findings failed to show an effect of ayahuasca on Stroop interference (arguably cognitive flexibility), there was some indication ayahuasca had an effect on Stroop task performance. Errors in the incongruent condition were significantly reduced post-ayahuasca which provides some indication that ayahuasca improved performance on interference trials of the Stroop and further research could help elucidate this relationship.

The improvements shown in cognitive flexibility in the current study are consistent with recent neurobiological research into ayahuasca, reporting changes to neural networks associated with cognitive flexibility, both acutely and in the afterglow period (and up to 2 months after use) (Sampedro et al., 2017). Changes to neural network functioning in this period may also alter neuropsychological functioning which could help to account for the changes in cognitive

flexibility shown in this study. This could be therapeutically significant because a loosening of "cognitive grip" may be helpful for example in depressed patients who suffer from ruminative thinking and are 'stuck' in certain patterns of thoughts and behaviours. Supporting patients to consider alternative behavioural strategies and broaden perspectives is a key therapeutic goal of cognitive behavioural therapy (Kuypers et al., 2016). Also interesting to note is that this is the first study to report changes in cognitive flexibility following ayahuasca use both through subjective self-report and more objective task based measures, lending support to the assertion that cognitive flexibility is another potential psychological mechanism of action involved in the therapeutic effects of ayahuasca.

Whilst this is the first known study to show improved cognitive flexibility in a sample of healthy ayahuasca users in the afterglow period and has replicated findings for mindfulness in this same period, it is not without its limitations. Firstly, drug use data is self-report. In addition, the dose of ayahuasca taken by participants was unknown to the researchers and not recorded. The absence of a control group also limits the generalisation of findings. Whilst the current study employed a good sample size of healthy users, they were largely experienced with some precious use of ayahuasca which limits the generalisability of the findings to psychedelic naieve and clinical samples. At the same time however, relative to other studies, our sample was considerably less experienced overall in terms of previous ayahuasca use which is a strength of the current study. The current study also helps to advance more recent work exploring ayahuasca in secular settings which suggests ayahuasca may have a therapeutic effect beyond the effect of a religion confound.

In conclusion, the present study provides further evidence of ayahuasca's ability to enhance mindfulness and highlights it as a potential psychological mechanism of the psychotherapeutic effects ayahuasca. The findings that ayahuasca increases cognitive flexibility in the afterglow period are preliminary and suggest it is also worthy of further exploration as another possible psychological mechanism of ayahuasca's therapeutic effects. Additionally, the afterglow effect is also significant therapeutically and understanding the psychological mechanisms which occur during the afterglow period will be essential in developing effective ayahuasca-assisted treatments. Given that prior ayahuasca exposure had no significant effect on gains made to mindfulness and cognitive flexibility in the afterglow period highlights the potential beneficial effects of this psychedelic to both naïve and experienced users.

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