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FACILITATION OF MEMORY RETRIEVAL BY THE "ANTI-ADDICTIVE" ALKALOID, IBOGAINE

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Abstract. Anecdotal observations in humans indicate that indole alkaloid ibogaine may have anti-addictive properties. It has been suggested that the therapeutic action of ibogaine may depend upon facilitated access to the past experiences, purportedly influencing the initiation of drug addiction. To determine if ibogaine may facilitate memory retrieval, rats were trained in the Morris maze spatial navigation task. It has been found that ibogaine (0.25 or 2.5 mg/kg) or O-desmethyl-ibogaine (2.5 mg/kg) but not t-Butyl ibogaine, administered just before the test trial, facilitated spatial memory retrieval compared to rats receiving placebo treatment. It is concluded that although previously described NMDA receptor antagonistic properties of ibogaine may represent a locus for at least some of its actions, other mechanisms, involving facilitation of memory retrieval may be of importance for its anti-addictive effects.

Key Words: **ibogaine, drug addiction, memory, learning, information processing**

Introduction

Ibogaine is a psychoactive indole alkaloid originally isolated from the roots of the West African shrub *Tabernanthe iboga*. Iboga extracts were used in the religious rites of several West African societies. Both anecdotal reports in humans and preclinical studies indicate that ibogaine interrupts addiction to a variety of abused substances including alcohol (1), opiates (2,3), nicotine, and stimulants (4) (for review, see ref. [5]). Despite recent findings which indicate that pharmacologically relevant concentrations of ibogaine can affect a number of neurotransmitter receptor and uptake systems implicated in the effects of drugs of abuse, the mechanism by which ibogaine exerts its "anti-addictive" actions remain controversial.

Ibogaine possesses profound and unusual psychoactive properties. In humans, it produces a state of "oneirophrenia", sometimes compared to a dream with full consciousness that could be easily manipulated by the psychotherapist (6). Others, (7) described the effects of ibogaine as a facilitation of fantasies, as a "*movie run at high speed*" or a "*slide show*". The fantasy-enhancing properties of ibogaine were employed in the 1960's and 1970's by psychotherapists to facilitate psychotherapy. Thus, Naranjo observed at least 40 sessions conducted with 30 patients, and reported that at doses of 4-5 mg/kg patients experienced an enhancement of fantasy without

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experiencing changes in the perception of the environment, delusions, depersonalization, or formal alterations of thinking (6). It was concluded that ibogaine could act as a psychological catalyst which could compress a long psychotherapeutic process (6).

It has been proposed that at the psychological level, ibogaine may facilitate memory retrieval and, that the "intellectual re-evaluation" of accessible past experiences (purportedly being the reason of the initiation of drug addiction) may be crucial to the therapeutic effect of this alkaloid (8). Therefore, the present experiment was designed to find out if ibogaine may facilitate memory retrieval in a controllable setting.

Methods

Male Wistar rats (200-250 g, Institute of Pharmacology breeding facility) were housed under standard laboratory conditions (lights on at 0600 h, lights off 1800 h; room temperature 23 ± 1°) with chow and tap water available *ad libitum*. Before and during the experiment the rats were housed in groups of 4. Experiments were carried out between 0900 and 1700 h. All animals were used only once. Ibogaine HCl was a generous gift from Dr. P. Potier, Institut de Chimie des Substances Naturelles, Centre National de la Recherche Scientifique, Gif, France. T-Butyl ibogaine and O-desmethyl-ibogaine (for synthesis see ref. [9]) were kindly donated by Dr. C. Bertha National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, USA.

A gray metal circular pool (180 cm of diameter, 50 cm of height) was filled to a height of 25 cm with lukewarm (22°C) tap water which was changed every day. Curtains, bright lamps, and other stimuli around and above the pool provided numerous stable extra-maze cues. The trial consisted of manually placing a rat into the water facing the wall of the pool, at one of the four starting positions (N, W, S, E) around the pool perimeter. Every day the starting position was changed. The rat was required to find a gray metal platform (10 x 10 cm) which was present inside the pool in the middle between its center and the wall, with the upper surface submerged 1 cm below the water surface. The total circular area of the pool was divided into 4 quadrants (NE, SE, SW and NW) by means of the signs on the TV screen on which the training was observed. For the half of subjects, the platform was placed initially in the SE quadrant, for the remaining half in the NW quadrant. If not indicated otherwise, during re-training trials, the platform was placed in the opposite location, i.e. in the NW and SE quadrants, respectively. If a rat escaped onto the platform, it was permitted to remain there for 30 s. If a rat failed to find the platform within 120 s it was placed onto the platform by hand and allowed to remain there for 30 s. For each trial, the time a rat needed to escape onto the platform (escape latency) was measured by a hand-held electronic timer. After completion of the trial, the rats were put in the "drying" cage and heated by infrared lamp.

The behavioral procedure consisted of 5 days of pre-training, a first probe trial, re-training to the new platform position and a second probe trial. During *pre-training*, (4 trials per day) rats were trained to find the platform located in the initial quadrant. On Day 6, during the *first probe trial*, rats were swimming for one minute in the pool without a platform. Their swimming behavior was video-recorded. On Day 7, rats were placed in the pool and *re-trained* to find the platform positioned in the opposite quadrant. If not indicated otherwise, 4 trials were given. On Day 8, the *second probe trial*, (again with the platform removed from the pool) was performed. Rats were injected with drugs 30 min. before the second probe trial to investigate effects on memory retrieval.

To examine the reliability of the present use of the Morris water maze, an experiment was carried out on a separate group of rats. After 5 days of pre-training and the first probe trial as described above, during re-training day, rats were injected with placebo and divided randomly into 4 groups that: a) swum 4 times to the platform positioned in the initial location; b) were handled and did not swum; c) swum 2 or, d) 4 times to the platform positioned in the opposite quadrant. This experiment was carried out to assess the relationship between the time of the exposure to a new spatial location and its memory. The data obtained from group (d) were pooled with data of placebo-injected rats used in experiments involving drug injection and served as a baseline for comparison. The experiments were carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals (publication No. 85-23, revised 1985).

Swimming behavior during probe trials was video-recorded using commercial VCR. Swimming paths were analyzed by a PC computer, using the EYE 1.3 (J. Długopolski, Kraków, Poland) and TRACK-ANALYZER (10) software. To measure the strength of spatial memory of a given platform position, during both the first and the second probe trials was calculated the time (in seconds) spent by a rat in pool quadrant that contained the platform during the pre-training trials as well as the time spent by the rat in the opposite quadrant (11). To assess the ultimate memory of the pre-training platform position resulting from either behavioral or pharmacological manipulations, the following calculations were performed. The time a rat spent in the quadrant containing the platform on pre-training (INITIAL) was added to the time a rat spent in the opposite quadrant (OPPOSITE). The time a rat spent in the INITIAL quadrant was divided by that sum. This value allowed the assessment of the *relative preference* for the quadrant of the pool that contained the platform on pre-training (0-100%). The difference between the relative preference for the first probe trial and for the second probe trial was used as the index of memory retrieval of the platform position used during re-training (see equation).

$$MEMORY_{INDEX} = \left[\left(100 * \frac{INITIAL(1)}{INITIAL(1) + OPPOSITE(1)}\right) - \left(100 * \frac{INITIAL(2)}{INITIAL(2) + OPPOSITE(2)}\right)\right]$$

This way of calculating and presenting data allowed to compare the relative strength of the "new" versus "previous" spatial memory in that it included the individual variance among subjects that otherwise would mask possible effects of treatment. For statistics, one way between subjects ANOVA, followed by LSD test was used.

Results

Rats demonstrated rapid acquisition of the spatial memory and on Day 5, the latencies to escape onto the platform were less than 10 s (data not shown). During the first probe trial, rats were swimming in the vicinity of the place that contained the platform during the acquisition trials. Table I demonstrates that drug-free rats preferred the quadrant in which the platform was present during pre-training whether on re-training subjects swum 4 times to the platform positioned in the initial location (group a) or just handled (group b). However, rats that swum 2 (group c) or 4 (group d) times to the platform placed in the opposite quadrant during re-training, exhibited a marked shift in the preference towards that quadrant. There was no difference in that preference between rats that swum 2 or 4 times to the opposite platform position on re-training.

The non-transformed data of control rats that swum 4 times to the opposite platform position on re-training are as follows. On the first probe trial, these subjects (N=33) spent 25 ± 1 s in the INITIAL

TABLE I.

The effect of re-training rats to the platform positioned in the new location on the expression of its memory

Training on the re-training trial	Memory index	N
4 x INITIAL	-2.96 ± 4.25 **	10
Handling	1.85 ± 4.35 **	11
2 x OPPOSITE	22.57 ± 4.88	8
4 x OPPOSITE	30.00 ± 4.13	33

After pre-training and the first probe trial, during re-training day, rats were injected with placebo and divided randomly into 4 groups. Subjects described as "4 x INITIAL" swum 4 times to the platform positioned in the "pre-training" quadrant, while subjects described as "Handling" were just handled and did not swum on that day. Other rats swum 2 (2 x OPPOSITE) or 4 (4 x OPPOSITE) times to the platform positioned in the opposite, "new" quadrant. Presented are Mean ± SEM memory retrieval indexes. ANOVA: $F(3,61)=10.19$, $p < 0.0001$. Asterisks denote significant ($p < 0.025$) difference from rats that swum 4 times to the platform positioned in a new location during re-training day, i.e., 4 x OPPOSITE group.

TABLE II.

Effects of ibogaine, O-desmethyl-ibogaine and t-Butyl ibogaine on the retrieval of spatial memory.

Treatment on re-training trial	Memory index	N
Placebo	30.0 ± 4.13	33
Ibogaine 0.025	35.65 ± 5.66	10
Ibogaine 0.25	47.59 ± 6.19 **	11
Ibogaine 2.5	43.32 ± 4.17 *	11
O-desmethyl ibogaine 0.025	31.88 ± 5.50	10
O-desmethyl ibogaine 0.25	36.81 ± 8.04	11
O-desmethyl ibogaine 2.5	50.96 ± 6.18**	11
t-Butyl ibogaine 0.025	35.16 ± 6.58	10
t-Butyl ibogaine 0.25	35.51 ± 5.60	11
t-Butyl ibogaine 2.5	25.25 ± 4.82	11

Rats were trained for 5 days to navigate into the hidden platform. The strength of this spatial memory has been measured subsequently during the first probe trial. This was followed by re-training rats to locate platform positioned in the opposite, "new" quadrant. Drugs were administered 30 min. before the second probe trial (doses are expressed in mg/kg). Presented are Mean ± SEM memory retrieval indexes. Asterisks denote significant (* $p < 0.05$; ** $p < 0.025$) difference from placebo-treated rats.

quadrant and 8 ± 1 s in the OPPOSITE quadrant (relative preference is 74 ± 3 %). On the second probe trial, these subjects spent 14 ± 1 s in the INITIAL quadrant and 18 ± 1 s in the OPPOSITE quadrant (relative preference is 43 ± 3 %). Since the relative preference for the quadrant that contained platform on the pre-training (INITIAL) dropped from 74 % to 43 %, (suggesting formation of a new memory trace) the memory index for this group is ~ 30 % (see Tables).

Table II demonstrates that compared to placebo-treated rats, subjects injected with 0.25 or 2.5 mg/kg of ibogaine showed a significantly higher shift in the searching behavior towards the quadrant containing the new platform during re-training. Similar effects were found for O-desmethyl-ibogaine (2.5 mg/kg). T-Butyl ibogaine did not affect the shift in the searching behavior towards the quadrant containing platform during re-training.

Discussion

Water maze offers a reliable way for assessing effects of drugs on spatial learning and memory processes (12). After the information is stored, the performance in that task requires its retrieval which may be regarded as a transfer of stored knowledge (11) between long-term memory and short- or intermediate-term (13) memory. If the cues upon which spatial task solving are based are changed, a new spatial map is formed and long-term memory is updated (12). Thus, treatments that facilitate memory retrieval are likely to affect information that has been stored most recently.

Ibogaine, an indole alkaloid derived from *Tabernanthe iboga*, has been claimed to decrease dependence and the severity of withdrawal symptoms produced by addictive substances including opiates, stimulants, ethanol and nicotine (7). While anecdotal, these claims are consistent with recent preclinical findings demonstrating that ibogaine decreases preference for cocaine and morphine, reduces morphine self-administration and attenuates symptoms of morphine withdrawal (for review, see ref. [5]).

Several hypotheses have been proposed to explain the inhibitory effects of ibogaine on drug seeking behavior. Although ibogaine affects a number of neurotransmitter systems (5), only few of them could be considered as a potential target of its anti-addictive properties. It has been estimated that at a typical dose of ~ 40-80 mg/kg administered to rats or mice, brain concentration of ibogaine is sufficient to affect σ (K_i for σ_2 sites ~ 250 nM), NMDA (K_i ~ 1 mM) or κ opioid (K_i ~ 2-3 mM) neurotransmitter systems (5). A likely explanation of ibogaine's anti-addictive effects was offered by linking evidences of anti-addictive properties of NMDA receptor antagonists (for review see ref. [14]) with NMDA – antagonistic properties of ibogaine (3).

The NMDA antagonistic activity of ibogaine fails to explain the present findings however. The previously reported inhibition of drug-seeking behaviors by ibogaine has been demonstrated at doses of ≥ 40 mg/kg of this alkaloid while ibogaine facilitated memory retrieval at much lower doses (0.25 - 2.5 mg/kg) that are unlikely to affect NMDA receptors in vivo. The NMDA-mediated action of ibogaine cannot explain also the facilitation of memory retrieval by O-desmethyl-ibogaine, a compound that affects NMDA receptor complex with affinity ~ 5 fold lower than that of ibogaine (9). Although O-desmethyl-ibogaine mimics inhibitory effects of ibogaine on drug seeking behaviors in some (15) but not other (9) studies, the doses needed for these effects (≥ 40 mg/kg) are ~ 20 times higher than doses needed for facilitation of memory retrieval effects. Similarly, at doses effective in the present experiments, ibogaine and O-desmethyl-ibogaine are unlikely to affect opioid κ receptors.

At commonly used doses, ibogaine may disrupt memory acquisition; in fact, Kesner and colleagues (16) found recently that 40 mg/kg of ibogaine had inhibitory effect on spatial learning. Learning and memory processes are typically affected by drugs in an inverted U shaped dose-response curve in that low doses have opposite effects from high doses (17). It may thus be assumed that at the doses effective in the present experiment (0.25-2.5 mg/kg) ibogaine may affect neurotransmitter system(s) for which it possesses an even greater affinity. At nanomolar concentrations, ibogaine binds to σ sites (K_i for σ_2 sites ~ 250 nM) (5,18). However, the inability to facilitate memory retrieval by the structurally related t-Butyl-ibogaine (ibogaine analog designed to resist O-dealkylation, a likely way of ibogaine's metabolic degradation [9]), a high affinity σ_2 ligand (K_i ~ 340 nM) indicates that σ sites are unlikely target. This conclusion is supported by the fact that another ibogaine derivative that was effective in the present experiments, O-desmethyl-ibogaine (ibogaine putative metabolite [19]) posses much lower (micromolar) affinity for σ sites (20).

According to Regan (8), the therapeutic "anti-addictive" action of ibogaine involves the release of repressed memories, intellectual re-evaluation of memories, and integration of new insights into the personality of the patient. Although this hypothesis remains highly speculative and so far was not deeply evaluated in the clinical settings, the ability of ibogaine and O-desmethyl-ibogaine to facilitate memory retrieval in rats seems consistent with it. Based on the comparison between potencies of ibogaine-related compounds to affect various neurotransmitter systems and to facilitate memory retrieval, it is likely that the potential target for at least some of ibogaine's effects remains to be identified.

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