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Short communication

Effects of chronic ibogaine treatment on cerebellar Purkinje cells in the rat

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Abstract

The present investigation assessed the chronic toxicity of ibogaine on cerebellar Purkinje cells in male Fischer 344 rats. A behaviorally active dose of ibogaine (10 mg/kg, i.p.) was administered to a group of six subjects every other day for 60 days while the control group received an equivalent volume of saline (1 ml/kg). Estimates of Purkinje cell number were determined using the optical dissector/fractionator technique. No significant differences in Purkinje cell number were observed between the ibogaine (243764[±32766]) and control groups (230813[±16670]).

Keywords: Ibogaine; Purkinje cell; Optical dissector; Chronic toxicity

Recent studies in both human and non-human subjects suggest a beneficial effect of ibogaine in the treatment of substance abuse. In rats, ibogaine blocks self-administration of morphine [3,4], heroin [2], cocaine [1,2,4], and ethanol [16]. Although clinical data in support of ibogaine's anti-addictive effects are limited [18], patents have been issued for its use in the treatment of opiate [8], cocaine [9], amphetamine [9], ethanol [10], and nicotine abuse [11]. Despite these encouraging results ibogaine remains a schedule I drug in the United States and thus not available for use in humans.

A major impediment to the approval of ibogaine for clinical use is the finding that this agent is neurotoxic to cerebellar Purkinje cells in rats [13,14]. These authors demonstrated that a single high dose of ibogaine ($\geq$ 100 mg/kg, i.p.) produced selective degeneration of Purkinje neurons. Although these findings have been confirmed by others [5,12], it appears that the neurotoxic effects of ibogaine are only seen following high doses ($\sim$ 100 mg/kg) as Molinari et al. [12] saw no evidence of toxicity following a single dose of 40 mg/kg. Because all of these studies focused on the neurotoxicity of acutely administered ibogaine the present investigation examines the effect of chronic administration of a behaviorally active dose of ibogaine (10 mg/kg) on cerebellar Purkinje cell number (Fig. 1). This dose was chosen for investigation because it is used in chronic behavioral experiments to induce stimulus control [6,17].

The method employed in the present study makes use of a state of the art stereologic technique known as the optical dissector/fractinator. This method allows for the determination of accurate estimates of cell number while eliminating confounding factors such as neuron size, shape, and orientation [19].

Male Fischer 344 rats were obtained from Harlan Sprague–Dawley Inc. (Indianapolis, IN). These subjects were approximately 60 days old at the beginning of this study. They were housed in pairs under a natural light-dark cycle and allowed free access to food and water in the home cage. Subjects were divided into two groups, ibogaine or control. The ibogaine group received ibogaine (10 mg/kg, i.p.) every other day for 60 days while the control group received an equivalent volume of saline (1 ml/kg, i.p) on the same schedule. Forty-eight hours after their last treatment, subjects were deeply anaesthetized with sodium pentobarbital (65 mg/kg) and perfused through the aorta with phosphate-buffered saline containing 1% sodium nitrite. This was followed by a 4% solution of paraformaldehyde in Sorensen's buffer (pH = 7.4). Brains were removed and the cerebellum was separated from the cerebrum and brainstem by transverse incisions through the

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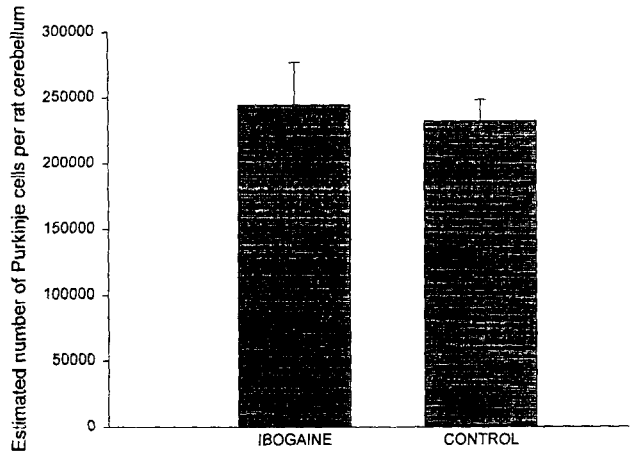

Fig. 1. The effect of ibogaine on cerebellar Purkinje cell number. Ibogaine (10 mg/kg) or saline (1.0 ml/kg) were administered every other day for a 60-day period. Each data point represents the mean value from six subjects. Error bars reflect S.E.M. values.

inferior colliculus and the medulla oblongata. Cerebella were then post-fixed for 3 days in 4% paraformaldehyde in Sorensen's buffer, dehydrated in a graded series of alcohols, and embedded in glycol methacrylate (JB-4 resin, Polysciences, Inc.) for coronal sectioning. Tissue blocks were completely sectioned on a rotary microtome at 25 μm. Every other section was discarded and the remaining sections were mounted on gel covered slides. These were stained with 0.5% toluidine blue in 1% sodium borate, dehydrated in alcohols, and coverslipped with Permount. The resulting slides were evaluated by one of us (SH) who was blind to the treatment group of the sample.

The total number of cerebellar Purkinje cells were estimated using the optical dissector/fractionator according to the method described by West et al. [19]. The dissector/fractionator consisted of a modified Olympus BH-2 microscope equipped with a motorized stage (x- and y-axis) (Marzhauser Corp., Germany) as well as an attached microcator (Ono Sokki, Japan) for measurements in the z-axis. Microscope images (viewed under oil, 100×) were displayed on a high resolution video monitor (Sony, Japan) with the use of a camera affixed to the microscope (MTI CCD C72). An unbiased counting frame which covered an area of 556.7 μm^2 was superimposed over the projected image. Twenty tissue sections were selected (representing a known fraction) from each cerebellum for counting. The first section in the series was determined by a number taken from a table of random numbers. Subsequent sections were spaced as dictated by the known fraction (e.g., if a given cerebellum yielded 150 sections and the number 5 was chosen at random then every seventh slide would be counted beginning with slide 5). Beginning at a random point outside of the region of interest, sections were moved through a raster pattern of x, y movement (steps) by the programmable stage shifter. Each section was sampled in its entirety in this manner. Using the microcator, the focus was moved through a fixed 10 μm depth of the section (3–13 μm), and those nuclei located within this depth were counted. In addition, Purkinje cell nuclei were counted only if they fell within the counting frame. For cells whose nuclei crossed the lines of the frame, only those touching the top and/or right line were counted whereas those crossing the bottom and/or left line were excluded.

The total number of Purkinje neurons, N, in each cerebellum was calculated using the following equation: $N = \Sigma Q^{-1} \cdot t/h \cdot 1/\text{asf} \cdot 1/\text{ssf}$ [19] where ΣQ^{-} represents the total number of Purkinje cells counted, t is the average section thickness, h is the height of the disector (10 μm), asf represents the areal sampling fraction, and ssf is the section sampling fraction.

Upon the completion of the counting studies the code was broken and group means were determined and compared using Student's t-test. No significant differences were observed between the ibogaine (2.4×10^5) and control (2.3×10^5) groups as shown in the figure below.

The findings of the present study suggest that repeated administration of a behaviorally active dose of ibogaine (10 mg/kg) does not produce Purkinje cell loss. Because Purkinje cells appear to be the neuronal population most sensitive to the toxic effects of ibogaine, it is assumed that other neuronal populations are likewise unaffected. Behavioral support for this assumption comes from a previous study in our laboratory [7] in which daily administration of 30 mg/kg of ibogaine failed to impair acquisition of a spatial learning task in the 8-arm radial maze. Instead the ibogaine-treated rats committed significantly fewer errors to criterion than control rats. A similar beneficial effect of ibogaine was observed in a recent study by Popik [15]. Currently we have a group of rats in our laboratory which are trained to discriminate ibogaine (10 mg/kg) from water in a two lever operant task [6]. Some of these subjects have been receiving ibogaine every other day for over 18 months. The fact that the performance by these subjects has remained stable for this period further demonstrates that chronic treatment with this dose of ibogaine does not produce behavioral toxicity. This, taken together with the results of the present study justifies the use of this compound in drug discrimination studies where this substance must be administered chronically without producing neurotoxic effects which may interfere with the stability of the ibogaine-trained discriminative stimulus.

The results of the present study together with those of Molinari et al. [12], who observed no significant Purkinje cell degeneration following a single dose of ibogaine of 40 mg/kg, suggest that ibogaine, at behaviorally significant doses in the rat, is devoid of neurotoxic effects. Caution is of course warranted in the extrapolation of these animal data to humans but it may well be that behaviorally active doses in man are likewise without neurotoxicity. Furthermore, even if ibogaine, because of its hallucinogenicity, never achieves status as a clinically useful drug, it may

serve as a lead compound in the development of more efficacious congeners. For example, 18-methoxycoranaridine, an ibogaine congener, has been shown to reduce morphine and cocaine self-administration in rats without producing Purkinje cell degeneration even at high doses [5]. The continuing societal need to develop effective treatments for substance abuse disorders makes it essential that no promising therapy be abandoned before it is thoroughly evaluated.

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