Neurochemical and behavioural interactions between ibogaine and nicotine in the rat

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1 *In vivo* brain microdialysis has been employed to investigate the effects of ibogaine on nicotine-induced changes in dopamine overflow in the nucleus accumbens (NAc) of freely moving rats. The effects of the compound on locomotor responses to nicotine and behaviour in the elevated plus-maze were also examined.

2 No changes were observed in the dopamine overflow or the locomotor activity of the animals following the administration of ibogaine (40 mg kg^{-1}, i.p.). However, ibogaine, administered 22 h earlier, significantly ($P < 0.01$) attenuated the increase in dopamine overflow but not the hyperlocomotion, evoked by nicotine.

3 In the elevated plus-maze test, significant reductions in the open:total runway entries in both saline-treated controls ($P < 0.05$) and nicotine-treated ($P < 0.01$) rats were obtained when the animals were tested 22 h after pretreatment with ibogaine (40 mg kg^{-1}, i.p.). The total activity was significantly ($P < 0.01$) greater in the nicotine-treated rats but this response was not affected by ibogaine pretreatment.

4 Administration of ibogaine was associated with reductions in the tissue levels of 5-hydroxyindoleacetic acid (5-HIAA) in the NAc ($P < 0.01$) and striatum ($P < 0.05$) and an increase in the level of this metabolite in the medial prefrontal cortex (mPFC) ($P < 0.01$) while the levels of dopamine and 5-hydroxytryptamine (5-HT) in the mPFC were reduced ($P < 0.05$). The DOPAC/dopamine ($P < 0.05$) and 5-HIAA/5-HT ($P < 0.01$) ratios were significantly increased in the mPFC for at least 7 days after a single treatment with ibogaine.

5 Ibogaine attenuates the nicotine-induced increases in dopamine overflow in the NAc and may, therefore, inhibit the rewarding effects of this drug. However, the long lasting anxiogenesis induced by ibogaine warrant further investigation before its use could be recommended for smokers.

Keywords: Ibogaine; nicotine; *in vivo* brain microdialysis; nucleus accumbens: medial prefrontal cortex; striatum; elevated plus-maze.

Introduction

Ibogaine (Endabuse; NH 10567), an indole alkaloid found in the root and bark of the West African plant, *Tabernanthe iboga*, has previously been reported to have CNS stimulant (Gershon & Lang, 1962), anxiogenic (Schneider & Sigg, 1957) and hallucinogenic (Clineschmidt *et al.*, 1978) activity. Recently, it has been reported (Cappendjik & Dzoljic, 1993; Dworkin *et al.*, 1995; Popik *et al.*, 1995) that U.S. patents have been awarded to Howard Lotsof (1985, 1986, 1989, 1991) for the use of ibogaine as an anti-addictive agent in the treatment of opiate (*Patent No.* 4,499,096) and stimulant abuse (*Patent No.* 4,587,243), alcoholism (*Patent No.* 4,857,523) and nicotine/cigarette smoking (*Patent No.* 5,026,697). However, preclinical studies to investigate the anti-addictive potential of ibogaine are seriously lacking.

The evidence available suggests that ibogaine can attenuate the self-administration of opiates (Glick *et al.*, 1991; Dworkin *et al.*, 1995) and cocaine (Cappendjik & Dzoljic, 1993; Dworkin *et al.*, 1995) by rats. Further support for the possible effectiveness of this alkaloid, at least in the treatment of opiate abuse, comes from the reports that morphine-evoked increases in nucleus accumbens dopamine overflow and locomotor activity are blocked by ibogaine-pretreatment (Maisonneuve *et al.*, 1991) and that some, though not all, of the naloxone-precipitated withdrawal symptoms are relieved following ibogaine (Dzoljic *et al.*, 1988; Glick *et al.*, 1992). With respect to the psychostimulants, it appears that ibogaine may potentiate rather than attenuate the hyperlocomotion and nucleus accumbens dopamine secretion in response to cocaine (Maisonneuve & Glick, 1992) and amphetamine (Maisonneuve *et al.*, 1992) in rats. Since self-administration of psychostimulants is thought to be dependent on potentiation of mesolimbic dopamine (Koob, 1993), these effects are contrary to those which might be expected. However, in view of the report that high doses of cocaine can lead to anxiety reactions (Cohen, 1975) Maissoneuve & Glick (1992) questioned whether the potentiation of the effects of cocaine by ibogaine would cause aversion and result in a decreased desire to continue cocaine use.

In common with morphine and the psychomotor stimulants, the rewarding and locomotor enhancing properties of nicotine appear to be related to its ability to increase nucleus accumbens dopamine overflow (Singer *et al.*, 1982; Clarke *et al.*, 1988; Di Chiara & Imperato, 1988; Reavill & Stolerman, 1990; Benwell & Balfour, 1992; Corrigall *et al.*, 1992). However, to date, there are no reports on the influence of ibogaine on the responses to nicotine, which, in the form of tobacco, is one of the most widely abused substances. Therefore, the present investigation has attempted to establish the extent to which ibogaine influences the stimulant effect of nicotine on mesolimbic dopamine systems and locomotion.

As a result of the intriguing possibility suggested by Maisonneuve & Glick (1992), that the combination of cocaine and ibogaine may be anxiogenic and aversive, we also sought to examine the possibility that ibogaine pretreatment induces an anxiogenic response when the animals are subsequently exposed to nicotine which could lead to a reluctance to continue self-administration.

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Methods

Subjects and pretreatments

Male Sprague Dawley rats, bred in the Biomedical Services Unit at Ninewells hospital, Dundee, from stock originally purchased from Interfauna Ltd were used throughout. All animals had free access to food and water and weighed 250–350g at the start of the experiments.

In the microdialysis study, all animals were pretreated with 5 consecutive daily subcutaneous injections of (−)-nicotine hydrogen tartrate (0.4 mg kg^{-1} free base in isotonic saline adjusted to pH 7.4 with NaOH) and were housed in pairs prior to and singly following surgery.

For the elevated plus-maze study, 24 rats were randomly assigned to 4 groups of 6 animals. Half of the animals received 5 consecutive daily subcutaneous injections of saline (1.0 ml kg^{-1}) and half received (−)-nicotine hydrogen tartrate (0.4 mg kg^{-1} free base in isotonic saline adjusted to pH 7.4 with NaOH). At least 3 h after their customary injection on day 5, half of the saline and half of the nicotine-treated groups received an intraperitoneal injection of ibogaine (40 mg kg^{-1}) while the remainder received the ibogaine injection vehicle (25% ethanol/sterile water solution administered in a dose of 1.0 ml kg^{-1}).

For the experiment involving the measurement of tissue levels, 24 animals were allocated randomly to 4 groups of 6. All animals were pretreated with 5 consecutive daily subcutaneous injections of saline. On day 5 half were injected with ibogaine (40 mg kg^{-1}, i.p.); the remainder with the vehicle. Half of the vehicle and half of the ibogaine-treated rats were killed by cervical dislocation 24 h after this i.p. injection while the remainder were killed 7 days later. The brains were rapidly removed and dissected over ice and stored at −70°C for subsequent analysis of the tissue levels of dopamine, DOPAC, 5-hydroxytryptamine (5-HT) and 5-hydroxyindole acetic acid (5-HIAA).

Microdialysis experiments

At least 3 h after their nicotine injections on day 5, the rats were anaesthetized with Halothane and dialysis probes (loop probe constructed from 200 μm cuprophan dialysis tubing with dimensions of 1.5 mm length (total unfolded length of 3 mm), 200 μm wide in the coronal plane and 400 μm wide in the anterior posterior plane protruding from a single 21 gauge stainless steel tube) stereotaxically implanted into the nucleus accumbens (NAc) using the co-ordinates of 1.7 mm rostral and 1.5 mm lateral with respect to bregma and 7.5 mm vertically from the dura as described in more detail elsewhere (Benwell & Balfour, 1992). At the end of the experiment the position of the probes was routinely determined histologically from sections prepared *postmortem*.

Eighteen hours following implantation of the probe, the animals were placed individually in an activity box (40 cm square × 25 cm high sides) where the locomotor activity was assessed by photocells located on adjacent sides of the box as described previously (Vale & Balfour, 1989). At this time the dialysis probe was connected to a syringe pump containing a Ringer solution (Composition mM: NaCl 147, KCl 4 and $CaCl_2$ 1.25 perfusing at a rate of 1.7 μl min^{-1}) via a liquid swivel. Following a 60 min equilibration period, samples of dialysate were collected at 20 min intervals for the duration of the experiment. When a stable baseline of dopamine and DOPAC levels had been obtained, all animals were challenged by an injection of saline followed 60 min later by an injection of nicotine (0.4 mg kg^{-1}, s.c.) after which a further 5 × 20 min samples were collected. At this time, on day 1 of dialysis, half of the rats were injected with ibogaine (40 mg kg^{-1} ip) and the remainder were injected with the injection vehicle (25% ethanol in sterile water administered at a dose of 1 ml kg^{-1}) and a further 4 samples collected. On the following day, all animals were again dialysed and challenged with saline followed 60 min later by nicotine as on the first dialysis day. The concentrations of dopamine and DOPAC in the dialysates were determined by h.p.l.c. in combination with a Coulochem detector as outlined by Benwell & Balfour (1992).

Elevated plus maze study

Twenty two hours following the injection of ibogaine or vehicle, the animals were given their customary daily subcutaneous injection of saline or nicotine (0.4 mg kg^{-1}) and, 3 min later, placed in the centre of a symmetrical elevated plus maze which was raised 0.6 metre from the floor and had runways 45 cm long × 9 cm wide, 2 of which had sides 15 cm high and 2 of which had no sides (Pellow *et al.*, 1985). The number of entries into each of the four arms was recorded automatically by photocells located in the arms of the maze.

Tissue levels of dopamine, DOPAC, 5-HT and 5-HIAA in discrete brain regions

NAc, striatum (Str) and medial prefrontal cortex (mPFC) were dissected according to the method described by Clarke & Pert (1985). The tissue levels were measured in ultrafiltered supernatants prepared from perchloric acid (0.1N; 100 mg ml^{-1}) homogenates of the brain regions. The concentrations of dopamine, DOPAC, 5-HT and 5-HIAA were assayed by h.p.l.c. and electrochemical detection.

Statistical analysis

This was carried out using the Statistics Package for Social Scientists (SPSS). The microdialysis data were analysed by analysis of variance for repeated measures where the time points used for analyses were 20–120 min for effects of saline, 80–220 min for the effects of nicotine and from 220–300 min for the effects of vehicle and ibogaine on day 1 of dialysis. The plus-maze data were analysed by a 2-way analysis of variance (ANOVA) with pretreatment with nicotine and ibogaine as the independent factors analysed and using the locomotor activity as a cofactor when analysing the open to total ratio. Data for the tissue level experiment were analysed by 2-way ANOVA with ibogaine and time after ibogaine as the factors analysed.

Drugs

Nicotine hydrogen tartrate was purchased from Sigma and ibogaine from Research Biochemicals Incorporated (RBI inc).

Results

The effect of ibogaine on extracellular dopamine, DOPAC and locomotion

The administration of ibogaine was not associated with any significant changes in the extracellular levels of dopamine (Figure 1a) or DOPAC (Figure 2a) in the NAc or in the locomotor activity (Figure 3a) of the animals over the 80 min period immediately following its administration as shown on day 1 of dialysis. However, all of the animals given ibogaine displayed marked ataxia and unusual body postures during the 60 min following administration of this drug. The vehicle-treated rats showed no such behaviour. There was a tendency for the basal levels of dopamine to be reduced on day 2 of dialysis compared with those obtained on day 1 (basal dopamine were, pre-vehicle 0.150 ± 0.038, 18 h post-vehicle 0.138 ± 0.038, pre-ibogaine 0.154 ± 0.036, 18 h post-ibogaine 0.130 ± 0.016 pmol 20 μl^{-1} dialysate, uncorrected for recovery through probe), an effect which proved significant (F_{day} (1,18) = 4.69, $P < 0.05$) with the extracellular levels of DOPAC (basal levels were pre-vehicle 23.49 ± 4.6, 18 h post-vehicle 16.49 ± 2.94, pre-ibogaine 18.75 ± 1.84, 18 h post-ibogaine 12.64 ± 2.56 pmol 20μl^{-1} dialysate, uncorrected for recovery

through probe). However, these changes were not influenced by the prior administration of ibogaine.

The effect of ibogaine on nicotine-induced changes in NAc dopamine and locomotion

The administration of nicotine caused a significant increase in the extracellular levels of dopamine ($F_{nic}(7,77) = 5.03$, $P < 0.001$) (Figure 1a), prior to the administration of ibogaine or vehicle on day 1 of dialysis. On day 2 of dialysis, the extracellular dopamine levels, obtained in response to nicotine, were significantly ($F_{ibo \times day}(1,22) = 5.52$, $P < 0.05$) influenced by the prior administration of ibogaine 22 h earlier. The vehicle-treated rats showed a significant increase in NAc dopamine ($F_{nic}(7,35) = 5.39$, $P < 0.01$) (Figure 1b) when challenged with nicotine on day 2, 22 h after an injection of vehicle. However, the nicotine-induced NAc dopamine response was significantly attenuated on day 2, 22 h after administration of ibogaine. This effect was different both in comparison to the response in the vehicle-treated group ($F_{ibo}(7,77)$-2.16, $P < 0.05$) as well as with their responses on day 1 ($F_{day}(7,77) = 2.27$, $P < 0.05$) of dialysis prior to the ibogaine injection. Analysis of the DOPAC data suggests that the administration of nicotine is associated with a significant ($F_{nic}(7,154) = 7.17$, $P < 0.01$) increase in the extracellular metabolite level in the NAcc (Figure 2a). This effect appears to be enhanced in the animals which had been treated with ibogaine 22 h previously; however, this only approached significance ($P = 0.077$) (Figure 2b). The locomotor responses elicited by nicotine ($F_{nic}(7,154) = 43.6$, $P < 0.001$) were unaffected by the ibogaine treatment and dialysis day (Figure 3a and b).

Effect of ibogaine and nicotine on plus-maze activity

There was a significant ($F_{ibo}(1,17) = 12.7$, $P < 0.01$) decrease in the open total arm entry ratio when the rats were placed in the plus-maze 22 h after the administration of ibogaine (Figure 4a). This occurred in both the saline and nicotine-pretreated rats. The injection of nicotine itself was without effect on the open/total arm entry ratio.

However, nicotine produced a very significant ($F_{nic}(1,18) = 23.7$, $P < 0.001$) increase in the spontaneous activity of the rats, as assessed by the level of total arm entries (Figure 4b), an effect which was not influenced by the prior administration of ibogaine.

The effect of ibogaine on brain tissue levels of dopamine, DOPAC, 5-HT and 5-HIAA

The concentrations of dopamine, DOPAC, 5-HT and 5-HIAA, in the NAc, striatum and mPFC, 1 day and 7 days after an intraperitoneal injection of ibogaine, are shown in Table 1.

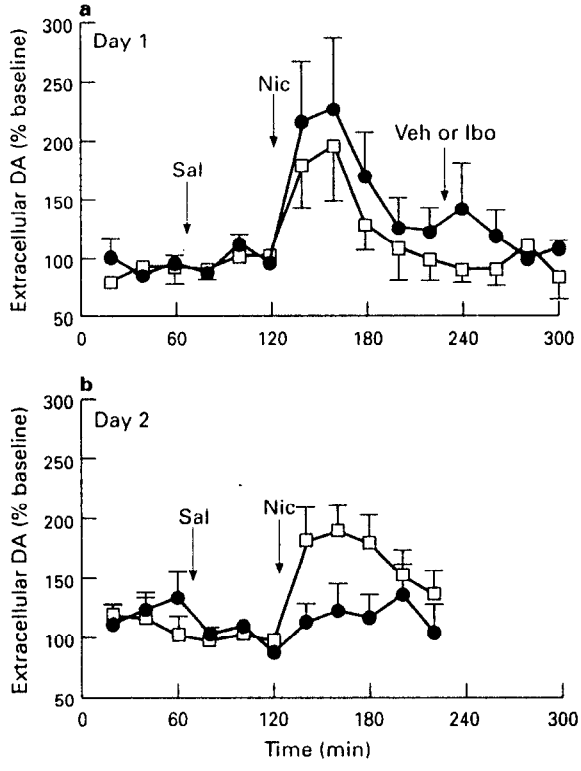

Figure 1 The influence of ibogaine on nicotine induced changes in NAc dopamine (DA). All animals received daily injections of nicotine (0.4 mg kg^{-1}, s.c.) for 5 days prior to implantation of dialysis probes in the NAc. On day 6 (a), all animals were challenged with saline (administered at the time shown by the first arrow) and nicotine (0.4 mg kg^{-1}, s.c. at the time shown by the second arrow) before receiving injections of either injection vehicle (□) or ibogaine (40 mg kg^{-1}, i.p.) (●) at the time indicated by the third arrow. On day 7 (b) the same animals were again challenged with saline and nicotine as on the previous day, 22 h after their injection of vehicle or ibogaine. The results are the means ± s.e.mean of 7 observations, expressed as percentages of the mean values obtained prior to the nicotine injection.

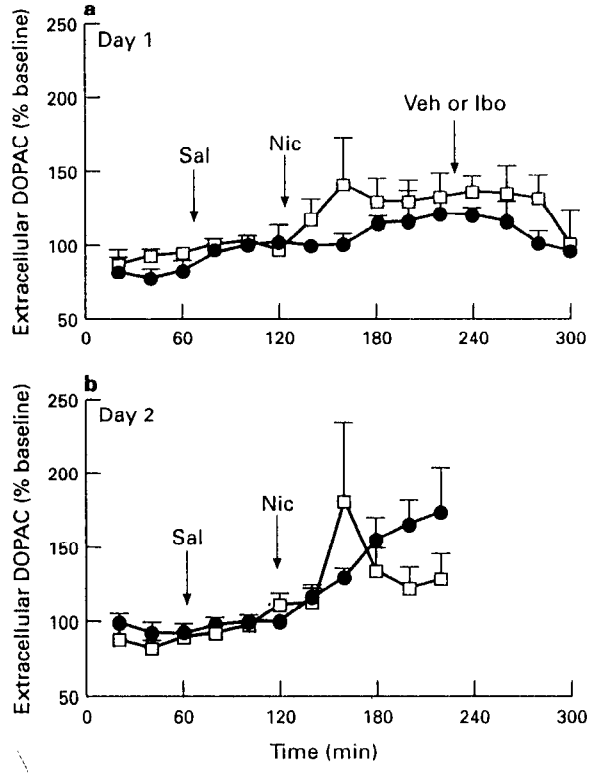

Figure 2 The influence of ibogaine on nicotine induced changes in NAc DOPAC. All animals received daily injections of nicotine (0.4 mg kg^{-1}, s.c.) for 5 days prior to implantation of dialysis probes in the NAc. On day 6 (a), all animals were challenged with saline (administered at the time shown by the first arrow) and nicotine (0.4 mg kg^{-1}, s.c. at the time shown by the second arrow) before receiving injections of either injection vehicle (□) or ibogaine (40 mg kg^{-1}, i.p.) (●) at the time indicated by the third arrow. On day 7 (b) the same animals were again challenged with saline and nicotine as on the previous day, 22 h after their injection of vehicle or ibogaine. The results are the means ± s.e.mean of 7 observations, expressed as percentages of the mean values obtained prior to the nicotine injection.

The concentration of dopamine in the mPFC was greatly ($F_{ibo}(1,20) = 9.4$, $P < 0.01$) reduced both 1 and 7 days following the ibogaine treatment. The 5-HT concentration in this tissue was also significantly ($F_{ibo}(1,20) = 7.2$, $P < 0.05$) less in the ibogaine-treated animals in comparison with the vehicle-treated controls which was not influenced by the day after treatment. However, this was accompanied by a significant ($F_{ibo \times day}(1,20) = 5.2$, $P < 0.01$) increase in 5-HIAA only 1 day after ibogaine. These changes resulted in an apparent increase in the turnover of both dopamine and 5-HT in the mPFC as indicated by the significant increases in the DOPAC/dopamine ($F_{ibo}(1,20) = 5.74$, $P < 0.05$) and 5-HIAA/5-HT ($F_{ibo}(1,20) = 16.00$, $P < 0.01$) ratios which were obtained (Figure 5), changes which persisted for at least 7 days. There were also significant reductions in the 5-HIAA levels of the NAc ($F_{ibo}(1,20) = 13.2$, $P < 0.01$) and striatum ($F_{ibo}(1,20) = 5.89$, $P < 0.05$) of the ibogaine-treated compared with vehicle-treated rats. However, there were no significant changes in the ratio of metabolites/transmitter levels in these brain regions associated with this treatment.

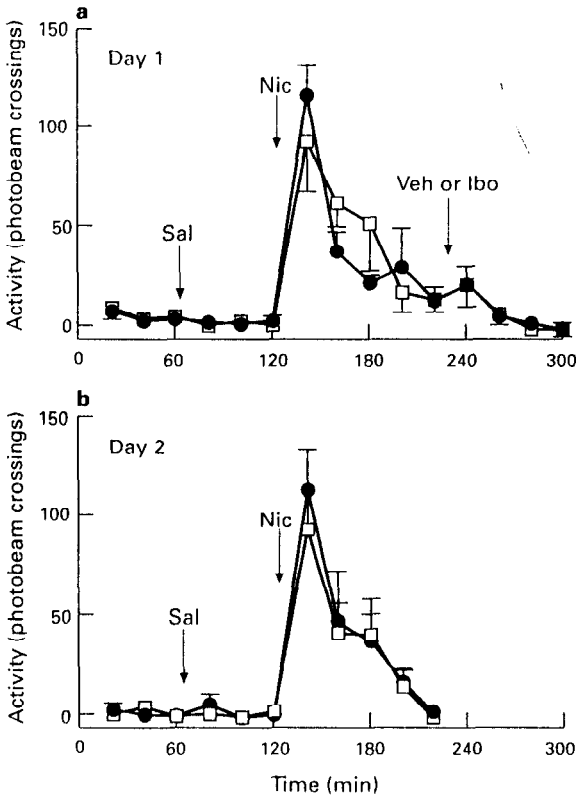

Figure 3 The influence of ibogaine on nicotine-induced changes in locomotion. All animals received daily injections of nicotine (0.4 mg kg^{-1}, s.c.) for 5 days prior to implantation of dialysis probes in the NAc. On day 6 (a), all animals were challenged with saline (administered at the time shown by the first arrow) and nicotine (0.4 mg kg^{-1}, s.c. at the time shown by the second arrow) before receiving injections of either injection vehicle (□) or ibogaine (40 mg kg^{-1}, i.p.) (●) at the time indicated by the third arrow. On day 7 (b) the same animals were again challenged with saline and nicotine as on the previous day, 22 h after their injection of vehicle or ibogaine. The results are the means ± s.e.mean of 7 observations.

Discussion

The present study investigated the effects of intraperitoneally administered ibogaine at only one dose level, 40 mg kg^{-1}. However, this route and dose has previously been shown to be behaviourally active (Glick *et al.*, 1991) and the dose is the one

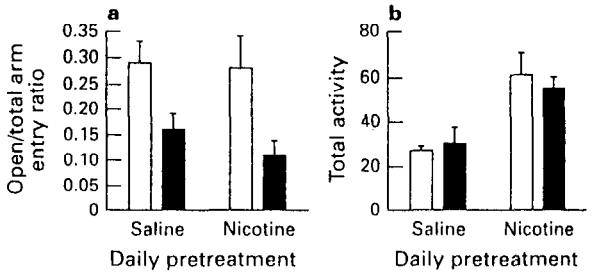

Figure 4 The influence of ibogaine and nicotine on plus-maze activity. The rats received a daily pretreatment of either saline or nicotine (0.4 mg kg^{-1}, s.c.) once/day for 5 days. On day 5, at least 3 h after their customary daily pretreatment, half of the saline and nicotine-treated rats received an injection of ibogaine (40 mg kg^{-1}, i.p., solid columns) while the remainder received the injection vehicle (open columns). The following day, 22 h after ibogaine or vehicle, the rats received their customary daily pretreatment 3 min before being placed in the plus-maze and their behaviour recorded for 15 min. Results are the means ± s.e.mean of 6 observations in each group.

Table 1 The effect of ibogaine on regional tissue levels of DA, DOPAC, 5-HT and 5-HIAA

Regional measure	*Day 1*		*Day 7*		*Statistics*
	Vehicle	*Ibogaine*	*Vehicle*	*Ibogaine*	
NAc					
Dopamine	4.52 ± 0.46	5.43 ± 0.67	5.08 ± 1.05	5.07 ± 0.70	
DOPAC	2.33 ± 0.22	1.8 ± 0.22	2.22 ± 0.41	2.29 ± 0.17	
5-HT	1.76 ± 0.06	1.59 ± 0.28	1.85 ± 0.27	1.59 ± 0.27	
5-HIAA	0.80 ± 0.04	0.53 ± 0.07	0.84 ± 0.05	0.70 ± 0.06	$F_{ibo}(1,20) = 13.2$, $P < 0.01$
Str					
Dopamine	7.15 ± 1.67	5.62 ± 0.86	7.45 ± 1.26	6.77 ± 2.04	
DOPAC	1.59 ± 0.28	1.16 ± 0.14	1.48 ± 0.22	1.23 ± 0.30	
5-HT	1.20 ± 0.10	0.92 ± 0.13	0.93 ± 0.13	1.13 ± 0.17	
5-HIAA	0.49 ± 0.03	0.37 ± 0.03	0.44 ± 0.04	0.39 ± 0.04	$F_{ibo}(1,20) = 5.89$, $P < 0.05$
mPFC					
Dopamine	0.175 ± 0.049	0.087 ± 0.015	0.200 ± 0.044	0.079 ± 0.011	$F_{ibo}(1,20) = 9.24$, $P < 0.01$
DOPAC	0.160 ± 0.034	0.164 ± 0.030	0.149 ± 0.013	0.108 ± 0.020	
5-HT	0.730 ± 0.120	0.490 ± 0.070	0.760 ± 0.120	0.470 ± 0.070	$F_{ibo}(1,20) = 7.2$, $P < 0.05$
5-HIAA	0.470 ± 0.060	0.690 ± 0.040*	0.470 ± 0.060	0.430 ± 0.060	$F_{ibo \times day}(1,20) = 5.2$, $P < 0.05$

Results are expressed as $\mu g\ g^{-1}$ wet weight and are the means ± s.e.mean of 6 observations in each case. *Post hoc* analysis, *$P < 0.05$ when compared with vehicle day 1.

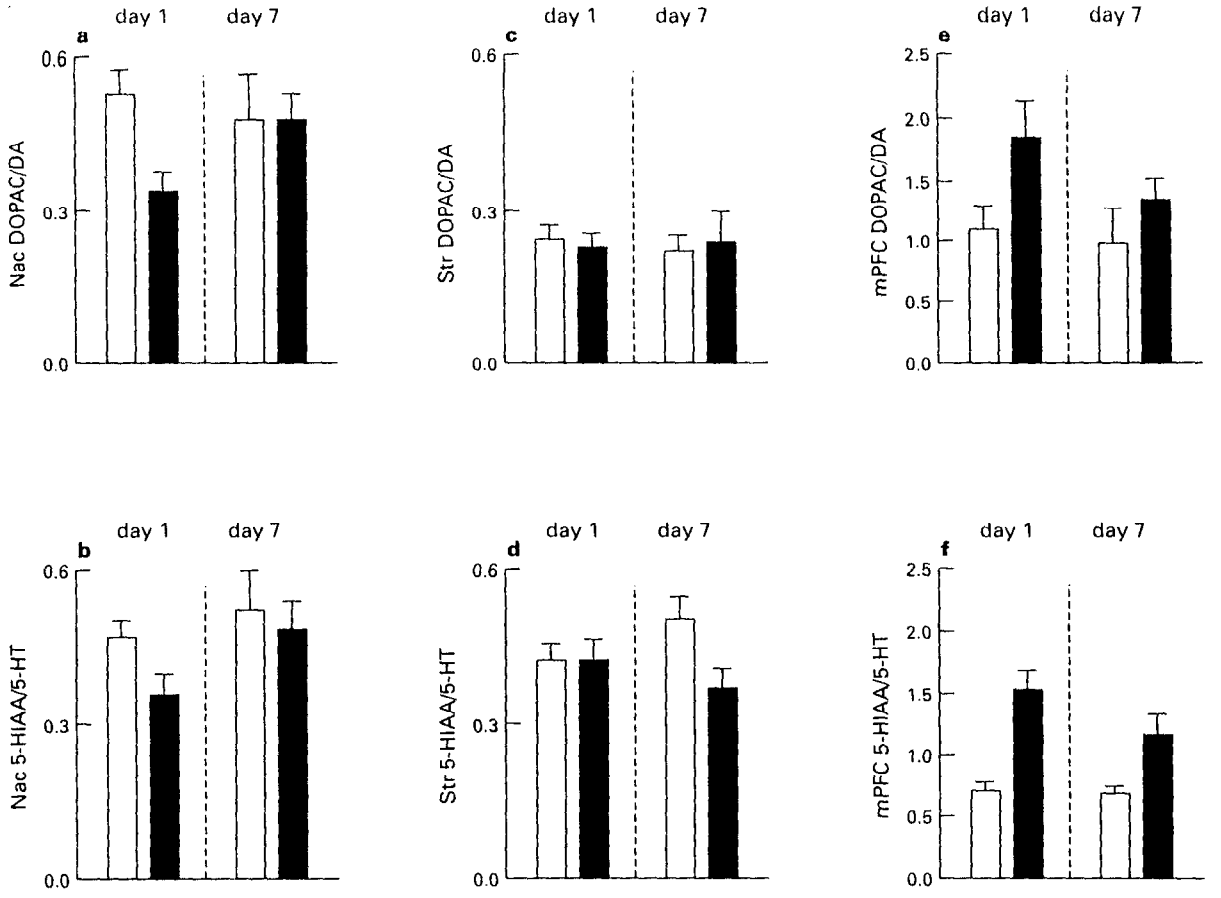

Figure 5 The effect of ibogaine on ratios of DOPAC/dopamine (DA) and 5-HIAA/5-HT in discrete brain regions. Ratios of *postmortem* tissue concentrations of DOPAC/dopamine in the NAc (a), striatum (c) and mPFC (e) and of 5-HIAA/5-HT in the NAc (b), striatum (d) and mPFC (f) at 1 and 7 days after receiving intraperitoneal injections of vehicle (open columns) or ibogaine (40 mg kg^{-1}, solid columns). Results are the means ± s.e.mean of 6 observations in each case.

most frequently used in studies reported by other groups (Dzoljic *et al.*, 1988; Maissoneuve *et al.*, 1991; Glick *et al.*, 1991; Maissoneuve & Glick, 1992; Cappendjik & Dzoljic, 1993; Dworkin *et al.*, 1995). The present results suggest that the acute injection of ibogaine has no immediate or long term effects on the extracellular dopamine levels in the NAc, a finding which is in agreement with that reported by Maisonneuve *et al.* (1991). However, the present study failed to detect the transient increase in extracellular DOPAC at 40 min or the decrease in this metabolite 19 h following ibogaine reported by these authors. The reason for these discrepancies is unclear. The administration of this dose of ibogaine did not significantly affect the relatively low levels of activity seen in rats under the conditions used in the present study, in which the animals have been allowed to habituate to the test environment. However, the fact that a behaviourally active dose had been administered was evident from the marked ataxia and abnormal body postures which developed shortly after the administration of ibogaine and which lasted for approximately 1 h. These effects have also been described by others (Schneider & Sigg, 1957; Dworkin *et al.*, 1995).

As reported previously (Di Chiara & Imperato, 1988; Benwell & Balfour, 1992), the subcutaneous administration of nicotine to rats caused an elevation in the extracellular levels of dopamine and DOPAC in the nucleus accumbens which was accompanied by an increase in the locomotor activity of the animals. However, when the animals were challenged with nicotine 22 h after receiving an injection of ibogaine, the increase in NAc dopamine was very significantly attenuated. Although ibogaine failed to produce a significant change in the nicotine-induced change in the DOPAC levels, the tendency was for this parameter to be potentiated rather than attenuated by ibogaine pretreatment. The explanation for this unusual finding is unclear. Nevertheless, ibogaine appeared to significantly reduce the ability of nicotine to stimulate mesolimbic dopamine secretion. Interestingly, there was no reduction in the locomotor activity evoked by nicotine, at this time, in the ibogaine-treated rats. This lack of effect of ibogaine on nicotine-induced locomotor activity was confirmed by the results of the plus-maze experiments in which the locomotion elicited by nicotine was similar in both the vehicle and ibogaine-pretreated groups. A number of previous studies have suggested a close correlation between the ability of nicotine to increase mesolimbic dopamine overflow and stimulate activity (Clarke *et al.*, 1988; Benwell & Balfour, 1992). This relationship, however, may not be as simple as once thought. The mesolimbic dopamine response to nicotine can be completely inhibited by a desensitizing dose of nicotine, while a residual level of nicotine-evoked locomotion persists (Benwell *et al.*, 1995). Moreover, the competitive NMDA antagonist, 3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphoric acid (CPPene), also appears to be able to inhibit the NAc dopamine response to nicotine while the locomotor response to the drug is unaffected (Shoaib *et al.*, 1994). These data are clearly difficult to reconcile with the hypothesis that the locomotor stimulant response to nicotine always corresponds closely with its effects on dopamine overflow in the extracellular space of the NAc. However, the explanation for these differences may lie in the heterogeneity of the neuronal nicotinic receptors present in brain (Wonnacott, 1990) such that more than one type of receptor is involved in mediating one or other of the mesolimbic dopamine and/or locomotor responses.

Maisonneuve & Glick (1992) suggested that the potentiation of the cocaine-induced mesolimbic dopamine overflow by ibogaine may be anxiogenic. Indeed, the ability of ibogaine to induce anxiogenesis, immediately after administration of the alkaloid, has been recognised for a long time (Schneider & Sigg, 1957). Therefore, using the elevated plus-maze, which has the ability to detect the anxiogenic as well as anxiolytic effects of drugs (Pellow *et al.*, 1985), the possibility that ibogaine-pretreated animals experience anxiety when they are subsequently exposed to nicotine was investigated. Consistent with previous reports (Balfour *et al.*, 1986), nicotine itself did not produce effects which would be indicative of anxiolysis nor anxiogenesis in this behavioural test although nicotine did result in a very significant increase in the overall activity of both the vehicle and ibogaine-pretreated rats. Furthermore, there was no change in the overall activity of the saline-treated control rats which could be attributed to their receiving an injection of ibogaine 22 h previously. However, there was a highly significant reduction in the proportion of open/total arm entries in the animals tested in the maze 22 h after ibogaine treatment. These results not only support the description of anxiogenesis induced by ibogaine administration as reported by Schneider & Sigg (1957), but also suggest that the anxiety may be relatively long lasting. Interestingly, tabernanthine, a structural analogue of ibogaine, appears to be an inverse agonist at the benzodiazepine receptor (Trouvin *et al.*, 1987). Therefore, if ibogaine shares this property, this mechanism may explain its putative anxiogenic effect. The administration of nicotine, to the ibogaine-treated animals, did not attenuate the decrease in the relative open to total arm entries, despite the hyperlocomotion seen in the nicotine-treated group. If anything, the magnitude of the open arm avoidance was greater in the nicotine-treated rats. This activity in an animal model for anxiety may have implications for the possible therapeutic use of ibogaine in humans.

In the present study, the influence of ibogaine on the postmortem tissue levels of dopamine and DOPAC in the 3 principal dopaminergic areas of the brain were measured. In addition, since ibogaine is an indole structure and could, therefore, be expected to have possible effects on 5-hydroxytryptaminergic systems in the brain, 5-HT and 5-HIAA were also assayed. Analysis of the data revealed that the 5-hydroxytryptaminergic system of the NAc, striatum and mPFC appeared to be particularly sensitive to ibogaine in that consistent reductions were observed in the 5-HIAA content of the NAc and striatum over the 7 day period studied. The 5-HIAA of the mPFC was significantly higher in the ibogaine-treated animals by 1 day but not 7 days after the ibogaine treatment. Of the three brain regions tested, the mPFC seemed to be most affected by the acute administration of this alkaloid with both dopamine and 5-HT being very significantly reduced even 7 days after treatment. The increase in the ratio of tissue metabolites to transmitter levels in the mPFC suggests that there may be a potentiation of the turnover of both dopamine and 5-HT in this brain region which lasts for at least 7 days. Interestingly, studies in other laboratories suggest that anxiogenic stimuli also increase the turnover and release dopamine and 5-HT in the mPFC (Abercrombie *et al.*, 1989; Moghaddam *et al.*, 1990; Pei *et al.*, 1990; Kawahara *et al.*, 1993). Therefore, if the changes in mPFC 5-HT and dopamine seen in the present study, play a role in mediating the apparent anxiogenic effects of the drug, the anxiety experienced following ibogaine may persist for a relatively long period of time. These longterm effects attributed to ibogaine, are difficult to explain since its plasma half-life in rodents is reported to be approximately 1 h (Dhahir, 1971). It is possibly converted to a long lasting active metabolite or produces a neurochemical change which is long lasting. The actual site at which ibogaine acts to produce its effects is not known. In a very recent review, Popik *et al.* (1995) suggest that its effects may be due to actions at multiple sites. However, they report that ibogaine acts as a non-competitive NMDA receptor antagonist and argue that the putative 'anti-addictive' properties and the longterm nature of the changes ibogaine produces may be explained by an action at the NMDA type of glutamate receptor. Interestingly, the finding in the present paper that ibogaine reduces the nicotine-induced dopamine response but not the nicotine-induced locomotor activity is similar to the ability of NMDA receptor antagonists to inhibit the nicotine-induced sensitization of the mesolimbic dopamine response, while having little (MK-801) or no (CPPene) effect on the locomotor responses to nicotine (Shoaib *et al.*, 1994). Therefore, the effects of ibogaine on nicotine-induced NAC dopamine may be due to inhibition of NMDA receptors.

In conclusion, the present results suggest that ibogaine reduces the ability of nicotine to induce mesolimbic dopamine secretion and thus has the potential to reduce the rewarding effects of nicotine and, presumably, smoking. The drug may also potentially evoke an anxiogenic response which is not reduced by subsequent exposure to dependence producing drugs and suggests that ibogaine may not only reduce the hedonistic effects of drugs but may also be aversive. However, there are some disturbing aspects to the psychopharmacological properties of ibogaine. Firstly, the marked degree of ataxia taken in conjunction with the intrinsic, potentially long-lasting, anxiogenic properties of the drug suggest that this may be a very unpleasant drug to take. Moreover, such longterm changes in the apparent activity of mPFC transmitters may indicate potential neurotoxicity. This possibility would be consistent with the findings of O'Hearn & Molliver (1993) who reported a loss of Purkinje cells in the cerebrum following treatment with ibogaine, albeit following higher doses than those employed in the present study. Thus, one would have to exercise a great degree of caution in recommending the use of ibogaine in the treatment of addictions such as the smoking habit.

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