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LETTER TO THE EDITORS

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On the anxiogenic and anxiolytic nature of long-term cerebral 5-HT depletion following MDMA

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The role of 5-hydroxytryptamine (5-HT) in anxiety-like behaviour in rats has been studied for over 30 years but still remains contentious. While a widely accepted view has been that a reduction in 5-HT can lead to an anxiolytic effect in rats (Iversen 1984), perusal of the comprehensive reviews of Griebel (1995) and Soubrie (1986) rapidly reveals that such a statement is now unsupportable: ample data are also available to support the hypothesis that lowering 5-HT function is anxiogenic. The large number of disparate animal models used to test "anxiety" including unconditioned (exploratory, social and stress-induced) and conditioned (conflict, conditioned fear and others) models might account for some of the apparent contradictory data. Further, the use of many different 5-HT receptor sub-type selective drugs has also made interpretation complicated as they can alter overall 5-HT neurotransmission in diverse and sometimes unexpected ways.

However, one might expect that techniques designed to decrease overall 5-HT function, that is, the use of synthesis inhibitors or selective neurotoxins, would produce consistent data, but this is also clearly not true. For example, administration of the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (PCPA) has been reported to decrease anxiety in 70% of studies, increase anxiety in 9% and cause no change in the rest, the total number of studies being 34 (Griebel 1995).

Studies which produced a serotonergic lesion with intracerebral 5,7-dihydroxytryptamine (5,7-DHT) also found an anxiolytic effect in the majority, but by no means all, of the investigations and one recent paper reported an anxiogenic effect following a moderate degree of 5-HT depletion (50–75%), but no effect when depletion was more severe (90%) (Hall et al. 1999). The latter result contrasts with that of Briley et al. (1990), who observed clear anxiolytic effects following a similar severe neurotoxic loss of 5-HT. A tryptophan depletion study in rats, involving a procedure designed to produce a rapid and moderate decrease in cerebral 5-HT concentration and function, observed the rapid appearance of an anxiogenic response (Blokland et al. 2002), although others have not found this (Brown et al. 1998). Further, when a different method of tryptophan depletion was used by the same authors, producing approximately 80% decrease in 5-HT, no effects on anxiety-like behaviour were seen, despite clear effects on cognition (Blokland et al. 2001). This pattern of results suggests the rather counter-intuitive principle that moderate 5-HT depletion may have a more pronounced effect on anxiety than near-total depletion.

Very recently, our two laboratories have been investigating the long term effects of a neurotoxic lesion induced by administration of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) to rats and have reported largely opposite results; despite the fact that the same paradigm was used for behavioural testing (elevated plus maze), a similar decrease in cerebral 5-HT concentration was produced (30–40% loss) and animals were evaluated after a similar delay in testing following administration of the MDMA, namely 70–90 days post-treatment (Morley et al. 2001; Gurtman et al. 2002; Mechan et al. 2002b). We have therefore re-evaluated our data in an attempt to understand this inconsistency.

Both the Morley et al. (2001) and Gurtman et al. (2002) studies used Wistar rats. Control rats of this strain spent between 30% (Morley et al. 2001) and 40% (Gurtman et al. 2002) of the total time on the open arm, a measure generally accepted as an index of the anxiety state of the animals. Another recent study revealed a figure of 20% in Sprague-Dawley rats (Mechan et al. 2002a). In contrast, the Dark Agouti strain spent only between 5–6% on the open arms (Mechan et al. 2002a) and it was this strain that was used in the MDMA study,

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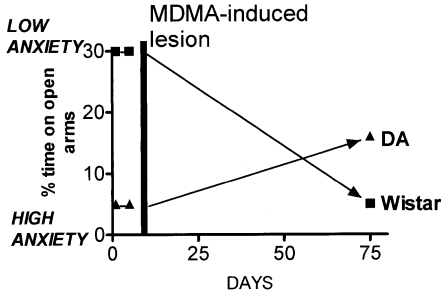

Fig. 1 Plot of the mean time on the open arms, as a percentage of total arm time, spent by Wistar and Dark Agouti (*DA*) rats on the plus maze both before and approximately 9 weeks after a MDMA-induced neurotoxic lesion. Data recalculated from results published by Morley et al. (2001) and Mechan et al. (2002a)

when a similar baseline level of open arm time was obtained (Mechan et al. 2002b).

At 9 or 12 weeks after the MDMA-induced neurotoxic lesion, the Wistar rats spent a significantly lower percentage of total time on the open arms, indicative of a long term anxiogenic effect (Morley et al. 2001; Gurtman et al. 2002), while the percentage of total time the Dark Agouti rats spent on the open arms following the MDMA treatment was increased significantly (Mechan et al. 2002b). Ruling out the possibility that this is yet another example of variability in the results obtained with the elevated plus maze model (Hogg 1996), our studies also found that other anxiety-related behaviours (the social interaction and emergence tests in Wistar rats and open field behaviour in the DA rats) were altered in a manner consistent with the plus maze outcomes. It thus appears that a 5-HT lesion may produce an anxiolytic response in an anxious strain, but an anxiogenic response in strains displaying low endogenous anxiety (see Fig. 1).

There are some further points. The lesion produced by MDMA treatment was relatively modest (30–40% loss of 5-HT) and no alteration of the behavioural responses was seen in the DA rats in the first few weeks after the lesion (Mechan et al. 2002b). While it is possible that the repeated handling or repeated testing may have altered basal levels of anxiety, or changed the nature of anxiety on the plus maze (File et al. 1993) in a way that allowed an anxiolytic effect of 5-HT depletion to emerge, a handling effect was largely discounted by Mechan et al. (2002b) as an explanation of their results. Therefore the delay in the appearance of the altered anxiety state suggests that the alteration in the behaviour on the plus maze may not be intimately associated with the 5-HT depletion, since this occurs within 4–7 days (Colado et al. 1993). Rather, it is probable that the alteration relates to adaptive responses produced as the result of the perturbation. To add to the complexity, there is a further recent study demonstrating that long-term anxiogenic effects can be seen in Lister rats in the social interaction test 29 days following MDMA administration, even in the absence of 5-HT depletion (Fone et al. 2002). This raises the possibility that currently unknown neural changes, rather than simple transmitter changes, may play a substantial role in altered long term behavioural responses seen after MDMA. As with tryptophan depletion, intracerebral 5,7-DHT injection and PCPA studies, there appears to be no simple or direct correlation between 5-HT depletion and anxiety.

These are not trivial issues: use of MDMA in human populations continues to escalate in many countries and there are well justified concerns about long term, perhaps permanent, emotional changes that may occur in users as a result of 5-HT depletion. Some clinical surveys suggest that heavy MDMA users may be more prone to pathological anxiety (Gamma et al. 2000; Parrott et al. 2000). However there are arguments about cause and effect and self-medication, as well as confounds resulting from the polydrug use that is typical of heavy users (Parrott 2001). Other studies have found no evidence for a changed anxiety state in recreational MDMA users (Morgan 1998), while McCann et al. (1999) found the anxiogenic effect of *m*-chlorophenylpiperazine to be blunted in MDMA users (McCann et al. 1999). Indeed, enthusiasts claim that the acute primary effect of MDMA in human users is that it is anxiolytic and the Food and Drug Administration have recently approved a small trial of MDMA as a possible treatment for post-traumatic stress disorder (see http://www.maps.org/research/mdma/). It should, however, be emphasised that the acute effects on mood may not be reflective of any change that might occur as a result of the long term neurotoxic damage.

In summing up the evidence from laboratory animals, we suggest that decreases in central 5-HT can produce an anxiogenic or anxiolytic response, dependent on the basal anxiety level of the rat strain being investigated, the amount of 5-HT depletion and the repeated nature of the testing. Further, altered emotional responses may not necessarily be due to the 5-HT loss per se but rather to the adaptive responses that occur in the brain following the lesion.

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References

Blokland A, Lieben CKJ, van Oorsouw KIM, Deutz NEP (2001) A new method for lowering plasma tryptophan concentrations: no effect on affective behavior but impaired object memory in rats. Soc Neurosci Abstr 27:319.311

Blokland A, Lieben C, Deutz NEP (2002) Anxiogenic and depressive-like effects, but no cognitive deficits, after repeated moderate tryptophan depletion in rat. J Psychopharmacol 16:39–49

Briley M, Chopin P, Moret C (1990) Effect of serotonergic lesion on "anxious" behaviour measured in the elevated plus-maze test in the rat. Psychopharmacology 101:187–189

Brown CM, Fletcher PJ, Coscina DV (1998) Acute amino acid loads that deplete brain serotonin fail to alter behavior. Pharmacol Biochem Behav 59:115–121

Colado MI, Murray TK, Green AR (1993) 5-HT loss in rat brain following 3,4-methylenedioxymethamphetamine (MDMA), *p*-chloroamphetamine and fenfluramine administration and the effects of chlormethiazole and dizocilpine. Br J Pharmacol 108:583–589

File SE, Zangrossi H, Viana M, Graeff FG (1993) Trial 2 in the elevated plus-maze – a different form of fear. Psychopharmacology 111:491–494

Fone KCF, Beckett SRG, Topham IA, Swettenham J, Ball M, Maddocks L (2002) Long-term changes in social interaction and reward following repeated MDMA administration to adolescent rats without accompanying serotonergic neurotoxicity. Psychopharmacology 159:437–444

Gamma A, Frei E, Lehmann D, Pascual-Marqui RD, Hell D, Vollenweider FX (2000) Mood state and brain electric activity in Ecstasy users. Neuroreport 11:157–162

Griebel G (1995) 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. Pharmacol Ther 65:319–395

Gurtman CG, Morley KC, Li KM, Hunt GE, McGregor IS (2002) Increased anxiety in rats after 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy"): association with serotonin depletion. Eur J Pharmacol (in press)

Hall FS, Devries AC, Fong GW, Huang S, Pert A (1999) Effects of 5,7-dihydroxytryptamine depletion of tissue serotonin levels on extracellular serotonin in the striatum assessed with in vivo microdialysis: relationship to behavior. Synapse 33:16–25

Hogg S (1996) A review of the validity and variability of the elevated plus-maze animal model of anxiety. Pharmacol Biochem Behav 54:21–30

Iversen SD (1984) 5-HT and anxiety. Neuropharmacology 23:1553–1560

McCann UD, Eligulashvili V, Mertl M, Murphy DL, Ricaurte GA (1999) Altered neuroendocrine and behavioral responses to *m*-chlorophenylpiperazine in 3,4-methylene methamphetamine (MDMA) users. Psychopharmacology 147:56–65

Mechan AO, Moran PM, Elliott JM, Young AMJ, Joseph MH, Green AR (2002a) A comparison between Dark Agouti and Sprague-Dawley rats in their behaviour on the elevated plus-maze, open-field apparatus and activity meters, and their response to diazepam. Psychopharmacology 159:188–195

Mechan AO, Moran PM, Elliott JM, Young AMJ, Joseph MH, Green AR (2002b) A study of the effects of a single neurotoxic dose of 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) on the subsequent long-term behaviour of rats in the plus maze and open field. Psychopharmacology 159:167–175

Morgan MJ (1998) Recreational use of ecstasy (MDMA) is associated with increased impulsivity. Neuropsychopharmacology 19:252–264

Morley KC, Gallate JE, Hunt GE, Mallet PE, McGregor IS (2001) Increased anxiety and impaired memory in rats 3 months after administration of 3,4-methylenedioxymethamphetamine ("Ecstasy"). Eur J Pharmacol 433:91–99

Parrott AC (2001) Human psychopharmacology of Ecstasy (MDMA): a review of 15 years of empirical research. Hum Psychopharmacol 16:557–577

Parrott AC, Sisk E, Turner JD (2000) Psychobiological problems in heavy "ecstasy" (MDMA) polydrug users. Drug Alcohol Depend 60:105–110

Soubrie P (1986) Reconciling the role of central serotonin neurons in human and animal behaviour. Behav Brain Sci 9:319–364