CORRESPONDENCE

Toxic effect of MDMA on brain serotonin neurons

Sir—U D McCann and colleagues (Oct 31, p 1433)[1] describe reduced [^{11}C]-McN-5652 binding, and by implication, serotonin transporter and serotonergic terminal loss, in users of (±)3,4-methylenedioxymethamphetamine (MDMA, ecstasy). This report provides some of the first biological evidence for the neurotoxic effects of MDMA in man, building up anatomical studies in non-human primates and complimenting indirect evidence from studies of neuropsychiatric symptoms in MDMA users.[2]

However, the results should be interpreted with caution because their meaning hinges on the accurate classification of participants in terms of cumulative use of MDMA. Unfortunately, this category depended on the participants' own estimates of their substance use, and, as the investigators acknowledge, the reliability of such estimates is questionable. The interpretation is also complicated by potentially confounding differences between the MDMA users and the controls. In our experience, heavy users of MDMA typically have quite different personalities from people who have never used the drug. Moreover, as Zuckerman and colleagues[3] discuss, personality traits associated with substance misuse (such as impulsivity and novelty-seeking behaviour) may be linked to serotonergic function. Heavy users of MDMA commonly use several other psychoactive substances concurrently, and the effects of amphetamine, lysergic acid diethlamide (LSD), cocaine, and cannabis on the distribution of [^{11}C]-McN-5652 in man are largely unknown. It is thus difficult to be sure that the differences in [^{11}C]-McN-5652 binding are secondary to MDMA use itself.

More specifically, it is unclear whether [^{11}C]-McN-5652 binds to other the molecular targets in addition to the serotonin transporter, and whether the kinetic model of [^{11}C]-McN-5652 distribution used by McCann and colleagues is the most appropriate for this ligand. The large range in [^{11}C]-McN-5652 binding among controls and the observation that most of the MDMA users fell within this range, questions the sensitivity and specificity of the technique. Furthermore, the investigators describe a negative correlation between cumulative MDMA dose and [^{11}C]-McN-5652 binding, but this correlation was evident only when the controls were included in the analysis: no relation is discernible when the MDMA users are considered.

Nevertheless, this study shows that neuroimaging has the potential to address the key question of whether MDMA use leads to neurotoxic effects in man. Our criticisms relate to the sensitivity and limitations of such ligand binding studies for an assessment of serotonin neuronal integrity and the functional consequences of impairments to this system. Neuronal systems may exhibit a wide range of adaptive and compensatory responses to cope with toxic effects or drug use. Such compensatory responses, perhaps resulting in increased serotonin transporter, might obscure the underlying toxic effects of MDMA. New imaging approaches that integrate cognitive function and neurotransmission,[4] are required to tackle the problem of whether MDMA use leads to a functional impairment of the serotonin system. Thus, in addition to studies of serotonin transporters and receptors, we also need to examine the effects of experimentally manipulating serotonin transmission on brain activity in MDMA users, this is the approach we have adopted.

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1 McCann UD, Szabo Z, Scheffel U, Dannals RF, Ricuarte GA. Positron emission tomographic evidence of toxic effect of MDMA ('Ecstasy') on brain serotonin neurons in human beings. *Lancet* 1998; **352:** 1433–37.

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Sir—The landmark study by U D McCann and colleagues provides convincing evidence of an association between decreased serotonin transporter binding sites and MDMA use. Nonetheless, it is important to note that this study does not allow comment on causal relations, and McCann and co-workers are over-eager to wring meaning from their findings.

The investigators briefly consider that MDMA users may pursue such use as a result of pre-existing serotonin function abnormalities, only to dismiss this on the grounds that "none of the MDMA users had a neuropsychiatric disorder in which serotonin has been implicated". Yet their exclusion of such disorders seems to have been based only on assessment of current axis-I disorder, as detected by the scheduled interview for diagnostic and statistical manual of mental disorders IV (SCID-IV). They did not exclude axis-I disorder before use of MDMA abuse, subclinical mood disorders, or previous or current axis-II diagnoses. With reference to the latter, abnormal serotonin binding sites may be particularly pertinent to personality disorders that involve impulsive or aggressive behaviour. Thus, some

people with mental disorders may self-medicate with MDMA.

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1 McCann UD, Szabo Z, Scheffel U, Dannals RF, Ricaurte GA. Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. *Lancet* 1998; **352:** 1433–37.

Sir—U D McCann and co-workers[1] show decreased brain serotonin transporter binding in MDMA users compared with age-matched and sex-matched controls who had never used MDMA. The investigators used a dual tracer approach: one radioligand [^{11}C](+)McN-5652 for imaging of composed specific binding, non-specific binding, and free ligand, whereas [^{11}C](−)McN-5652 imaging consists of only non-specific and free ligand. The latter technique is mainly affected by tissue perfusion.

Distribution volume of specific binding in the users of MDMA was found to be decreased in all regions except the thalamus. McCann used logarithmic transformations of distribution volumes corrected for non-specific binding to achieve a normal distribution in the control and MDMA groups. This approach raises the question: are the presented reductions based on the different kinetics of the non-specific radioligand [^{11}C](−)McN-5652 between controls and MDMA users? Figure 1 of the original article shows that the time-activity curve of this non-specific radioligand significantly differs between the study participants. An MDMA user had a more delayed inflow and outflow of this tracer into and from the brain than a control. We reanalysed the representative time-activity curves by means of Logan-Patlak plot.[2]

Results of the Logan-Patlak plots showed no difference in global distribution volume between an MDMA user (1·46 mL/mL) and a control (1·47 mL/mL) if corrected for different non-specific binding and free ligand.* If no such correction were applied, the figures are similar to those reported by McCann: 1·18 mL/mL and 1·47 mL/mL, respectively. The only difference was a completely different kinetics of non-specific binding and free ligand between an MDMA user and a control. The conclusion is that the main affective factor is different perfusion between the study participants, not the global serotonin specific binding. Regionally, it might be different.

A lesson of McCann and co-workers' report is that the biochemical fate of the tracers has to be known completely, and in this case multiple chemical species ([^{11}C](+)McN-5652 and [^{11}C](−)McN-5652) must be modelled simultaneously[3,4] to analyse the effects of various tissue components on the specific binding. One cannot say whether the findings of McCann are based on the different kinetics of the non-specific binding and free ligand or on the true serotonin transporter binding in MDMA users.

*Full details are available from the authors.

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1 McCann UD, Szabo Z, Scheffel U, Dannals RF, Ricaurte GA. Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. *Lancet* 1998; **352:** 1433–37.
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Sir—The finding of U D McCann and colleagues[1] of reduced serotonin transporter binding in heavy users of MDMA lends support to evidence of the harmful effect of this drug on the serotonin system, which was previously reported in animals.[2] However, as the investigators acknowledge, little is known of the functional effects of MDMA-induced brain serotonin neurotoxic lesions. We report the effect of long-term consumption of MDMA on cognitive function.

We recruited participants who had a history of substantial and predominant MDMA use (mean estimated number of tablets consumed 235 [range 12–2600]) through popular magazines and the internet. The MDMA group (n=36; age 24·1 [SD 4·9] years; duration of MDMA use 4·3 [2·6] years) was compared with a control group (n=19; age 22·7 [2·3] years) who had never used illicit or prescribed psychoactive substances. The MDMA users had not taken the drug or any other psychoactive drug for some days before testing (79 days [2–400 days]). We took a detailed history of each participant's drug use together with current and past psychiatric state (Present State Examination). Four drug users met criteria for a diagnosis of neurotic depression, but there were no group differences between groups on the Beck depression inventory (p=0·304), and congnitive impairment was unrelated to diagnosis and depression scores.

A multivariate analysis of variance of cognitive scores showed significant impairment of MDMA users on tests of learning, recognition, and recall.* Compared with controls, users of MDMA had worse immediate recall of words from a list, showed impairment in recognition memory for faces and in learning a repeatedly administered word list and a sequence of digits. Associative learning tasks revealed impairment in learning spatial information. By contrast, executive (frontal lobe) functions (measured by verbal fluency tests, and verbal and non-verbal working memory) were not impaired. Compared with normative data for the learning and memory tests, three MDMA users scored below the 2 SD limit on two tests, as did an additional eight users on one test, whereas only one control scored lower than the 1 SD limit on one test (p=0·021).

Most users of MDMA have also taken other illicit psychoactive drugs that may contribute to cognitive impairment and to serotinergic depletion. We selected individuals whose use of MDMA was more frequent and regular than of any other drug; we also recorded the frequency and use of other drugs. Other drugs did not correlate significantly with the deficits found with MDMA, although other deficits were implicated, such as frontal impairment with cannabis use. Our results do not represent a global impairment of cognitive function, instead we found discrete deficits in verbal and non-verbal memory and learning while other cognitive functions were intact. Accordingly, the deficits could not be explained as the result of impaired attention or poor motivation, which substantiates evidence of patterned cognitive impairment.[3,4]

Chronic disruption of serotonergic transmission by MDMA is a possible explanation for the memory and learning deficits we found.

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1 McCann U, Szabo Z Scheffel U, Dannals RF, Ricaute GA. Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. *Lancet* 1998; **352:** 1433–37.

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Sir—U D McCann and colleagues equate self-reported use of ecstasy with the use of MDMA. Tablets described by users or dealers as ecstasy may contain one or more of various substituted amphetamines, including MDMA, amphetamine, ephedrine, ketamine, tiletamine, or other compounds.[2,3] These drugs do not have identical neuropharmacological properties.[4] Thus, what these investigators have shown is a difference in serotonin transporter activity between a group of individuals who thought they had taken MDMA in the past, compared with a group of people who said they had never taken MDMA before.

The MDMA users were tested for psychiatric disorders, such as anxiety and depression, and all proved normal. So, these differences in transporter activity relative to the control group existed without any anxiety and depression, as established by the investigators themselves.

Although MDMA may cause some brain changes, the evidence for chronic depression and anxiety disorders as a result of these changes is unconvincing. The midweek mood dip (which usually returns to normal by the end of the week) that follows weekend use of MDMA is due to an acute fall in serotonin, not to structural brain changes.

Were the MDMA users selected entirely at random from all the persons who replied to their advertisements, rather than from a highly selected subsample? None of the 14 cases reported includes people who described taking more than 400 pills. The manner in which the 14 cases seem to have been selected from a large initial sample raises questions about the statistics used. The investigators should clarify their selection procedure.

The statement that the absence of neuropsychiatric disorder means that the low concentrations of transporter could not have been pre-existing is unproven. People with lower concentrations of transporter and other neurochemical differences may experience drives to take drugs of this nature, or to seek stimulation in other ways, without necessarily having a neuropsychiatric disorder. That the participants were tested and proved free from such disorders confirms the point that low serotonin transporter activity can co-exist with a normal mental state.

We believe that it would be valuable to compare serotonin transporter activity in heavy cocaine users with MDMA users. Cocaine is a stimulant that may be sought out by people who might have a pre-existing underactivity of some brain systems, such as parts of the dopamine and serotonin systems. Cocaine is not, however, a ring-substituted amphetamine and does not cause specific changes to serotonin fine terminals. So if heavy cocaine users proved to show similar changes in serotonin transporter mechanisms to MDMA users, we could conclude that these deficits, relative to non-drug use, are pre-existing and not caused by MDMA. We are not convinced that the differences reported by McCann were not pre-existing, because the putative use patterns and doses of MDMA were generally well below those that cause persistent changes in most animal studies. We agree with McCann and colleagues that some people take MDMA at levels equivalent to those used in some animal studies.[5]

Conclusive proof requires following a group of individuals who continue to use large amounts of MDMA alone, and a comparison of the progression of changes in their serotonin transporter activity with cocaine users and a group of people who do not use drugs.

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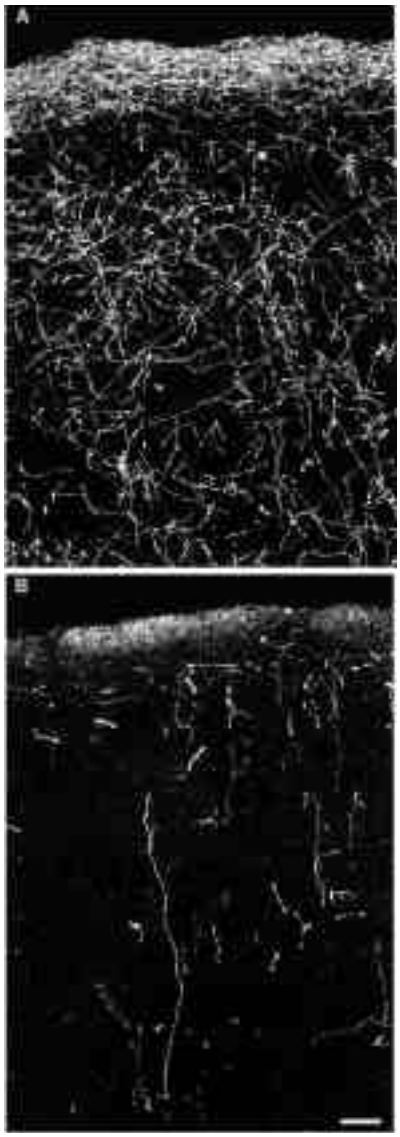
Authors' reply

Sir—Laurence Reed and co-workers underscore the inherent difficulties of retrospective studies in individuals who have used illicit substances, and



Serotonin transporter immunoreactive fibres in prefrontal cortex of a control monkey (A) and a monkey treated with MDMA 2 weeks previously (B)
Scale bar 50 μm. MDMA was given subcutaneously 5 mg/kg twice daily for 4 days.

question whether amphetamine, LSD, cocaine, and cannabis might also lead to reductions in the serotonin transporter. Neither cocaine nor amphetamine is toxic toward brain serotonin neurons and the structural requirements for substituted amphetamine neurotoxicity are quite strict. As to the selectivity of [^{11}C]McN5652 binding, neither dopamine nor norepinephrine transporter blockers inhibit specific [^{11}C]McN5652 binding, whereas serotonin reuptake inhibitors do.[1] Reed and colleagues are incorrect when they state that the negative correlation between extent of MDMA use and [^{11}C]McN5652 binding is lost when controls are dropped from the

analysis ($r=-0{\cdot}51$, $p<0{\cdot}05$, without controls). It is not yet known if functional neuroimaging has the requisite sensitivity or specificity to show MDMA-induced serotonin neurotoxicity.

John Morgan and two of the other correspondents suggest that pre-existing low concentrations of serotonin transporters could be the cause of MDMA use, rather than the result of serotonin neurotoxicity. Although an intriguing possibility, it does not take into account animal data that provide compelling evidence for a longlasting neurotoxic effect of MDMA on brain serotonin neurons (figure).

Jyrki Kuikka and Aapo Ahonen question whether the observed reductions in specific [^{11}C]McN5652 binding might not be based on different kinetics of [^{11}C](−)McN-5652 in controls and MDMA users. Analysis of the data with only the active enantiomer, [^{11}C](+)McN5652, shows that the differences are still significant (ANOVA, $p<0{\cdot}014$), mitigating against a confounding effect of non-specific binding. As to the analysis of our representative time-activity curves with a Logan-Patlak plot, it is inappropriate for several reasons. First, one should use the actual input function derived from metabolite-corrected arterial blood samples, as was done in our work. Second, we do not believe it is appropriate to derive general conclusions from time-activity curves of single individuals. Third, simple graphical analysis with [^{11}C](−)McN-5652 instead of the actual input function is inappropriate because the slow dissociation of [^{11}C](−)McN-5652 from non-specific binding sites leads to a gross underestimation of the actual distribution volume, as is evident from Kuikka and Ahonen's estimated values. These reservations notwithstanding, to assess the possibility of perfusion effects, we have calculated the K_1 indices (brain uptake) in MDMA users and controls and find that the two do not differ significantly (K_1, control 0·312 [SD 0·032]; K_1, MDMA 0·303 [0·053]; ANOVA, $p=0{\cdot}475$).

Karl Jansen and A R Forrest question whether MDMA tablets used by people in our study might have contained other illicit substances. While this is possible, it is noteworthy that among recreational drugs, only ring-substituted amphetamines like MDMA have been shown to cause serotonin neurotoxic injury.[2] Jansen and Forrest also suggest that, since none of our participants had anxiety or depression, our findings are clinically irrelevant. However, we deliberately excluded such individuals from our study because of potential confounds on our outcome measures. The selection of MDMA users for PET studies was purely random and subject to participants meeting predetermined inclusion and exclusion criteria. All MDMA users who had undergone PET imaging by the time our paper was submitted are included in our report, including those kindly referred by Jansen. We expressed extent of previous exposure to MDMA in terms of the number of times or occasions participants reported having used MDMA, rather than the number of pills they recalled having taken, because the former approach is likely to provide a more accurate measure than the latter. Our finding that participants with lower degrees of MDMA exposure have evidence of reduced serotonin transporters should be viewed as further cause for concern, rather than as a reassuring observation.

We were interested in the findings of Anthony Klugmann and colleagues which accord with recent findings in other studies.[3]

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Does variant Creutzfeldt-Jakob disease have an Achilles heel?

Sir—A F Hill and co-workers' report (Jan 16, p 183)[1] of variant Creutzfeldt Jakob disease (CJD) in tonsillar lymphoid tissue, and an earlier similar finding in an appendix,[2] raise concerns about the safety of current procedures for sterilisation of surgical instruments. The outcome may be introduction of universal precautions to disinfect surgical instruments by means of six 3 min autoclave cycles at 134°C, or one 18 min cycle at 134°C. This method would require additional autoclaves, provision of extra space to accommodate them, and the purchase of additional surgical instruments because of the longer turn-around time. A less costly way to inactivate the CJD agent is urgently needed.

A highly heat-resistant replicating agent (IFDO),[3] may provide a model for the development of new strategies for sterilisation of CJD. Like CJD agent, IFDO can survive autoclaving at 134°C for 3 min, as well as exposure to formaldehyde and ionising radiation, and is of similar size to CJD.[3] This agent occasionally survives autoclaving for 30 min at 134°C,[4] but I found IFDO is inactivated by boiling in concentrated salt solutions that contain a weak acid or alkali. I used three different batches of IFDO with viable counts between $1{\cdot}2\times10^9$ and 3×10^9 for these experiments. After autoclaving at 121°C with a holding time of 15 min the viable counts were reduced by less than 1 log. In 13 experiments in which 55 samples were tested for viability after boiling for 15 min in 2·0 mol/L sodium chloride, 0·02 mol/L disodium orthophosphate, the residual counts were: less than $0{\cdot}5\times10^2$ in 35 samples, $0{\cdot}5\times10^2$ in 16, $1{\cdot}0\times10^2$ in three, and $1{\cdot}5\times10^2$ in one.

The mode of action of sodium chloride in enhancing inactivation of IFDO is unknown. However, when IFDO is suspended in concentrated salt solutions it partly disaggregates, which may render acid or alkali susceptible targets more accessible, and raises the possibility that other chemicals and disinfectants might also be effective when combined with high concentrations of salts. The validity of IFDO as a model for sterilisation of CJD should be tested by observing the effect of the above procedure on the viability of CJD agent.

If confirmed as a valid model for sterilisation of CJD, IFDO would provide a simple and rapid tool for the development of new ways to inactivate CJD agent.

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