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How strong is the evidence that brain serotonin neurons are damaged in human users of ecstasy?

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Abstract

Understanding the diverse functions of serotonin in the human brain can be obtained through examination of subjects having a lower than normal number of brain serotonin neurons. Behavioral abnormalities consistent with brain serotonergic damage have been reported in some polydrug users who also use the neurotoxin ecstasy (methylenedioxymethamphetamine, MDMA). This review evaluates the evidence from neuroimaging studies that brain serotonergic damage is a feature of human users of ecstasy. To date, neuroimaging studies designed to establish whether levels of brain serotonin neurons are lower than normal in ecstasy users have employed radioligands that bind to one component of the serotonin neuron, the serotonin transporter (SERT). Because these studies are methodologically flawed in terms of reliability or validity of the SERT measurement and appear to have employed polydrug users, no definitive information is yet available on the question of ecstasy toxicity to human brain serotonin neurons. Until these issues are resolved, it cannot be assumed that ecstasy exposure represents a chronic serotonin deficiency condition. © 2002 Published by Elsevier Science Inc.

Keywords: Ecstasy; MDMA; Serotonin; Brain; Serotonin transporter; Neurotoxicity

1. Introduction

Experimental animal studies of the role of monoamine neurotransmitters in brain function typically assess the influence of experimental surgical and pharmacologic manipulation of the activity or concentrations of brain monoamine neurons on behavior. In the human, such information can also be obtained through pharmacological investigation of drugs that reversibly or irreversibly alter neurotransmitter function and, for some neurotransmitter systems, study of behavior in humans chronically deficient in number of brain monoamine neurons due either to a neurodegenerative condition of unknown etiology or to damage by a selective neurotoxin. In the case of the brain dopamine neurotransmitter system, the results of human investigations showing that degeneration of brain dopamine neurons is the fundamental characteristic of both idiopathic Parkinson's disease (Ehringer and Hornykiewicz, 1960) and parkinsonism caused by a dopaminergic neurotoxin, MPTP (Vingerhoets et al., 1994), and that human parkinsonism can be reversed by dopaminergic agents (Sano, 1960; Birkmayer and Hornykiewicz, 1961) have established the role of nigrostriatal dopamine in the control of movement.

Serotonin neurons originate, in the human, in the lower brain stem raphe nuclei, including the dorsal and median, and project to all regions of the brain (Tork, 1990). In humans, the results of a variety of pharmacological studies have suggested a role for the brain serotonin system in regulation of such biological processes as mood and appetite (cf. Staley et al., 1998). However, to date, no human behavioral disorder has been described for which the etiological involvement of the brain serotonin system has been clearly established. As in the case of the dopamine neurotransmitter system, further information on the function of serotonin in human brain could be obtained through behavioral examination of subjects deficient in number of serotonin neurons due either to a neurodegenerative disorder or to damage caused by a neurotoxin. The objective of this review is to assess the strength of the evidence, involving examination of the brain, that chronic exposure to the serotonergic neurotoxin ecstasy (methylenedioxymethamphetamine, MDMA) represents a state of decreased brain serotonin neuron concentration in human users of the drug.

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2. Some behavioral abnormalities reported in chronic users of ecstasy could be explained by ecstasy-induced damage to brain serotonin neurons

Animal data indicate that ecstasy causes elevation of extracellular levels of brain serotonin and, to a lesser extent, dopamine (Nichols et al., 1982; Kankaapää et al., 1998). Since this action is blocked by selective serotonin reuptake inhibitors (SSRIs), the elevation of serotonin is generally considered to involve serotonin transporter (SERT)-mediated release (Hekmatpanah and Peroutka, 1990; Rudnick and Wall, 1992; Gudelsky and Nash, 1996), although inhibition of serotonin reuptake might also be a contributory factor (Iravani et al., 2000). Pharmacological data in the human showing that some of the behavioral effects of ecstasy (positive mood, extroversion, heightened sensory perception) are blocked by the SSRI citalopram (Liechti et al., 2000a) support the involvement of serotonin and SERT in the mechanism of action of ecstasy. The findings that some of the effects of ecstasy are blocked by ketanserin (perceptual changes, emotional excitation; Liechti et al., 2000b) and haloperidol (euphoria; Liechti and Vollenweider, 2000) also suggest some involvement of the serotonin $5HT_2$ and (probably via ecstasy-induced dopamine release) dopamine receptors. These findings, taken together with the observations, in ecstasy users during drug withdrawal, of behavioral disturbances involving sleep, memory, and mood (Curran and Travill, 1997)—behavioral functions considered to be under partial serotonergic control (Staley et al., 1998; Meneses, 1999; Portas et al., 2000)—support the notion that some of the acute effects of ecstasy are probably mediated by enhanced serotonergic stimulation of as yet unidentified serotonin receptor subtypes. Conversely, the withdrawal syndrome might be due to a serotonin deficiency caused by excessive release, metabolism, and depletion of the neurotransmitter (see Kish et al., 2000).

Animal data also indicate that, depending on the dose, ecstasy can damage brain serotonin nerve terminals, but with a sparing of cell bodies, as indicated by a persistent reduction in serotonin nerve terminal markers (serotonin, tryptophan hydroxylase, SERT; Stone et al., 1987; Battaglia et al., 1987; 1991; Commins et al., 1987; Schmidt, 1987; Ricaurte et al., 1988) and by immunohistochemical evidence of silver staining and presence of swollen, fragmented axons (Commins et al., 1987; O'Hearn et al., 1988) and abnormal reinnervation pattern (Hatzidimitriou et al., 1999). The mechanism of neuronal damage is unknown, but may be related to ecstasy-induced release of dopamine (Stone et al., 1988) and oxidative stress (Sprague and Nichols, 1995; Colado et al., 1997; Aguirre et al., 1999; Jayuanthi et al., 1999; Shankaran et al., 1999; Yeh, 1999). Parenthetically, however, it should be noted that reactive gliosis, a usual consequence of neurotoxic damage, has not been reported in animals exposed to ecstasy, leading some to suggest that the evidence of actual damage to serotonin neurons by ecstasy is equivocal (Grob, 2000; Kalia, 2000).

The animal data describing a long-term reduction of brain serotonin markers following high-dose exposure to ecstasy suggest that ecstasy might also damage serotonin neurons in the brain of some human ecstasy users. In this regard, McCann and Ricaurte (2001) argue that the dose of ecstasy which causes neurotoxicity in animal studies is close to that used by human users of the drug (but see Aghajanian and Lieberman, 2001). Numerous reports have now appeared describing changes in behavior (mood, cognition, sleep), which might be serotonin-related, in chronic polydrug users who use ecstasy (Krystal and Price, 1992; Pallanti and Mazzi, 1992; Solowij et al., 1992; Allen et al., 1993; Curran and Travill, 1997; Bolla et al., 1998; Parrott and Lasky, 1998; Parrott et al., 1998; Schifano et al., 1998; McCann et al., 1999a; Morgan, 1999; Gouzoulis-Mayfrank et al., 2000; Parrott et al., 2000; Reneman et al., 2000; Rodgers, 2000; Wareing et al., 2000; Bhattachary and Powell, 2001; Croft et al., 2001; Verkes et al., 2001; Zakzanis and Young, 2001; for comprehensive review see Morgan, 2000). The major limitations of these investigations, which will be discussed in detail elsewhere in this volume, are the continued failure of the investigators to establish, by forensic drug analysis, whether any of the subjects had ever actually used ecstasy, and the use, in the "ecstasy group" of subjects who also used other drugs (especially psychostimulants, opiates, alcohol) which also affect the same behaviors. Thus, a recent systematic study of 100 drug users recruited from the "dance scene" showed a concordance of only approximately 50% between self-report and forensic drug hair analysis (Cooper et al., 2000). Furthermore, behavioral abnormalities, especially cognitive impairment, have been described in users of drugs commonly used by ecstasy users (e.g., cannabis: Croft et al., 2001; cocaine: Berry et al., 1993; Tarter et al., 1995; Bauer, 1996; Bolla et al., 1999; Smelson et al., 1999; methamphetamine: Simon et al., 2000; Ornstein et al., 2000; Volkow et al., 2001a,b, opiates: Darke et al., 2000).

The animal toxicity data and human findings of behavioral changes in self-reported polydrug users of ecstasy raise the possibility that ecstasy might be neurotoxic to humans. However, a more definitive conclusion on this public health question will require: (1) forensic confirmation in studies of ecstasy users that the self-report data on drug use are accurate; (2) behavioral examination of "pure ecstasy users" (which may not be possible as almost all ecstasy users use other drugs [e.g., Schifano et al., 1998]) or, if not possible, demonstration of a persistent behavioral syndrome unique to polydrug users who use ecstasy; and (3) documentation of serotonin neuron damage in human ecstasy users by postmortem brain examination (the gold standard) or by neuroimaging studies of living brain. As described below, only scanty information is available on the status of brain serotonin neurons in human ecstasy users.

3. Measurement by neuroimaging procedures of brain levels of the SERT is one approach to establish whether ecstasy might damage serotonin neurons in the human

3.1. Why have so few brain studies of ecstasy users been conducted?

Ecstasy use is now recognized as a major public health issue worldwide. However, to date (October 2001), only three investigations (two neuroimaging, one postmortem) have been conducted in which components of serotonin neurons have been measured in the brain of human ecstasy users. The lack of autopsied human brain investigations is probably due to the low number of ecstasy users who are autopsied each year as compared to the number of users of other neuroactive drugs such as cocaine, methamphetamine, and heroin in which death from drug toxicity is a more likely event (Kish, unpublished observations). The small number of neuroimaging studies of ecstasy users might be explained by the relative (compared with the dopaminergic system) absence of neuronal targets for assessment in living brain and the reluctance of many neuroimagers to use the presently available radiolabeled probes ([^{123}I]β-CIT; [^{11}C](+) McN-5652/[^{11}C](−) McN-5652), which appear to have uncertain reliability or validity for measurement of the integrity of serotonin neurons (see below). Finally, there may be the general perception that damage to brain serotonin neurons in ecstasy users has already been conclusively demonstrated in the McCann et al. (1998a) positron emission tomography (PET) investigation (see below).

3.2. Most studies of ecstasy neurotoxicity in human brain will continue to employ only one marker of serotonin nerve terminal integrity (SERT)

Because of the relative absence of postmortem human brain material, and the availability, at the present time, of only one marker of brain serotonin neuronal number that can be assessed in living human brain, namely, the SERT (the site on serotonin neurons which takes released serotonin back into the neuron), investigations of brain serotonin neuronal integrity in ecstasy users will continue to involve primarily neuroimaging studies employing radioligands that bind to this transporter. Although the use of the radioligand α[^{11}C] methyl-L-tryptophan has been proposed to measure the rate of serotonin synthesis, and by implication, serotonin neuronal integrity in living human brain (Chugani et al., 1998; Nishizawa et al., 1998), it continues to be a controversial issue whether α[^{11}C] methyl-L-tryptophan uptake in brain reflects serotonin synthesis vs. simple tryptophan uptake (Shoaf et al., 2000). Thus, it is likely that most future neuroimaging studies of brain serotonin neurons in ecstasy users will employ SERT radioligands. This is in contrast with studies of brain dopamine neuronal integrity, in which a variety of radioligands that bind to different components of the dopamine neuron (dopamine transporter, VMAT2 in dopamine-rich striatum, dopa decarboxylase) can be employed.

3.3. Strengths and weaknesses of employing SERT as a marker of the integrity of serotonin neurons by neuroimaging: what is definitive proof of serotonergic brain damage?

As shown in Table 1, the two neuroimaging studies (PET: McCann et al., 1998a; single photon emission computed tomography [SPECT]: Semple et al., 1999) designed to assess serotonin neuronal damage in the brain of ecstasy users have employed radioligand-based methodology designed to detect binding to SERT, with the assumption that decreased levels of SERT will reflect decreased number of serotonin neurons/nerve endings. Is this assumption correct?

As mentioned above, SERT is a protein, localized to the membrane of serotonin neurons, which is responsible for taking released serotonin back into the neuron. Although SERT has traditionally been assumed to be localized to serotonin nerve endings, recent animal data suggest that SERT might also be localized to serotonin axon membranes far from the synapse where it could function to collect and conserve serotonin that has escaped from the synaptic cleft (Zhou et al., 1998). The use of SERT as an index of serotonin neuron integrity is indicated by findings of decreased brain SERT levels in experimental animals, including nonhuman primates, exposed to serotonergic neurotoxins (Battaglia et al., 1987; Scheffel et al., 1998; Brown and Molliver, 2000). However, it has now been established that drug-induced changes in levels of brain neurotransmitter transporters can occur independently of any changes in the number of serotonin neurons. Thus, cocaine exposure to experimental animals (Wilson et al., 1994) and, possibly, in humans (cf. Wilson et al., 1996b), can cause increased or decreased levels of the striatal dopamine transporter. This lack of a direct correspondence between brain dopamine transporter and neuron concentration indicates that the dopamine transporter can be up- or down-regulated by some drugs and possibly even in the drug-free state (e.g., during aging, behavioral states of chronic dopamine excess or deficiency) and that caution should be exercised in inferring loss of dopamine neurons from decreased levels of the dopamine transporter. This concern has led to the difficulty in interpretation of the findings of striatal dopamine transporter reductions in human users of the dopaminergic neurotoxin methamphetamine (Wilson et al., 1996a; McCann et al., 1998b; Volkow et al., 2001a) in which the decrease could be explained as either drug-induced reversible down-regulation of transporter levels, damage to the dopamine transporter but with normal number of dopamine nerve terminals, or a neurotoxic event in which dopamine transporter loss is accompanied by an actual loss of dopamine nerve terminals. In this regard, Volkow et al. (2001b) recently reported that levels of the striatal dopamine transporter, assessed by PET, are low

Table 1
Neurochemical studies of brain serotonin markers in human users of ecstasy

Reference	Subject number	Brain areas	Serotonergic marker	Mean % change vs. controls	Comments
McCann et al., 1998a	14	cerebral cortex, cerebellum, diencephalon, midbrain, striatum, pons	SERT [^{11}C](+) McN-5652, PET	No percentage changes reported in text. Approximately 50% (cerebellum) to 85% (hypothalamus) reduction calculated from log scores in Fig. 3.	Nonspecific binding calculated using [^{11}C](−) McN-5652 (see Kuikka and Ahonen, 1999, and Laruelle et al., 2000 for discussion). Data were log-transformed because of high scatter of subject values (approximately 30-fold range of control levels — see Fig. 4). No test–retest data provided in normal subjects. Extent of past use of other neuroactive drugs not stated. No hair analysis.
Semple et al., 1999	10	cerebral cortex, striatum, thalamus, caudal midbrain/pons	SERT [^{123}I]β-CIT, SPECT	− 10% to − 13% (calcarine, occipital, and cingulate cortices) normal in other brain areas	[^{123}I]β-CIT is not specific for SERT, with validity especially uncertain in areas of low SERT density (cerebral cortex; for comments see Heinz and Jones, 2000). Hair analysis confirmed presence of ecstasy in 7 of 10 subjects; no hair analysis for other drugs of abuse.
Kish et al., 2000	1	caudate, putamen, nucleus accumbens	serotonin (postmortem brain)	− 60% to − 77%	Single case study only. Blood and hair analysis confirmed recent and past use of ecstasy but also use of cocaine and opiates. Low serotonin could be due to reversible and/or irreversible effects of ecstasy.

only in human methamphetamine users examined during early drug detoxification and recover to normal levels after more prolonged withdrawal, and concluded that the dopamine transporter loss was most likely due to a ''functional downregulation.''

Although less attention has been devoted to studies of SERT regulation, postmortem human brain (Little et al., 1998; Mash et al., 2000) and SPECT (Jacobsen et al., 2000; Staley et al., 2001) studies have reported above-normal levels of brain SERT in human users of cocaine and in tobacco smokers. These findings suggest that brain levels of SERT, like the dopamine transporter, might change following exposure to some drugs independently of any changes in levels of nerve terminals.

Experimental animal and/or human data suggest that SERT levels might also vary as a function of estrogen status, gender, and variant in a SERT promoter gene polymorphism in a manner that might be unrelated to serotonin neuron number.

Estrogen influences brain SERT levels in studies of female animals, suggesting that this might occur in humans. Although the data are not entirely consistent, brain SERT levels and/or mRNA in ovariectomized animals are increased the day after estrogen (estradiol) exposure (McQueen et al., 1997; Sumner et al., 1999) whereas levels are lower after chronic treatment (Mendelson et al., 1993; Pecins-Thompson et al., 1998 (but see Rehavi et al., 1987). In a recent primate study, brain SERT mRNA levels were similarly decreased after estrogen or estrogen plus progesterone supplementation, suggesting that estrogen, but not progesterone, might influence brain SERT levels in women (Pecins-Thompson et al., 1998).

Most studies of brain SERT in humans still do not consider that gender might influence levels of the transporter. This is probably due to increased cost and difficulty of obtaining a representative number of both female and male subjects for neuroimaging studies and the unstated assumption that gender probably does not affect brain SERT levels in humans. However, in a recent postmortem brain investigation, females had approximately 25% lower SERT levels in prefrontal cortex (the only area examined) as compared with those in males (Mann et al., 2000). Clearly, insufficient data are available to establish conclusively whether estrogen and gender influence brain SERT levels in the human. However, these preliminary data suggest that some consideration should be given that both factors might influence SERT levels independently of any change in the levels of serotonin nerve terminal number.

A body of animal and human data also suggest that a genetic factor might influence brain SERT concentration without any alteration in neuron number. The SERT gene (SLC6A4) has been cloned and is localized on chromosome 17q11.1–12 (Lesch et al., 1993; Ramamoorthy et al., 1993). The SLC6A4 has a polymorphism located in the promoter region (5-HTTLPR) that consists of 44 base pairs insertion/deletion (Heils et al., 1996; Lesch et al., 1996). Lesch et al. (1996) found allele frequencies of 57% for the long (*l*) or 16-repeat allele and 43% for the short (*s*) or 14-repeat allele in a population of 505 subjects of Caucasian origin. Lesch et al. made the exciting discovery that the SERT gene polymorphism appears to be highly functional. Both SERT expression (transcriptional activity and protein levels assessed by radioligand binding) and activity were 30% to more than threefold higher in transformed lymphoblastoid

cells that have two copies of the long variant (*l*) as compared with those that have the short (*s*) variant (Heils et al., 1996; Lesch et al., 1996). Little et al. (1998) subsequently reported postmortem human brain data consistent with the Lesch et al. findings, with brain levels of SERT in *l* carriers approximately twice that of *s* carriers. New data, however, allow no clear consensus. Thus, in a more recent postmortem brain study, SERT levels were found to be unaffected in frontal cortex of normal subjects by the different variants (Mann et al., 2000) whereas the results of two SPECT studies that employed the radioligand [^{123}I]β-CIT are contradictory (Heinz et al., 2000; Willeit et al., 2001). Although these data do not yet allow any firm conclusion to be made regarding the influence of variants in SERT gene polymorphism on brain SERT levels in the human, the available genetic findings suggest that brain SERT levels might be influenced by genetic makeup and that the results of studies of drugs on brain SERT in humans might be confounded to some extent if the control and drug-exposed groups are not matched with respect to such variables.

The above considerations indicate that a finding of decreased concentration of brain SERT by a neuroimaging procedure in the brain of a subject exposed chronically to a neuroactive drug can be suggestive, but should not be taken as definitive proof of reduced number of serotonin neurons.

More "definitive" proof of neuronal damage in a condition restricted to damage to nerve terminals (no cell body loss) can only be obtained by postmortem brain examination, in which levels of all markers of serotonin nerve terminal integrity—serotonin, tryptophan hydroxylase, and SERT—are decreased if nerve terminal loss has occurred. In addition, if the interval between last exposure to the drug and death has not been prolonged, histopathological procedures can be utilized to demonstrate the qualitative signs of acute neuronal damage (the gold standard), namely silver staining (Switzer, 2000) and swollen, fragmented axonal elements. At the same time, however, it needs to be emphasized that all morphological procedures to assess such signs of neuronal damage rely on the very neurochemical markers (serotonin, tryptophan hydroxylase, and SERT) that, as discussed above, can be regulated independently of changes in neuronal number. Because of this potential confound it can be argued that, on theoretical grounds, interpretation of studies of serotonin nerve terminal damage (without loss of cell bodies) employing neurochemical serotonergic markers will always be difficult.

4. Assessment of strength of neuroimaging data on ecstasy and brain SERT in human brain

SERT has been measured by neuroimaging in a PET investigation (McCann et al., 1998a) and in a SPECT study (Semple et al., 1999) of polydrug users of ecstasy. The major difficulty with both investigations is the uncertainty that SERT was ever reliably measured.

4.1. McCann PET investigation

The 1998 McCann PET investigation employed binding of [^{11}C](+) McN-5652 (Suehiro et al., 1993; Szabo et al., 1995) to measure brain SERT and reported decreases in binding levels in all 12 examined brain areas (10 of which were statistically significant) in 14 users of ecstasy (minimum reported use of 70 times) as compared with levels in 15 control subjects.

The major limitation of this study is the uncertainty regarding the reliability and the validity of the SERT measurement by the PET procedure selected by the investigators. Although [^{11}C](+) McN-5652 is recognized as being problematic for SERT measurement because of its high nonspecific binding and suboptimal pharmacokinetic profile, it appears that the radioligand can be used to measure SERT in regions of the human brain that contain high density of the transporter (midbrain, thalamus, and striatum) if the quantitation of the nonspecific binding is conducted using the cerebellum as a reference region (Parsey et al., 2000; but see Buck et al., 2000). However, the study of McCann employed a controversial procedure for the measurement of nonspecific binding, namely, binding of the inactive enantiomer [^{11}C](−) McN-5652, which likely yielded an overestimate of specific binding, especially in cortical regions (Parsey et al., 2000). This prompted one group to argue that "... the results of this [McCann] study should be viewed with caution." (Parsey et al., 2000). Much more seriously, and perhaps related to the difficulty in quantitation of nonspecific binding, the individual subject binding values for both the controls and the ecstasy users in the McCann investigation were so scattered that the data had to be logarithmically transformed, even in regions of high SERT density. Thus, examination of the individual control binding values (see Fig. 4 in McCann et al., 1998a) revealed a wide range of [^{11}C](+) McN-5652 binding values in which the highest SERT concentration is approximately 30–35 times the lowest. The need to logarithmically transform in vivo neuroimaging data for measurement of a component of a monoamine neurotransmitter neuron (it is reasonable to assume that concentrations of such components should be distributed normally) appears to be without precedent. (For comparison, see a much tighter distribution of SERT brain levels in normal human subjects using a radioligand with a higher specific/nonspecific binding and in which the nonspecific binding was calculated using a more conventional procedure (Houle et al., 2000)). The additional methodological difficulties with the study are the absence of any published test–retest data in normal human subjects (which would address significantly the issue of the wide scatter of the data) and, based on more recent data employing experiments with an SSRI; uncertainty as to whether [^{11}C](+) McN-5652 binding in regions of very low SERT density (cerebral and especially cerebellar cortices) actually reflects binding to SERT (see Parsey et al., 2000).

The percentage changes between mean control and ecstasy group values are not stated in the McCann publication, an omission that has caused some confusion in the literature. Since many readers do not appreciate that the mean values provided in the McCann figures have been log-transformed, the magnitude of the differences have been incorrectly calculated from the summary figure (Fig. 3, McCann et al., 1998a) as representing only a modest (e.g., 22%: Holland, 1999; 25%: Gamma, 2000) decrease in brain [^{11}C](+) McN-5652 binding in the ecstasy group. However, after taking into account the log-transformation of the Fig. 3 data, it is apparent that the average extent of loss of [^{11}C](+) McN-5652 binding in the polydrug ecstasy users is actually quite severe, ranging from approximately 50% loss of binding in the cerebellum (a region with very low SERT levels) to a profound 85% depletion in the hypothalamus. The extent of reduction actually becomes even more striking if one accepts the argument of the investigators that "... PET imaging with [^{11}C] McN-5652 tends to underestimate the magnitude of reductions in 5-HT transporter density by about 50%." (McCann et al., 1999b). This would appear to suggest a global loss of about 75% to 95% of [^{11}C](+) McN-5652 binding/SERT concentration in brain of ecstasy users who, presumably, do not display any clinical signs of neurotoxicity.

Because of the serious methodological concerns in the PET measurement related to the high scatter of the values for the control and drug groups and lack of test–retest results, the data derived from the McCann investigation can only be considered, at most, "semiquantitive." Nevertheless, since the mean binding values in the ecstasy group are lower than that of the controls in all brain regions examined, it is not unreasonable to suggest that some reduction of [^{11}C](+) McN-5652 binding had probably occurred in the brain of the ecstasy users.

4.2. Semple SPECT investigation

The second neuroimaging investigation of SERT in ecstasy users employed the radioligand [^{123}I]β-CIT by a SPECT procedure in 10 male ecstasy users (minimum reported use of 50 tablets) and 10 control subjects (Semple et al., 1999). The assumption in the Semple investigation was that brain [^{123}I]β-CIT binding in striatum measured 1 day after injection of the radioligand reflects binding to the dopamine transporter (a procedure widely used in studies of patients with Parkinson's disease) whereas binding in all brain areas (including striatum) measured 90 min following injection reflects binding only to SERT. Mean [^{123}I]β-CIT binding in the striatum (caudate, putamen) measured 1 day after radioligand injection was normal in the ecstasy users, suggesting that ecstasy was not toxic to dopamine neurons in the ecstasy users of this study. Analysis of 90-min scan data revealed normal [^{123}I]β-CIT binding levels of striatum, midbrain/pons, thalamus, and cerebral cortex of the ecstasy users, with the exception of a slight (10–13%) reduction in binding in several cerebral cortical brain areas (occipital, cingulate, and calcarine). In contrast to the data of the McCann investigation, the binding data in the Semple study were not log-transformed.

The primary difficulty with the Semple SPECT investigation is the use of a radioligand for measurement of SERT, [^{123}I]β-CIT, which is not specific to SERT as it also binds to both dopamine and noradrenaline transporters. In fact, the uncertainty regarding the validity of [^{123}I]β-CIT for SERT measurement in human brain is clearly acknowledged by most investigators who employ this nonselective radioligand in human investigations (e.g., Malison et al., 1998; Dahlström et al., 2000; van Dyck et al., 2000; Laruelle et al., 2000; Pirker et al., 2000; Staley et al., 2001). Because of the lack of selectivity of [^{123}I]β-CIT for SERT, the radioligand is not usually used for measurement of SERT in areas of high dopamine transporter concentration such as the dopamine-rich striatum. A controversial issue is whether [^{123}I]β-CIT should ever be employed for measurement of SERT in those extra-striatal brain areas of the human such as the midbrain/brain stem and diencephalon which contain, in addition to SERT, dopamine (e.g., in substantia nigra) and noradrenaline transporters. In this regard, proponents of the use of [^{123}I]β-CIT in these areas cite data demonstrating some displacement of [^{123}I]β-CIT binding to brainstem/thalamus in humans exposed to the SSRI citalopram (Pirker et al., 1995). However, even at high doses of the SSRI (20–60 mg), the extent of maximal displacement in humans is only about 50% (Pirker et al., 1995; see also Tauscher et al., 1999), suggesting that a substantial proportion of [^{123}I]β-CIT is probably binding to non-SERTs.

An equally controversial issue regarding the utility of [^{123}I]β-CIT for SERT measurement is whether the radioligand should be employed for measurement of SERT in areas of very low SERT density, such as the cerebral cortex, in which the measurement might not be reliable or valid (e.g., presence of multiple transporters), with some SPECT groups concluding that it should not be utilized for this purpose in the human (Heinz and Jones, 2000; Laruelle et al., 2000). Although administration to humans of the SSRI citalopram does alter washout of [^{123}I]β-CIT from cerebral cortex (Kuikka et al., 1995; see Ebmeier et al., 2000) the results of this SPECT investigation do not allow assessment of the proportion of cortical [^{123}I]β-CIT binding attributable to SERT vs. that to other transporters.

Finally, the Semple investigation has been criticized because of the selection of a short time point (90 min after injection of the radioligand) for measurement of the binding that probably was not at equilibrium (see Heinz and Jones, 2000 for discussion).

The major limitations of the Semple study are the use of a radioligand recognized for not being selective for measurement of SERT in brain areas in which non-SERTs are known to be present and uncertainty regarding the extent to which the signal in the SERT-poor cerebral cortex (in which

slight changes in binding were observed in the ecstasy users) reflects binding to SERT.

The above considerations suggest that methodological problems related to the reliability and/or validity of the procedure for SERT measurement cast doubt on the conclusions in the McCann and Semple investigations that brain SERT levels and, by implication, serotonin neuron concentration, are lower than normal in chronic users of ecstasy.

In addition to these issues related to SERT measurement, there are also other potentially complicating factors, generic to such retrospective studies, which also affect interpretation of the data. The most difficult issue to address in all such investigations is the possibility, often raised by users of ecstasy, that the subjects of the ecstasy group in the investigations might have had a condition (e.g., major depressive disorder) preexisting drug use which was associated with decreased number of brain serotonin neurons. Although this possibility must be acknowledged, and can never be ruled out, other than in a prospective study in which measurements are made before and after drug exposure, this potential confound should not be used to dismiss arbitrarily the results of all retrospective investigations, especially when animal data suggest the possibility of ecstasy neurotoxicity in human brain.

A surprising failing of almost all studies of brain and behavior in ecstasy users is the lack of any forensic data to establish whether the ecstasy users had ever actually taken the drug (on even a single occasion) or whether the ecstasy users had used other drugs of abuse that could have affected brain SERT levels. This is related to concerns that ecstasy users are often unaware of the contents of the drugs that are self-administered and that other psychoactive drugs (e.g., cocaine) often taken by ecstasy users affect SERT levels in human brain (see above). In the McCann investigation, for example, not only was there the lack of any drug testing to prove that any of the subjects in the ecstasy group had ever used ecstasy, but also lacking was any statement on the results of an administered questionnaire on use of drugs other than ecstasy. In contrast, the Semple investigation included the results of drug testing in hair. However, in this study insufficient hair was taken to establish use of drugs beyond approximately 1 month or use of non-amphetamine (e.g., cocaine) drugs of abuse, and with three "ecstasy users" who tested negative for ecstasy in hair still included in the ecstasy group.

Regarding the potential confounds of gender and variants in SERT promoter gene polymorphism, the issue of gender was dealt with in the Semple (but not McCann) investigation by inclusion of only males in the study. Unfortunately, however, such a design would not permit testing of the hypothesis, based on findings in a prospective study that the psychoactive effects of ecstasy are more intense in women than in men (Liechti et al., 2001), that ecstasy might be more neurotoxic to the brains of women than that of men.

Neither neuroimaging investigation took into account the possible influence of variants in SERT promoter gene polymorphism.

4.3. Kish postmortem brain study

Finally, Kish et al. (2000) recently described low levels (-60% to -77%) of serotonin, but generally normal dopamine concentration, in autopsied striatum of a long-term ecstasy user who had also used cocaine and opiate drugs (drug history confirmed by sequential hair analysis) and had died of toxicity to one or more of these drugs. Although a limitation of this single case study is the coabuse in the single case of cocaine and heroin, investigations in the same laboratory have not disclosed any reduction of serotonin in chronic users of cocaine (Wilson et al., 1996b) or heroin (Kish et al., 2001), suggesting that the decreased serotonin was caused by the ecstasy exposure. These data are compatible with either an acute, reversible effect (serotonin depletion) of ecstasy and/or actual toxic degeneration of serotonin nerve endings.

5. Recommendations

It can be expected that many of the important issues affecting the interpretation of studies of serotonin neuronal markers in living brain of ecstasy users will be satisfactorily addressed in future studies, in part by the use of more selective radioligands for SERT measurement by SPECT or PET, appropriate quantitation of the data, confirmation of drug use by forensic drug analysis, and, if at all possible, selection of a group of "pure ecstasy users" for study. Furthermore, as neuroimaging data relying on a single marker of serotonin neurons (SERT), even in subjects withdrawn (e.g., 6–12 months) from ecstasy, can only be suggestive of brain damage, some postmortem confirmation of toxicity using established histopathological procedures will also be required. Until this is accomplished, most neuroscientists and ecstasy users will continue to consider the question of possible toxicity of ecstasy to serotonin neurons in human brain as unresolved.

6. Conclusions

1. It is likely, on the basis of animal data, that ecstasy, at some dose, will damage serotonin neurons in human brain. However, because of methodological problems in the limited number of studies conducted in the human, no conclusions can yet be established on ecstasy toxicity in human brain or whether ecstasy exposure represents a chronic serotonin deficiency syndrome.

2. The theoretical limitation of studies relying on neurochemical markers of the integrity of serotonin neurons, which can be up- and down-regulated independently of

neuronal number, to assess serotonin neuronal damage should be recognized.

Appendix

Since submission of this review, Reneman et al. (2001a,b) recently reported slightly decreased [^{123}I] β-CIT binding in brain of human ecstasy users. As discussed in detail in section 4.2, interpretation of the SPECT data is made difficult by the uncertainty that SERT was never reliably measured.

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