Neurotoxicity Research in Humans

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Introduction and Overview

This chapter reviews the studies that explore the possibility of neurotoxicity in ecstasy users. The primary purpose of this review is to aid in assessing risks of clinical MDMA studies. This chapter attempts to interpret findings in a manner that produces a conservative risk assessment. Studies of illicit ecstasy users are useful in risk assessment because they identify possible areas of toxicity and identify the possible severity of toxic changes. Studies of ecstasy users are limited because it is not always possible to distinguish the effects of MDMA exposure from other factors and many questions have not been adequately studied. Studies in ecstasy users are important because research in animals (reviewed in a separate chapter) shows that MDMA can cause neurotoxicity, damaging serotonergic axons and permanently changing their distribution in the brain. It is likely that some MDMA exposures cause similar or identical changes in humans.

Most research on ecstasy users can be categorized into two areas of study: neurofunctional measures and neurocognitive measures. In this document, "neurofunctional" is loosely used to indicate measures of how the brain is working and measures of the concentration or density of neurochemicals. "Neurocognitive measures" refers to performance on standardized tests of mental abilities. Research on ecstasy users supports associations between MDMA exposure and alterations in both neurofunctional and neurocognitive measures. Measures that do not cleanly fit either of these categories include those examining mood and personality in ecstasy users. These measures are also reviewed, even though they are difficult to interpret and have questionable relevance for neurotoxicity risk assessment.

Reported neurofunctional differences between ecstasy users and nonusers include concentration of a serotonin metabolite in cerebrospinal fluid (CSF 5HIAA levels), serotonin transporter (SERT) density, $5HT_{2A}$ receptor density, neuroendocrine response to serotonergic drugs, EEG measures, altered sleep architecture, cerebral myo-inositol concentration, cerebral glucose utilization, and cerebral blood flow/volume. There is insufficient evidence to assess the permanence or reversibility of most reported neurofunctional differences.

Statistically significant correlations have been reported between ecstasy exposure and specific neurofunctional measures, such as CSF 5HIAA levels, SERT density, global brain volume, myo-inositol increases, $5HT_{2A}$ receptor density, and EEG alterations. The most conservative interpretation of these correlations is to regard them as evidence that ecstasy exposure caused the neurofunctional differences. A less conservative interpretation would be that differences in these neurofunctional measures predate ecstasy exposure and indicate a tendency to use ecstasy. The authors find this interpretation to be implausible.

In some cases, there are questions as to whether these changes can be considered evidence of serotonergic neurotoxicity rather than responses to the nontoxic pharmacological effects of MDMA. To distinguish between neurotoxicity and responses to pharmacological effects of MDMA, it is helpful to consider (1) animal data on the effects of MDMA and other serotonergic neurotoxins and (2) human data on the effects of drugs that are not serotonergic neurotoxins, particularly stimulants. When these additional data are considered, some neurofunctional differences can conservatively be regarded as evidence of serotonergic neurotoxicity in users, because they are documented in animals after neurotoxic regimens of MDMA and other serotonergic neurotoxins. These differences include decreases in serotonin transporter and CSF 5HIAA.

Nonetheless, most neurofunctional differences are not clear evidence of selective serotonergic neurotoxicity. Some, such as increased alpha and beta EEG and altered sleep architecture, occur in users of stimulant drugs that do not cause serotonergic neurotoxicity. Others, such as cerebral blood flow/volume and cerebral glucose utilization, are altered in the opposite direction in ecstasy users compared to neurotoxin-exposed animals. Still others, such as increased $5HT_{2A}$ receptor density, have not been seen in animals exposed to serotonergic neurotoxins. These reported differences are of unknown significance.

Neurocognitive performance studies suggest that, under some conditions or patterns of use, ecstasy exposure can decrease performance in some measures of neurocognitive functioning into the lower range of what is considered clinically normal. There is no conclusive evidence that a specific domain of cognitive functioning is impaired in ecstasy users, although some have suggested that a category of mental abilities called “executive function” that includes the ability to plan ahead may be specifically altered. Measures of verbal memory have most consistently detected differences between ecstasy users and nonusers, but many other measures have also sometimes detected differences.

We do not know the relationship between these neurocognitive changes and serotonergic neurotoxicity. Decreased neurocognitive performance can occur in users of other drugs of abuse, such as cocaine or marijuana. Only 2 of at least 11 studies have found evidence of long-term impairment in the neurocognitive performance of animals exposed to neurotoxic MDMA regimens. This suggests that serotonergic neurotoxicity might occur in the absence of neurocognitive performance changes and that neurocognitive performance changes in ecstasy users may or may not be caused by serotonergic neurotoxicity.

There are not yet sufficient data to conclude whether these neurocognitive differences would lessen after the discontinuation of frequent ecstasy exposure. One study and one analysis described in this document found evidence of recovery, while another study and a second analysis in this document found no evidence of recovery. The issue of recovery is worrisome because even small changes in illicit users could be important if they were permanent.

While statistically significant, the reported differences between matched groups of ecstasy users and nonusers are subtle and can for the most part only be detected with sensitive neurofunctional and neurocognitive measures. The limited data from volunteers in clinical MDMA studies have not shown evidence of toxicity. This suggests that cautious clinical MDMA research can be conducted with low risks. Nonetheless, the possibility of currently undetected chronic toxicity cannot be excluded and ethics requires discussing this matter with volunteers. It is important to be aware that published studies have compared, at most, groups of 30 individuals and excluded those with histories of serious psychiatric or neurological problems. This has at least two implications. First, there are only anecdotal data on whether individuals with a history of psychiatric or neurological illness are affected differently by MDMA exposure than other ecstasy users. Second, the limited sample sizes in these studies means that only common adverse effects of MDMA will have been detected. There may be rare adverse events that are not yet identified.

It is possible that impairment will manifest as users age. Some hypothesize that serotonergic neurotoxicity could lead to depression and anxiety disorders as individuals' serotonergic systems undergo age-related decreases in functioning. Although age-related decreases in serotonergic functioning appear relatively modest compared to those seen in the dopaminergic system, age-related changes in the brain are not sufficiently understood to make predictions about the possible long-term consequences of serotonergic neurotoxicity with any confidence.

Furthermore, there is currently no direct evidence on this issue. There are no published studies with rodents or other animals with short lifespans suggesting MDMA exposure causes significant toxicity that only becomes apparent in the aged animal. There are also no published studies or other evidence of problems developing in humans. MDMA has been widely used for over 20 years and similar drugs with similar capacity for long-term serotonergic changes (e.g., 3,4-methylenedioxyamphetamine, MDA) have been used since the 1960s without evidence of dramatic age-related toxicity. Methamphetamine, which produces both long-term serotonergic and dopaminergic changes, has been used clinically for over 60 years without reported incidents of neurocognitive deficits appearing with age. This lack of evidence of problems developing with age is reassuring, but not conclusive. Until appropriate studies are conducted, lack of evidence of problems cannot be taken as evidence of lack of problems.

Overall, it is very likely that repeated ecstasy exposure causes neurofunctional changes in some illicit users. It is also very likely that some of these changes are due to serotonergic neurotoxicity. Nevertheless, the reported differences between matched groups of ecstasy users and nonusers are clinically subtle and can, so far, only be detected with sensitive neurofunctional and neurocognitive measures. Studies of illicit ecstasy users give no indication that one or two exposures to MDMA in a clinical setting would produce significant or lasting toxicity. Preliminary data from clinical MDMA studies support this conclusion. However, the risks and benefits of proposed MDMA studies must be assessed on an individual basis.

Limitations of The Research Literature

Differences may be Pre-existing. In retrospective studies, correlation cannot be taken as a demonstration of causality. While ecstasy exposure may be associated with user-nonuser differences in a given study measure, ecstasy exposure may simply be a marker for some other variable that causes the observed differences between ecstasy users and nonusers. This other variable may be a habitual behavior (e.g., repeated sleep and nutrition deprivation from attending raves) or a pre-existing trait (e.g., genetic serotonergic differences influencing personality). People who prefer drugs may simply be different from those who do not. For example, Uhlenhuth et al. (1981) found that volunteers (with no history of drug abuse) who preferred amphetamine to placebo had significantly higher baseline scores on POMS subscales for anxiety, depression, fatigue, and confusion than other volunteers. Such an association between preexisting dysphoric mood and preference for euphoric drugs could easily be mistaken for stimulant-induced dysphoria in a retrospective study of stimulant abusers.

One report (Schifano 2000) recently described currently unpublished survey data from high school students in Italy that found students attending less academic secondary schools were 2.89 times more likely to have used MDMA than those attending more academic schools. In another survey of 737 Italian MDMA users, cited in the same publication, there was reportedly evidence of inverse relationships between the tendency to take higher MDMA doses and both lower schooling level and family income (Schifano 2000). These findings provide evidence that there are differences between MDMA users and nonusers that predate illicit drug use.

The possibility of such pre-existing differences is particularly significant when studying serotonergic differences between drug users and nonusers. It is known that there are genetic influences on the serotonergic system. Increasing evidence suggests that these differences may influence response to drugs of abuse. For example, genetic variation of the promoter for the serotonin transporter (SERT) gene has been associated with level of SERT activity. *In vitro* studies show carriers of a short allele of the SERT promoter may have significant reductions in SERT activity (Lesch et al. 1996). Recently, Heinz et al (2000) suggested that homozygous carriers of the long allelic variant of the serotonin transporter gene promoter region may have increased susceptibility to the neurotoxic effects of ethanol, which were measured as decreased serotonin reuptake transporter (SERT) availability in the raphe. In addition, decreased serotonergic functioning is thought to be related to impulsivity and sensation-seeking (Zuckerman 1996). Individuals with greater tendency to use illicit drugs may therefore have a higher prevalence of serotonergic abnormalities. Furthermore, it is not implausible to hypothesize that individuals with lower serotonergic tone might be specifically vulnerable to self-administration of drugs that are serotonin releasers, such as MDMA (Laviola et al. 1999). Thus, when correlations between ecstasy use and altered serotonergic functioning or psychopathology are detected in retrospective studies, it is impossible to determine the direction of causality.

Table 5.1: Summary of Common Study Limitations

Reference	Volunteer Sampling			Group Matching		Method of Confirming and Duration of Ecstasy Abstinence		Other Issues	
	Non-Random Sampling	Possible Pre-existing Difference	Non-Representative Users or Controls	Fails to Control for Polydrug Use	Poor or Imperfect Matching			Non-independent samples	Lack of Adequate / Established Norms
Allen et al, 1993	X	X		X	X	U, B	2 w +	1	
Bolla et al, 1998	X	X			X	U, B	4 (2-36) w	1	
Brody et al, 1998	X	X		X		S	NA		
Chang et al, 2000	X			X		U	6.6 ± 7.7 (0.5-26) m	3	X
Chang et al, 1999	X	X		X		?	4.0 (0.5-26) m *	3	
Croft et al, 2001	X	X				S	2 d +		
Dafters et al, 1999	X	X			NA	S	7 d +		X
Gamma et al, 2000	X	X			X	S	1 wk +	9	X
Gamma et al, 2001	X	X			X	S	1 wk +	9	X
Gerra et al, 1998	X	X	X	X		U	3 w	4	
Gerra et al, 2000	X	X	X	X		U	3 w-12 m	4	
Gouzoulis-Mayfrank et al, 2000	X	X				U	41 ± 71.1 (7-356) d	5	
Krystal et al, 1992	X	X		X	NA	S	66 ± 50 (20-180) d	2	
McCann et al, 1994	X	X				U, B	17.9 ± 24.7 (2-104) w	1	
McCann et al, 1998	X	X			X	U, B	19 (3-147) w		X
McCann et al, 1999a	X	X				U, B	14 ± 29 (3-139) w	6	
McCann et al, 1999b	X	X				U, B	13.91 ± 6.54 (3-147) w	6	
Morgan et al, 1998 - Study 1	X	X				S	20.4 ± 33.6 d **		
Morgan et al, 1998 - Study 2	X	X				S	65.1 ± 85.7 d	7	
Morgan et al, 1999	X	X				S	65.1 ± 85.7 d	7	
Obrocki et al, 1999	X	X	X	X	X	U	(2-16) m		X
Parrott & Lasky, 1998	X	X				S	7 d +		
Parrott et al, 1998	X	X		X		S	NA		
Parott et al, 2000	X	X		X	X	S	1 d +		
Peroutka et al, 1987	X	X			X	S	6 w +		
Price et al, 1989	X	X		X	X	S	66 ± 50 (20-180) d	2	

Abbreviations: B = blood drug screen; d = day; H = hair drug test; m = month; NA = not applicable; S = self report; U = urine drug screen; w = week.

Numbers in column on Non-Independent Samples indicates studies that contain some of the same individuals.

Some study limitations discussed in text are not indicated here due to space constraints.

* gives median, rather than mean, time since last use.

** 7 of 16 used 3-7 d before testing.

Table 5.1 continued: Summary of Common Study Limitations

Reference	Volunteer Sampling			Group Matching		Method of Confirming and Duration of Ecstasy Abstinence		Other Issues	
	Non-Random Sampling	Possible Pre-existing Difference	Non-Representative Users or Controls	Fails to Control for Polydrug Use	Poor or Imperfect Matching			Non-independent samples	Lack of Adequate / Established Norms
Reneman et al, 2000a	X	X			X	U	4.6 (2-11) m	8	X
Reneman et al, 2000b	X	X				U	Current: 7 ± 5 w; Former: 18 ± 15 w	8	X
Ricaurte, 1990	X	X	X	X	X	S	16.7 ± 23.3 (2-104) w	2	
Rodgers et al, 2000	X	X			X	S	2 m		
Schifano et al, 1999	X	X	X	X	X	U	NA		
Semple et al, 2000	X	X				H	18 ± 8.0 d		X
Tuchtenhagen et al, 2000	X	X				U	41 ± 71.1 (7-356) d	5	X
Verkes et al, 2000	X	X			X	U	1 w		
Wareing et al, 2000	X	X		X		S	Current: 8.20 ± 5.75 d; Former: 323.25 ± 130.05 d		
Zakzanis et al, 2001	X				NA	U, B	4 (2-36) w		

Abbreviations: B = blood drug screen; d = day; H = hair drug test; m = month; NA = not applicable; S = self report; U = urine drug screen; w = week.
Numbers in column on Non-Independent Samples indicates studies that contain some of the same individuals.
Some study limitations discussed in text are not indicated here due to space constraints.
* gives median, rather than mean, time since last use.
** 7 of 16 used 3-7 d before testing.

Recruitment and Matching of Volunteer Groups is Poorly Described. The procedures with which user and nonuser groups were recruited and matched are often poorly described. When attempting to detect subtle effects of drug exposure, it is crucial that comparison groups be as similar as possible. For example, if one comparison group has more education, it would not be surprising if they also perform better on neurocognitive tests. Unless education is comparable between groups or the difference is taken into consideration during statistical analysis, then a performance difference may be erroneously attributed to drug use when it is actually related to education. In this report, groups are described as matched with respect to a given variable if researchers indicated that there was no statistically significant difference between groups for that variable. Unless otherwise noted, this should not be taken to imply that matching was part of the inclusion/exclusion criteria or recruitment process.

Self-reports of Drug Use are Inaccurate and Many Ecstasy Users are Polydrug Users. A third significant limitation to this literature is the reliance on user self-reports of past ecstasy (and other drug) exposure. Both the accuracy of user recollections and the contents of illicit drug preparations are questionable. The inaccuracy of attempts to measure past ecstasy use is likely to make it more difficult to detect significant relationships between ecstasy exposure and user-nonuser differences. For example, given that one 'brand' of illicit ecstasy pills contained between 19 and 140 mg in one published

report (Sherlock et al. 1999), two individuals reporting ecstasy use of a single tablet of a single type once or twice per month for the last year may have actually consumed between 228 and 3360 mg MDMA total. This will obviously make attempts to correlate repeated drug use and other indices difficult. Furthermore, this estimate does not take into consideration the fact that some ecstasy pills contain other potentially neurotoxic compounds, such as ketamine (Shewan and Dalgarno 1996). As a result of these adulterants, even ecstasy users who do not report other drug use may actually be polydrug users.

Very few regular ecstasy users report restricting their substance use to ecstasy alone, and a large number of ecstasy users have also used other drugs. Several researchers have attempted to control for the effects of polydrug use by either employing drug matched controls (Bolla et al. 1998; Semple et al. 1999) or employed drug-naïve and drug-using controls, with cannabis users often serving as the drug-using controls (Croft et al. 2001; Gouzoulis-Mayfrank et al. 2000; Morgan 1998; 1999; Rodgers 2000). However, ecstasy users report a greater number of exposures to substances such as psychostimulants and hallucinogens and report having used a wider variety of substances than cannabis users or polydrug users who have not used ecstasy. Hence differences between ecstasy users and drug-matched controls may be due in part to differences in exposure to other drugs.

Polydrug use is often associated with more severe consequences than use of individual drugs of abuse (Selby and Azrin 1998). This may partially reflect the type of person who uses multiple drugs, but it also probably reflects the fact that simultaneous use of multiple drugs can lead to increased toxicity beyond that predicted from the individual drugs. Numerous studies in rats have documented the ability of other pharmacological agents to increase MDMA-induced neurotoxicity. For example, hallucinogens appear to increase MDMA-induced serotonergic neurotoxicity (Gudelsky et al. 1994). Thus, even if researchers attempt to control for other drug use in their analyses, it is possible that the apparent toxicity of ecstasy may be exaggerated in polydrug users due to drug interactions.

Short-term Effects are not distinguished from Long-term Effects. A related and probably more serious concern is the difficulty of separating short-term from long-term ecstasy effects. Unless hair samples or repeated urine samples are collected, it is impossible to confirm abstinence from MDMA for more than a few days. In many published studies, no attempts to confirm user reports of recent drug use appear to have been made. In other investigations, participants report their last use of ecstasy or MDMA to be fewer than seven days before the study day. User surveys have documented dysphoric mood for several days after ecstasy use in some users (Croft et al. 2001; Curran and Travill 1997; Parrott and Lasky 1998). Dysphoric mood has also been reported in clinical studies where MDMA has been administered to participants in a controlled setting (Liechti and Vollenweider 2000b; Vollenweider et al. 1998a). These short-term residual effects therefore presents a possible explanation for some of the reported changes in ecstasy users in studies where volunteers may have ingested ecstasy only a few days before testing (e.g., Gamma et al. 2000b; Parrott et al. 1998). It is not clear how long an abstinence period is required to exclude this possibility with MDMA. While withdrawal-

induced dysphoria is seldom significant in studies administering psychostimulants to healthy volunteers, recovery from psychostimulant use can reportedly take as long as 1 month in chronic stimulant abusers. In a recent study, dependent cocaine users improved in mood and self-reported cognitive skills over 28 days of abstinence (Coffey et al. 2000).

Subjects may be Aware of Research Hypotheses. The media has reported on research findings concerning the neurotoxic effects of MDMA in non-human animals and deficits in neurocognitive function in ecstasy users. It seems likely that ecstasy users have encountered this information, with awareness increasing over the last ten years. The impact of such widespread awareness of research hypotheses concerning the effects of MDMA is unknown. It is possible that individuals who perceive themselves as affected by regular ecstasy use may be more likely to participate in research studies than individuals who do not perceive any effects arising from regular ecstasy use. Some participants may strive to demonstrate their competence on neurocognitive tasks in order to refute hypotheses concerning the detrimental effects of ecstasy on cognitive function. However, they may also strive to be "good subjects" by attempting to confirm the researcher's hypothesis by performing less well or by searching for and exaggerating any psychiatric symptoms they have experienced.

Same Volunteers are used in Multiple Publications. Another limitation of the literature comparing ecstasy users and nonusers is the low independence of publications. Multiple papers appear to use largely the same groups of volunteers. For example, Krystal et al. (1992) use the same group of ecstasy users as Price et al. (1989) which appears to be a subset of volunteers from Ricaurte et al. (1990). Similarly, ecstasy users in Bolla et al. (1998) appear to be mostly, but not completely, a subset of those in McCann et al. (1994). While it may be reasonable to separately publish different measures from the same volunteers, it is important to clearly state that this is being done. When the same volunteers are used in multiple studies, consistent findings of serotonergic differences may say more about the convergent validity of each publication's serotonergic measures than about the effects of MDMA exposure.

Serotonergic and Neurofunctional Measures do Not Indicate Toxicity Per Se. There is a lack of well-validated measures of serotonergic toxicity. Serotonergic measures indicate altered serotonergic functioning, but may not indicate toxicity. Thus, measures are not clearly selective for toxic changes. One proposed method of deciding whether differences in biological markers indicate toxicity involves statistically defining an abnormal level based on the distribution characteristics in the study population. This interpretive method has been used in nonhuman MDMA toxicity studies to interpret neurochemical data (Gaylor and Slikker 1990) and is similar to the approach frequently used in clinical neurocognitive assessment. In neurocognitive assessment, it is common practice to consider test scores falling at least 1.3 standard deviations (SD) below the normative mean as "borderline" while those at least 2.0 SD below the mean are considered "impaired" (Lezak 1995). It should be noted that this approach relies on an adequately characterized study population. In some papers, measures are used which are sufficiently new (e.g., PET measures of estimated cortical SERT density) that values for the ecstasy-free comparison group in the paper(s) present the only available data on

"normal" values for the measure. As a result, it is not clear whether the full range of healthy, normal values is being represented. This is particularly unfortunate with SERT measures since increasing evidence suggests that there are genotypic and phenotypic variations in SERT functioning that are possibly associated with different vulnerabilities to substance abuse (Heinz and Jones 2000).

Table 5.2: Comparison of PET and Autoradiography Measures of SERT Density

Region	PET Estimate using [11C]McN5652 (% of Control)	Post-Mortem [3H]Paroxetine Measure (% of Control)	% Difference in Measures
Frontal Cortex	37.9	14.9	23
Parietal Cortex	22.45	14.8	7.65
Occipital Cortex	26.73	26.9	-0.17
Temporal Cortex	136.64	21.2	115.44
Hypothalamus	223.46	171.2	52.26
Midbrain	200.59	255.1	-54.51
Thalamus	145.69	67.6	78.09
		Mean Absolute Difference:	47.30
		Stand. Dev.:	40.93

Data are from Scheffel et al. (1998) and compare two methods of assessing SERT changes in a baboon administered 5 mg/kg s.c. MDMA twice daily for 4 days 14 weeks. A single MDMA-treated animal was compared to a single control animal.

Non-invasive Serotonergic Measures Have Unclear Sensitivity. In addition to the interpretive problems of trying to measure toxicity with neurochemical or neurofunctional markers that are not selective for toxic changes, putative *in vivo* measures of the serotonergic system appear to have unclear or only moderate sensitivity. Nonhuman animal studies attempting to establish the use of potential clinical *in vivo* serotonergic measures consistently find that these measures underestimate central serotonergic changes. For example, **Table 5.2** shows data from an animal study (Scheffel et al. 1998) comparing *in vivo* estimates of regional serotonin reuptake transporter (SERT) density (made using PET and [^{11}C]McN5652) to post-mortem measures of SERT density (which employed [^{3}H]Paroxetine binding). In most brain regions, actual SERT density is underestimated by the PET measure. The researchers suggest that differences in the temporal cortex and thalamus may be due to differences in the boundaries of the measured regions with the two methods. Even if these two regions are excluded from consideration, it can be seen that the PET measure of SERT density tends to overestimate changes in comparison to autoradiography. Similarly, primate studies of cerebral spinal fluid (CSF) concentrations of the serotonin metabolite, 5-hydroxyindoleacetic acid (5HIAA) show that MDMA-induced changes in CSF 5HIAA are about 20% less than either brain serotonin or brain 5HIAA changes (Ricaurte et al. 1988b).

Interpreting Studies for Risk Assessment

The above limitations complicate interpretation of retrospective studies. For the purpose of risk assessment, user-nonuser differences that correlate with ecstasy exposure or are consistent with the nonhuman animal toxicity literature should be accepted as possible effects of MDMA exposure. This approach differs from a purely scientific one in that the

standards of proof are lowered and correlation is taken to imply causality. This should produce a conservative risk analysis.

It should be noted that some of the apparent changes seen in ecstasy users are in the opposite direction of those reported in nonhuman animals. Such discrepancies between nonhuman neurotoxicity studies and studies of human ecstasy users may be due to (1) differences in MDMA administration patterns or time from last exposure, (2) actual species differences in response to MDMA, or (3) differences between ecstasy users and nonusers which predate ecstasy use. There is unfortunately little information about the time course of neurofunctional changes after MDMA exposure in nonhuman animals. Thus, reported increases at one time point cannot be taken to exclude decreases at another time point (or vice versa). In nonhuman MDMA studies, serotonin depletions in some brain regions are followed by increases as axons regrow. Similarly, human cerebral blood flow (CBF) data suggest a biphasic response to some MDMA exposures with decreases for several weeks followed by possible increases (Chang et al. 2000). One approach is to consider animal studies as demonstrating whether, in principle, MDMA exposure can alter a given index, ignoring discrepancies in the direction of changes.

It is more difficult to interpret studies that measure aspects of functioning in ecstasy users that have not been studied in MDMA-exposed animals. When these studies find differences between ecstasy users and nonusers, it is unclear whether these differences are due to serotonergic neurotoxicity or the repeated pharmacological effects of ecstasy. In evaluating these possibilities it is helpful to consider whether the possible changes have been seen in animals exposed to other serotonergic neurotoxins, such as dihydroxytryptamine (DHT). This can provide evidence that serotonergic neurotoxicity can alter this aspect of functioning. However, MDMA is thought to selectively damage axons originating in the dorsal raphe nucleus, while DHT will damage serotonergic (and noradrenergic, if no blocking agent is used) axons wherever it is infused. Unless DHT is infused into the dorsal raphe nucleus, it can be expected to produce a different pattern of serotonergic damage than MDMA, which may limit comparisons.

It is also helpful to consider whether the possible changes in ecstasy users have been documented in users of stimulant drugs that are not selective serotonergic neurotoxins, such as cocaine and amphetamine. If these other drugs can cause such changes, then it is possible that they can occur in ecstasy users in the absence of serotonergic neurotoxicity. This does not mean that the putative changes are not evidence of toxicity. It simply means that they are not evidence of selective serotonergic neurotoxicity. It is well established that cocaine, amphetamine, and other stimulant drugs can cause clinically significant toxicity, including impaired neurocognitive performance and ischemic damage to the brain.

A final important consideration concerns to magnitude of the apparent changes. In neurocognitive assessment, it is common practice to interpret test scores following at least 1.3 SD units below the normative mean as “borderline” and those 2.0 SD units below the mean as “impaired” (Lezak 1995). This approach is most useful for comparing two populations, such as ecstasy users and the general population. Such an approach can

establish whether individuals are still within the "normal" range on a given measure. However, remaining in the normal range of functioning can be a rather lax test, if changes are permanent or long lasting. Therefore, evidence of recovery must also be considered when assessing risks.

In summary, there are many limitations in existing studies of ecstasy users. Given the partially unknown long-term risks of MDMA exposure, it is important to be conservative in risk assessment. This can be achieved by assuming that there were no preexisting differences between ecstasy users and nonusers. One must then tentatively evaluate whether these differences are evidence of serotonergic neurotoxicity, some other type of toxicity, or neurofunctional changes of unknown significance. Nonhuman MDMA studies are obviously an important source of evidence for these assessments, although the lack of time course information makes it difficult to reconcile differences in the apparent direction of changes between human and nonhuman studies. The most conservative course appears to be to ignore these directional differences. In addition to nonhuman MDMA studies, it is helpful to consider nonhuman studies employing other serotonergic neurotoxins and studies of users of non-neurotoxic stimulant drugs. Finally, one must consider both the magnitude of apparent changes and any evidence of recovery.

Evidence of Serotonergic Differences between Ecstasy Users and Nonusers

Cerebral Spinal Fluid Levels of 5HIAA. The earliest attempts to detect possible serotonergic changes in ecstasy users involved measuring cerebral spinal fluid (CSF) concentrations of the serotonin metabolite 5-hydroxyindole acetic acid (5HIAA) (Peroutka et al. 1987). As summarized in **Table 5.3**, CSF 5HIAA appears to be modestly reduced in very experienced ecstasy users (McCann et al. 1999b; McCann et al. 1994; Ricaurte et al. 1990).

Table 5.3: Cerebral Spinal Fluid Levels of 5HIAA in Ecstasy Users and Nonusers

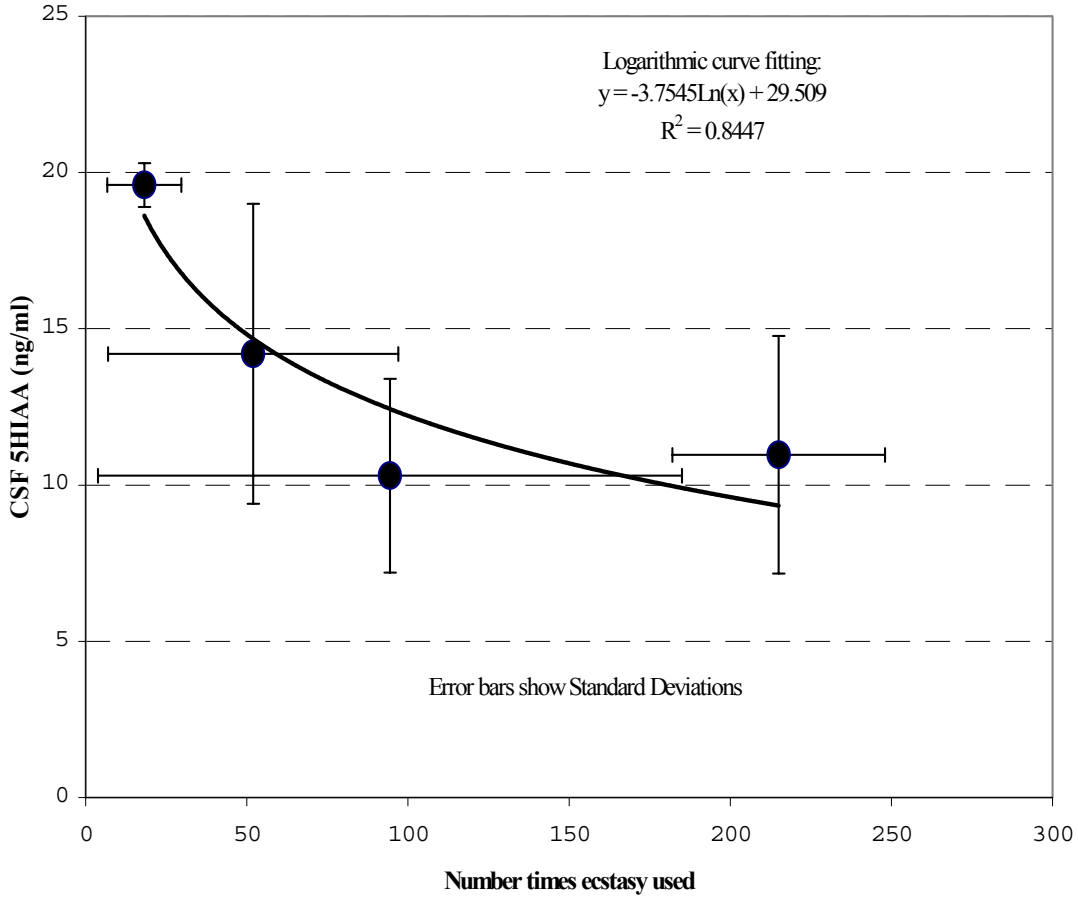
Study	Controls	Ecstasy Users
Peroutka et al., 1989	22.5 ± 9.8	19.6 ± 0.7 n.s.
	N = 17	N = 5
z-score=	**0 ± 1**	**-0.296 ± 0.07 n.s.**
Ricaurte et al., 1990	19.1 ± 4.3	14.2 ± 4.8*
	N = 24	N = 33
z-score=	**0 ± 1**	**-1.14 ± 1.1***
McCann et al., 1994	15.2 ± 7.9	10.3 ± 3.1*
	N = 28	N = 30
z-score=	**0 ± 1**	**-0.62 ± 0.39***
McCann et al., 1999b	14.77 ± 7.1	10.97 ± 3.8*
	N = 23	N = 22
z-score=	**0 ± 1**	**-0.535 ± 0.54***

Raw values are means ± SD expressed in ng/ml of CSF.
* indicates values are significant less than those of controls, P <= 0.05.

McCann et al. (1994) were unable to detect a significant correlation between CSF 5HIAA levels and ecstasy exposure. In contrast, Bolla et al. (1998) describe a significant correlation between CSF 5HIAA and estimated milligrams of ecstasy per month in what appears to be largely a subset of the volunteers from McCann et al. (1994). No other significant correlations between exposure and this index have been reported. However, comparison of mean CSF 5HIAA values and ecstasy exposure across studies (**Figure 5.1**) suggest such a correlation might exist if all individual data collected by McCann and Ricaurte were pooled. Thus, while no significant decrease was noted by Peroutka et al. (1987) in volunteers who had 18.2 ± 11.5 (1-33) exposures to ecstasy, significant decreases were noted by McCann and Ricaurte in studies using volunteers with 52 ± 45 (11-219) exposures (Ricaurte et al. 1990), 215 ± 33 (30-725) exposures (McCann et al. 1999b), and 94.4 ± 90.6 (25-300) exposures (McCann et al. 1994). In contrast to this suggested relationship between exposure and decreased CSF 5HIAA, no apparent pattern exists when evidence of recovery is sought by comparing average time from last exposure to CSF 5HIAA levels. This may be due to the narrow range of times from last exposure in published studies.

MDMA-related reductions in CSF 5HIAA are consistent with nonhuman primate data that have documented long-lasting changes. Ricaurte et al. (1988b) reported that CSF 5HIAA in squirrel monkeys was 40% of controls (or approximately 2.8 SD units), brain 5HIAA 21% of controls, and brain serotonin was approximately 15% of controls at 2 weeks after administration of 5 mg/kg s.c. MDMA twice daily for 4 days. A second study (Insel et al. 1989) reported that CSF 5HIAA decreases were detectable up to 14 weeks after MDMA exposure in rhesus monkeys administered 10 mg/kg i.m. MDMA twice daily for 4 days. Figure 5 of the paper by Insel et al. graphically indicates that CSF 5HIAA levels were reduced to approximately 60% of controls (approximately 1 SD units) at 14 weeks post-exposure.

Figure 5.1: Relationship between ecstasy exposure and CSF 5HIAA in 4 studies



Serotonin Reuptake Transporter Density. Two studies using positron emission tomography (PET) have reported reduced serotonin transporter binding in ecstasy users (McCann et al. 1998; Semple et al. 1999). These reports have proven controversial and interpretations have been disputed (Heinz and Jones 2000; Kuikka and Ahonen 1999; Reed et al. 1999). The novelty of PET measures of cortical serotonergic functioning makes their interpretation difficult.

In contrast to these studies of ecstasy users, preliminary unpublished results from Dr. Franz Vollenweider and colleagues did not detect changes in SERT density (using the same PET ligand as McCann et al.) at four weeks after six MDMA-naïve volunteers were administered either 1.5 or 1.7 mg/kg po (Vollenweider et al. 2001; personal communication). Thus, it would appear that single doses up to 1.7 mg/kg MDMA do not produce detectable long-term effects on the serotonin transporter when administered once in a clinical setting.

However, comparisons of illicit ecstasy users and nonusers have detected significant differences in estimated SERT density. One study (Semple et al. 1999) reported significant SERT decreases in the left occipital, left and right calcarine, and right posterior cingulate cortices when results were analyzed by ANCOVA using cerebellar activity as a covariate. These decreases were moderate in magnitude and ranged from -0.629 ± 0.8 standard deviation (SD) units in the left occipital cortex to -0.971 ± 0.83 SD units in the left calcarine cortex. Significant decreases in other brain regions were detected using statistical parametric mapping. McCann et al. (1998) graphically depict but do not numerically report their results. Figure 4 in their paper, a graph of estimated SERT binding (i.e., "in distribution volume"), indicates that data from only one ecstasy user is obviously lower than those of control volunteers and data from other ecstasy users remain within the range of nonuser volunteers.

Both research groups reported correlations between ecstasy exposure and SERT binding. Specifically, Semple et al. (1999) reported significant correlations between time from last ecstasy exposure and apparent SERT binding, suggesting an unexpectedly fast recovery of SERT levels over a several week period after ecstasy exposure. McCann et al. (1998) did not detect evidence of recovery but did report a correlation between lifetime number of ecstasy exposures and apparent SERT binding. However, this correlation has been criticized for its inclusion of control volunteers (Kuikka and Ahonen 1999).

Using PET to estimate SERT density requires complex modeling (Buck et al. 2000), and methodological criticisms have been made of the PET studies of ecstasy users (Heinz and Jones 2000; Kuikka and Ahonen 1999). Most significantly, it has been argued that there is little evidence that the binding of [^{125}I]beta-CIT or [^{11}C]MCN-5652 in the cerebral cortex reflect specific binding to serotonin transporters (Heinz and Jones 2000). This suggests that the findings of altered cortical binding by these radioligands may be partially or completely due to differences in blood flow, blood-brain barrier permeability, or other factors. In fact, Chang et al. (2000) have reported decreases in regional cerebral blood flow 10-21 days after MDMA administration in a prospective controlled clinical trial. Decreased cerebral blood flow in ecstasy users could potentially produce a lowering in apparent SERT density. Because decreases in regional cerebral blood flow were not detectable in two subjects who were measured at 43 or 80 days after drug administration, this phenomenon may be an important confounding variable in PET studies only when MDMA exposure has occurred in the last few weeks. Thus, a normalizing of cerebral blood flow may explain Semple et al's (1999) dramatic correlation (see Figure 3 in Semple's paper) between apparent SERT density and time since last ecstasy use (which was an average of 18 ± 8 days). It does not appear that reduced rCBF can explain the findings of McCann et al. (1998) since time from last ecstasy exposure was reportedly an average of 19 (range: 3-147) weeks in that study.

An additional issue is that neither PET study controlled for genetic influences on SERT density. Polymorphisms in the SERT gene and encoder regions have been reported (Heils et al. 1996; Lesch et al. 1996) and may be associated with anxiety-related traits (Lesch et al. 1996) and vulnerability to drug abuse (Heinz and Jones 2000). These genetic variations influence the expression and activity of the SERT. For example, a

postmortem study of suicide victims and controls reported that the density (Bmax) of [3H]-paroxetine-labeled SERT in the prefrontal cortex of individuals who were homozygous or heterozygous for the long allele variant of the SERT gene was only about 46% of individuals who were homozygous for the short allele (Du et al. 1999). Although studies on this issue have found conflicting results, it would appear helpful in future retrospective studies of ecstasy users to confirm that genotype distributions are in Hardy-Weinburg equilibrium within each study group.

In summary, one small, still unpublished, prospective study did not find changes in estimated SERT density after up to 1.7 mg/kg MDMA. On the other hand, SERT binding appears modestly lower in ecstasy users than nonusers. One study suggests that there is rapid recovery of SERT density while another finds no such evidence. There is some doubt that SERT binding is being accurately measured. As discussed earlier and depicted in **Table 5.2**, a nonhuman animal study also suggests that PET measures may be somewhat insensitive to SERT changes.

Neuroendocrine Measures of Serotonergic Functioning. Alterations in neuroendocrine response to $5HT_{1A}$ agonist 8-OH-DPAT have been reported in rats at 2 weeks after a non-neurotoxic dose (2.0 mg/kg) of MDMA (Poland 1990), suggesting that such neuroendocrine changes are not necessarily evidence of neurotoxicity. Altered neuroendocrine response to serotonergic drugs has also been shown in a variety of psychiatric disorders and may be related to personality. For example, humans with high scores on a measure of "sensation seeking" have been shown to have blunted neuroendocrine response to the partial $5HT_{1A}$ agonist ipsapirone (Netter et al. 1996). Thus, altered neuroendocrine response in ecstasy users cannot be assumed to have been caused by MDMA exposure nor can it be considered a measure of toxicity. Despite these caveats, there is evidence that psychostimulant abuse can lead to alterations in the neuroendocrine response to serotonergic drugs. For example, the prolactin response to fenfluramine in a group of cocaine-dependent individuals was significantly increased between the first and third weeks after discontinuing cocaine use (Buydens-Branchey et al. 1999), suggesting a drug-induced decrease followed by recovery.

A number of studies have measured the neuroendocrine response to serotonergic compounds as a method of probing the serotonergic systems of ecstasy users. Former ecstasy users (with confirmed 12 months of abstinence from ecstasy) in substance abuse treatment programs have been found to differ from healthy nondrug users in the neuroendocrine response to the serotonin releaser, d-fenfluramine (Gerra et al. 2000). Despite obvious limitations in comparing individuals in treatment to healthy controls, these results are consistent with two other studies reporting decreased neuroendocrine response to serotonergic drugs in ecstasy users (McCann et al. 1999a; Verkes et al. 2001). In an earlier study, Price et al. (1989) reported a nonsignificant trend towards decreased response to L-tryptophan in ecstasy users whom had been preselected for low CSF 5HIAA compared to control volunteers (R. Doblin, personal communication). A subsequent report also failed to detect effects of ecstasy exposure on the neuroendocrine response to L-tryptophan (McCann et al. 1994). The findings of these studies differ somewhat from the nonhuman animal literature. The prolactin response to dl-

fenfluramine in rats is reportedly enhanced at 4 months and normal at 12 months after a neurotoxic MDMA regimen (Poland et al. 1997).

Serotonin-Related EEG Differences. Tuchtenhagen et al. (2000) reported that ecstasy users had increased stimulus dependence for event related potential EEG N1/P2 amplitudes, a measure that may indicate low serotonergic activity. The selectivity of this measure for serotonergic changes was cast in doubt by one study that was unable to find a significant effect of acute 5HT depletion (achieved with acute dietary tryptophan depletion) on the stimulus dependence of auditory evoked potentials (Dierks et al. 1999). Thus, the difference between ecstasy users and controls may not reflect a specific serotonergic difference, although it undeniably represents a neurofunctional difference. Results of two other studies investigating EEG differences between users and nonusers are discussed below.

$5HT_{2A}$ Receptor Density Differences. A recent study by Reneman et al. (2000a; Reneman et al. 2000b) suggested that ecstasy users have altered $5HT_{2A}$ receptor density in the cortex. While the first published report from this study suggested increased $5HT_{2A}$ receptors in the occipital cortex of five ecstasy users, the second published report from the same group suggested that cortical $5HT_{2A}$ receptors may be initially decreased then increased in ecstasy users. Although not reported in either paper, increased $5HT_{2A}$ density correlated with the extent of previous ecstasy exposure in the five ecstasy users whose data appeared in both papers (Reneman, personal communication to R. Doblin).

In contrast, animal studies have found evidence of only transient changes in $5HT_{2A}$ receptor density after serotonergic neurotoxicity. A rodent study found that neurotoxic MDMA exposure was associated with a transient decrease in $5HT_{2A}$ receptor density, which normalized by 21 weeks (Scheffel et al. 1992). In another study (Granoff and Ashby 1998), researchers were unable to detect any change in a behavioral measurement of central $5HT_{2A}$ functioning (DOI-induced head twitch and locomotion) in rats one month after a neurotoxic regimen of MDMA. Finally, serotonergic neurotoxicity achieved using dihydroxytryptamine (DHT) has generally not been found to cause alterations in $5HT_{2A}$ receptors (Compan et al. 1998; Fischette et al. 1987). These studies suggest that changes in $5HT_{2A}$ receptor density may not be the result of neurotoxicity, but may be a response to the pharmacological effects of MDMA.

Other (Possibly Nonserotonergic) Neurofunctional Differences between Ecstasy Users and Nonusers

If much of the evidence of serotonergic differences between frequent illicit ecstasy users and nonusers can be disputed, it is only because the measurements may not be specific to serotonergic toxicity and may reflect some more general neurofunctional differences (which may or may not be caused by MDMA exposure). There is strong evidence of such neurofunctional differences between ecstasy users and nonusers. These reported neurofunctional differences are summarized in **Table 5.4**.

Cerebral Blood Flow and Volume Differences. In the retrospective study discussed above, Reneman et al. (2000a, 2000b) recently reported preliminary data that two ecstasy users had increased regional cerebral blood volume (rCBV) in some areas compared to three ecstasy users with more recent exposure and six nonuser volunteers. In contrast, two larger studies have failed to find significant regional cerebral blood flow (rCBF) differences between users and nonusers (Chang et al. 2000; Gamma et al. 2001). Thus, there is mixed evidence for lasting alterations in rCBF/V.

More compelling evidence of rCBF changes after MDMA use comes from a prospective clinical study measuring rCBF before and after volunteers were administered MDMA. As mentioned above, Chang et al. (2000) reported that 8 volunteers had decreased cerebral blood flow in several brain regions 10-21 days after participating in a study employing two oral doses of 1.0 to 2.5 mg/kg MDMA given two weeks apart. Apparent increases in rCBF were found in an additional 2 volunteers who were measured 43 and 80 days, respectively, after MDMA, suggesting that the decreases in rCBF are not lasting or that a biphasic response with decreases followed by increases may have occurred.

Given this evidence of short-term rCBF decreases, the conflicting results in the retrospective studies may be due to the different ecstasy exposure patterns for the volunteers, with short-term decreases in rCBF influencing the results. The use patterns and time from last exposure to ecstasy are not described for the five ecstasy users in the Reneman study. However, the larger groups of 10 recent and 5 less recent users (called "ex-users" in the paper) from which these volunteers were selected had reportedly been last exposed to ecstasy an average of 1.75 ± 1.25 and 4.5 ± 3.75 months previously, respectively. Thus, some of the volunteers had reported abstinence times in which decreases are expected (i.e., 2 weeks). The abstinence times in the Reneman et al. study are less than those of the larger study by Chang et al. (1999) which examined 21 nonusers and 21 ecstasy users with times from last ecstasy exposure of 6.6 ± 7.7 (0.5-26) months and failed to detect any differences. In total, the overall evidence appears to suggest that these rCBF/V changes are not persistent, although further research is needed.

Possible late increases in CBF are consistent with the animal literature. McBean et al. (1990) examined young rodents for changes in CBF 6 to 9 weeks after exposure to a neurotoxic MDA regimen and found significant increases in CBF and the ratio of CBF-to-cerebral glucose utilization in some regions. In a subsequent report, the same investigators described enhanced cerebrovascular responsiveness to hypercapnia (excessive carbon dioxide) in the frontal cortices of similarly treated animals (Kelly et al. 1994). Given the reportedly separate regulation of cerebral blood flow from peripheral cardiovascular functioning, it seems unlikely that this phenomenon is related to the altered cardiovascular regulation reported in ecstasy users by Brody et al. (1998). Decreased cerebral blood flow has been previously noted in cocaine abusers (Volkow et al. 1988) with some limited evidence of abstinence-related recovery (Herning et al. 1999; Holman et al. 1993; Kosten et al. 1998).

Table 5.4: Reported Neurofunctional Differences Between Ecstasy Users and Nonusers

Measure	Selective for Serotonergic Differences?	Relevant Animal Literature?	Correlated with MDMA Exposure?	Evidence for Recovery?	References
Putative Serotonergic Measures					
Decreased CSF 5HIAA in 3 of 4 studies	Yes	**Decreased** up to 2 weeks after MDMA in squirrel monkeys (Ricaurte et al., 1988) and 14 weeks after MDMA in rhesus monkeys (Insel et al., 1989).	No	No	Decreased in McCann et al.,1999, 1994; Ricaurte et al.,1990. Unchanged in Peroutka et al., 1989
Decreased then **Increased** $5HT_{2A}$ receptor density in 1 of 1 study	Yes	**Decreased** at 24 hr, **normal** at 21 d after MDMA in rats (Scheffel et al., 1992).	Yes	Not reported	Increased in Reneman et al., 2000a; Decreased then increased in Reneman et al., 2000b
Decreased neuroendocrine response to serotonergic drugs, in 4 of 6 studies	Yes	**Increased** at 2 months, normal at 12 months in rats (Poland et al. 1997)	Yes in Gerra et al., 2000; Verkes et al., 2001.	No	Decreased in Gerra et al., 2000, 1998; McCann et al., 1999a. Unchanged in Price et al., 1989; McCann et al., 1994.
Decreased SERT density, estimated with PET, in 2 of 2 studies	Disputed - ligand kinetics may be altered by other changes (Kuikka & Ahonen 1998).	PET measures apparently **decreased** in one baboon up to 14 weeks after MDMA (Scheffel et al., 1998).	Yes, though McCann included controls.	Mixed (Yes in Semple; No in McCann)	Semple et al., 1999; McCann et al., 1998.
Increased stimulus dependence for ERP EEG N1/P2 amplitudes in 1 of 1 study	Disputed – 5HT depletion did not change measure in one study (Dierks et al., 1999).	Unknown	No	Not reported	Tuchtenhagen et al., 2000
Nonspecific Neurofunctional Measures					
Increased brain myo-inositol measured as by 1H MRS in 1 of 1 study	-	Unknown	Yes	Not reported	Chang et al., 1999
Altered sleep patterns in 2 of 2 studies	-	Unknown	No	Not reported	Allen et al., 1993; McCann et al., 2000.
Altered cerebral blood flow or volume in 2 of 4 studies	-	**Increased** blood flow 6-9 wks after MDA in rats (McBean et al., 1990)	Yes	Yes	Altered in Reneman et al., 2000a,b and in Chang et al., 2000 (prospective study). Unchanged in Chang et al., 2000 (user-nonuser study) and Gamma et al., in press.
Decreased cerebral glucose utilization in 1 of 1 study	-	**Increased** in some hipppocampal areas 2 wks (Sharkey et al., 1991) after MDMA and 6-9 wks after MDA (McBean et al., 1990) in rats	No	No	Obrocki et al., 1999
Increased alpha and beta EEG power in 2 of 2 studies	-	Unknown	Yes	Not reported	Dafters et al., 1999; Gamma et al., 2000

Electroencephalogram Differences. In addition to the report by Tuchtenhagen (2000) discussed above, two other studies have looked for evidence of EEG alterations in ecstasy users (Dafters et al. 1999; Gamma et al. 2000b). Both studies reported increased alpha and beta EEG power in ecstasy users. Although no control group was included in the report by Dafters et al., the authors detected a significant association between ecstasy use in the previous year and EEG measures. Whether these apparent changes reflect neurotoxicity (as suggested by Dafters et al.) or nontoxic functional differences (a possibility raised by Gamma et al.) is unclear. Average time from last ecstasy exposure was not reported in these studies. Dafters et al. state that no volunteers reported use within 7 days. Thus, it is also not clear to what extent short-term changes are being detected. Beta increases have been reported 4 to 15 days from last exposure in cocaine dependent individuals and correlated with 30 day cocaine exposure in one study (Herning et al. 1997), suggesting a possible role for short-term effects in these changes. However, the fact that Dafters et al. found that EEG measures correlated with ecstasy use in the previous year (which was 14.04 (1-60) tablets) suggests that long-term changes may have contributed to the findings. Gamma et al. reported increased depressiveness and anxiety (measured using self-report EWL ratings) in the ecstasy users, which may be related to the EEG changes. Both alpha and beta increases have been reported in depressed individuals (Pollock and Schneider 1990), while increased alpha power is generally associated with decreased brain activation.

Sleep Differences. Allen et al. (1993) reported that 23 ecstasy users with 79.4 (25-300) previous exposures had decreased total sleep time, NREM sleep time, and stage 2 sleep time in comparison to nonusers. For example, ecstasy users slept approximately 20 minutes less than nonusers. Twelve of the 23 ecstasy users but only 5 of the 22 nonusers had traveled from two or three time zones to participate in the study. However, the authors reported that the differences were still significant when these possible jetlagged volunteers were removed. In a recent review of evidence for MDMA-induced neurotoxicity in humans, McCann et al. (2000) referred to a currently unpublished study of ecstasy users that found increased sleep efficiency and sleep time with specific increases in stage 3 and stage 4 sleep. These increases in stage 3 and stage 4 sleep are somewhat consistent with nonsignificant trends toward increases in the Allen et al. study. However, there seems to be no consistent pattern of changes in sleep efficiency or sleep time in ecstasy users.

Changes in sleep architecture are not necessarily caused by serotonergic neurotoxicity. Evidence for this claim comes from rat locomotor studies and a study of chronic amphetamine users. One study found that rats had decreased diurnal and nocturnal locomotor activity at 7 to 14 days after a neurotoxic MDMA regimen (Wallace et al. 2001). One possible explanation for this is that serotonergic neurotoxicity led to the dysregulation of the sleep-wake cycle. However, the rats appeared to have a generalized decrease in locomotor activity that is more consistent with drug withdrawal than sleep-wake alterations. Decreased locomotor activity has been reported in rats that have been sensitized to non-neurotoxic doses of *d*-amphetamine (Robinson and Camp 1987). DHT lesions in a number of brain areas produce hyperactivity (Williams and Azmitia 1981),

while selective dorsal raphe lesions do not alter activity (Geyer et al. 1976). In a study of 6 female volunteers in withdrawal from chronic amphetamine use, researchers found changes in sleep architecture lasting 3 to 8 weeks (Oswald and Thacore 1963). Because amphetamine does not produce serotonergic neurotoxicity, this suggests that long-lasting changes in sleep architecture may be produced in chronic ecstasy users by mechanisms other than serotonergic neurotoxicity.

Magnetic Resonance Spectroscopy Differences. Proton Magnetic Resonance Spectroscopy (MRS) was used by Chang et al. (1999) to compare cerebral metabolite concentrations in ecstasy users and nonusers. N-acetyl-aspartate concentrations, an indicator of cell damage or death, which is sensitive to the effects of methamphetamine abuse (Ernst et al. 2000), were unchanged. However, myo-inositol (MI) concentrations were raised in the parietal white matter of ecstasy users. This appears to be related to ecstasy exposure since the increase correlated with the logarithm of lifetime ecstasy use. MI is found in high concentration in astrocytes where it appears to act as an osmolyte (a regulator of cell volume). Increased MI therefore suggests possible astrocyte proliferation in ecstasy users. This may be the result of neurotoxic insult (chemical markers of glial activation are used as measures of neurotoxicity). This possibility cannot currently be excluded. However, in an earlier report, Chang et al. (1997) found a similar degree of MI elevation in abstinent cocaine users, suggesting that elevated MI may be a nonspecific effect of psychostimulant (including MDMA) use and not necessarily linked to serotonergic damage. Chang et al. point out that *in vitro* research has demonstrated that MDMA and 5HT can increase glial glycogen phosphorylase activity (Poblete and Azmitia 1995), indicating the possibility of non-neurotoxic effects of MDMA on glial processes. Because $5HT_{2A}$ receptors are expressed on astrocytes and increased by astrocyte activation (Wu et al. 1999), it is intriguing to speculate that the preliminary report of increased $5HT_{2A}$ expression in ecstasy users (Reneman et al. 2000a) may also be related to glial activity.

Cerebral Glucose Utilization Differences. Changes in local cerebral glucose utilization have also been reported in ecstasy users with most recent ecstasy exposures ranging from 2 to 16 months ago (Obrocki et al. 1999). Using FDG-PET, significant decreases were noted in the left hippocampus with trends towards decreases in some other brain regions. Studies using rats have reported increases rather than decreases in glucose utilization. Increased glucose utilization in the hippocampus and several other brain regions was detected in rats 6-9 weeks after neurotoxic MDA exposure (McBean et al. 1990). Similarly, increased glucose utilization was found in some hippocampal areas in rats treated 14 days earlier with neurotoxic MDMA regimens (Sharkey et al. 1991). Examined by brain region, these changes did not seem to correlate with SERT changes, and thus may be a response to the serotonergic changes in other areas. It has been reported that local cerebral glucose utilization was not altered in adult rats at 3 weeks after neurotoxic 5,7-DHT exposure, despite an 80% decrease in 5HT levels (Cudennec et al. 1988). This suggests that serotonin neurotoxicity per se may not cause local cerebral glucose utilization alterations.

Mood, Personality, Behavioral, and Neurocognitive User-Nonuser Differences

Mood Differences. Ecstasy use has been associated with increased self-reported dysphoria in comparisons between ecstasy users and nonusers and surveys of users. This dysphoric mood appears to peak several days after ecstasy use (Curran and Travill 1997; Liechti and Vollenweider 2000b; Parrott and Lasky 1998; Vollenweider et al. 1998a), but is likely more lasting in chronic users. Because dysphoria is a known residual effect of psychostimulant drugs, it is likely that ecstasy use plays a causal role in this phenomenon. **Table 5.5** summarizes studies of mood in ecstasy users and comparison groups. Interestingly, mood alterations are not always seen in chronic ecstasy users, despite serotonergic or neurofunctional differences in some cases (Krystal et al. 1992; McCann et al. 1994; Verkes et al. 2001).

The most extensive characterization of mood and psychological differences between illicit ecstasy users and nonusers was carried out by Parrott et al. (2000). Using several self-report instruments (the SCL-90R, Eysenck's IVE, and an Uplifts/Hassles questionnaire), the authors found significant increases in dysphoric mood and reported psychological problems in a group of poydrug users with an average of 371 (30-1000) ecstasy exposures compared to a group of ecstasy-naïve volunteers. In contrast, less experienced ecstasy (and polydrug) users with an average of 6.8 (1-20) ecstasy exposures differed from nonusers in only two SCL-90R subscales (psychoticism and paranoid ideation), although their scores tended to be nonsignificantly higher than nonusers in other measures as well. The primary limitation of this study is that time from last drug exposure was not recorded (volunteers had reportedly not used any drugs on the day of assessment). This is a particularly important point because the standard instructions on the SCL-90R ask volunteers to report how they have felt in the last week, a time period during which the volunteers may have used drugs. Thus, it is not clear to what extent residual effects of a number of different drugs were detected rather than chronic ecstasy effects.

Current research cannot adequately address whether ecstasy use is associated with increased risk of mood or anxiety disorders, such as major depression. Case reports (discussed in a subsequent chapter) have described severe depressive episodes and anxiety disorders that seemed to be associated with ecstasy use. However, it is impossible to distinguish between ecstasy triggering symptoms in vulnerable individuals and ecstasy creating problems de novo. A review of case reports of psychiatric complications after ecstasy use concluded that there was insufficient evidence to conclude that ecstasy use was a main responsible factor for the reported psychiatric symptoms (Bango et al. 1998). Similarly, Curran noted that the majority of reports concerning ecstasy-related psychiatric complications involve polydrug users who are self-referred to psychiatric or drug abuse services (Curran 2000). These individuals may not be representative of the broader population of ecstasy users. However, given the apparent role of serotonergic dysfunction in affective disorders, further research comparing abstinent ecstasy users and properly matched controls is warranted.

Table 5.5: Mood Alterations in Ecstasy Users

Ecstasy Use (Mean ± SD)				Different from comparison group in…			
Frequency (per mo)	Exposures (pills or occasions)	Duration (mo)	Time Since Last Use (days)	Mood?	Anxiety?	Anger/ Hostility?	Reference
..	6.8	..	..	N	..	..	Parrott et al., 2000
..	1 to 9	..	7	N	..	..	Parrott and Lasky, 1998
..	>10	..	7	N (VAS Mood)	..	..	Parrott and Lasky, 1998
..	271	..	..	N	Y (SCL90)	Y (SCL90)	Parrott et al., 2000
..	222 ± 358.4	..	> 7	Y (HAMD)	N (EWL)	..	Gamma et al., 2000
..	235	51.6 ± 31.2	79	N (BDI)	..	..	Klugman et al, 1999
..	270 ± 397.2	..	..	Y (EWL, HAMD)	N (EWL)	..	Gamma et al, 2000
0.5	73 ± 68	52.8 ± 28.8	15.7 ± 9.5	N (BDI)	N (STAI-DY)	N (BDHI)	Verkes et al, 2001
1.9 ± 1.7	..	61 ± 27.6	66 ± 50	N (BDI, HAMD)	..	..	Krystal et al, 1992
2.94 ± 0.93	35.6 ± 17.5	25.44 ± 16.32	20.4 ± 33.6	N (Mood Likert)	N (STAI)	N (STAXI)	Morgan, 1998b
3	230 ± 170	54 ± 21.6	9.0 ± 7.5	N (BDI)	N (STAI-DY)	N (BDHI)	Verkes et al, 2001
4.16 ± 4.79	94.4 ± 90.6	59.7 ± 35.52	125.3 ± 172.9	..	..	N (BDHI)	McCann et al., 1994
4.36 ± 1.15	49.6 ± 33.2	49.4 ± 15.2	65.1 ± 85.7	Y (GHQ)	..	..	Morgan, 1998b
4.7 ± 2.7	62.7 ± 34.2	14 ± 8	21	Y (HAMD, MMPI-D)	..	Y (BDHI)	Gerra et al., 1998
4.7± 2.7	69.3 ± 38	15 ± 9	365	Y (HAMD, MMPI-D)	..	N (BDHI)	Gerra et al., 2000
5 ± 1	196 ± 24	60 ± 36	98 ± 203	N (Lader Mood, VAS); Y (NIMH Sympton)	N (NIMH Panic)	..	McCann et al, 1999
8.43 ± 3.33	..	49.2 ± 16.44	8.20 ± 5.75	..	Y (Anxiety Arousal)	..	Wareing et al., 2000

Table was adapted from Morgan (2000).
Abbreviations: BDHI = Buss-Durkee Hostility Index; GHQ = General Health Questionnaire; HAMD = Hamilton Depression; MMPI-D = Minnesota Multiphasic Personality Inventory depression subscale; NIMH Panic = National Institute of Mental Health Self-Report Panic Scale; NIMH Symptom = National Institute of Mental Health Self-Report Symptom Scale; SCL90 = Symptom Check List 90; STAXI = State Traits Anxiety Scale; VAS Mood = Visual Analog Mood Scale.

Impulsivity and Other Personality Differences. Studies yield some mixed evidence that ecstasy use may be associated with increased impulsivity. It is not yet clear to what extent this represents a preexisting trait in individuals vulnerable to frequent ecstasy ingestion, a value espoused by the "rave culture" with which ecstasy is associated, or an effect of ecstasy exposure. It should be noted that, within limits, impulsivity is not itself an indicator of psychopathology (although research has examined links between impulsivity and the tendency to use drugs). Self-report measures of impulsivity include items that are likely to be viewed as positive traits by some.

Many of the personality differences between ecstasy users and control volunteers who do not use illicit drugs likely reflect preexisting differences. Increased novelty-seeking (Gerra et al. 1998), venturesomeness and impulsivity (Morgan 1998) can be expected in users of illicit drugs compared to nonusers. This interpretation has been advanced by several authors including Gerra et al. (2000) who suggested that the enhanced Novelty Seeking (measured with the self-report Tridimensional Personality Questionnaire) in ecstasy users undergoing substance abuse treatment reflected a preexisting psychobiological trait. Similarly, the increased Buss-Durkee Hostility Index (BDHI) Direct Aggression scores of ecstasy users in substance abuse treatment (Gerra et al. 2000) and the decreased BDHI Indirect Hostility in untreated ecstasy users (McCann et al. 1994) may be partially explained by social circumstances and subcultural values, respectively.

Comparisons of (1) ecstasy users with different total ecstasy exposures or (2) polydrug users with and without ecstasy experience can provide a potentially stronger basis for concluding that ecstasy use is specifically associated with certain personality traits. There is only limited support for this possible conclusion. Morgan (1998) reported that a post-hoc comparison of more (30+ tablets ingested) and less experienced (20 – 30 tablets ingested) ecstasy users revealed heightened Impulsivity (measured with Eysenck's self-report IVE questionnaire) in the more experienced group. Parrott, Sisk, and Turner (2000) reported a nonsignificant trend towards greater IVE Impulsivity in polydrug-using ecstasy users with an average of 371 (30-1000) exposures compared to a group of users with an average of 6.8 (1-20) exposures. Tuchtenhagen et al. (2000) found that ecstasy users with an average of 93.4 ± 119.9 (20-500) exposures have significantly higher scores for Nonplanning Impulsivity (measured with the self-report Barratt Impulsiveness Scale) compared to controls matched for other drug use. The researchers also noted a trend towards increased Experience Seeking (measured with the self-report Sensation Seeking Scale) that reached statistical significance only when ecstasy users were compared to nondrug users. These findings differ from those of McCann et al. (1994) who compared ecstasy users with an average of 94.4 ± 90.6 (25-300) exposures compared to nonusers (but did not control for other drug use). McCann et al. reported decreased Impulsivity (measured as increases in the Control subscale of the Multidimensional Personality Questionnaire) but failed to find significant differences in self-reported Impulsivity with a second questionnaire (the self-report Eysenck Personality Questionnaire). Thus, there is mixed evidence that ecstasy use is associated with increases in self-reported impulsivity.

There are less data and consistency in findings on behavioral impulsivity, which is thought to be different from self-reported impulsivity (Evenden 1999). Gouzoulis-Mayfrank et al. (2000), using the same volunteers as in the Tuchtenhagen et al. (2000) report, did not find evidence of behavioral impulsivity in ecstasy users undergoing a cognitive test battery. In contrast, Morgan (1998) reported that ecstasy users made increased errors in a Matching Familiar Figures task, a difference he interpreted as evidence of increased impulsivity. Morgan suggested that his behavioral findings were an indication of decreased capacity to cope with high levels of cognitive demands. The evidence for such impairments is discussed below.

Neurocognitive Differences between ecstasy users and nonusers

As summarized in **Table 5.6**, repeated ecstasy exposure is associated with decreased performance on measures of neurocognitive function. Tests of verbal memory have been frequently used to detect this decrease (Gouzoulis-Mayfrank et al. 2000; Morgan 1999; Parrott 1998; Parrott and Lasky 1998; Reneman et al. 2000a). The results of these tests are summarized in **Table 5.7** and **5.8**. However, user-nonuser differences have been detected with a broad range of neurocognitive tasks (Gouzoulis-Mayfrank et al. 2000; McCann et al. 1999b; Rodgers 2000), including tests of executive function, visual memory, selective attention, and logic. Some have suggested that specific alterations in executive function and working memory may explain the observed differences (Dafters et al. 1999; Gouzoulis-Mayfrank et al. 2000; Wareing et al. 2000), but evidence for this is not conclusive. Results from tests of executive functioning are summarized in **Table 5.9**. The possibility that ecstasy users are impaired in a specific area of neurocognitive functioning is discussed below.

Table 5.6: Summary of Significant Neurocognitive Findings

Function	Significant group differences found in:
Executive Function	22.8% (13/57) of measures in 11 studies
Memory:	42.9% (30/70) of measures in 13 studies
Verbal	55.8% (24/43) of measures in 13 studies
Visual	16% (4/25) of measures in 7 studies
Attention	23.7% (9/38) of measures in 7 studies
Information Processing	22.2% (2/9) of measures in 3 studies
Logic / Problem Solving	50% (3/6) of measures in 1 study
Psychomotor Speed	14.3% (1/7) of measures in 3 studies
Intelligence	50% (3/6) of measures in 2 studies
Time Estimation	0% (0/3) of measures in 1 study

Tests were categorized as measuring one of the above neurocognitive functions. However, no test relies entirely on one area of functioning and other categorization schemes are possible.

Correlations across studies. In order to explore possible relationships between ecstasy exposure and neurocognitive performance, we correlated average performance in different studies with average time since last ecstasy use (in days), duration of ecstasy use (in months), frequency of ecstasy use (in times per month), average dose per use (in tablets) and number of exposures (in occasions or tablets). Each ecstasy exposure parameter was separately correlated with performance. Executive function and verbal memory were selected for analysis from the various areas of neurocognitive performance because these areas had been most frequently assessed. Neurocognitive performance of current ecstasy users was normalized in comparison to performance of the volunteer group in each study having the least drug use. Only one measure per volunteer group was used in each analysis. When a published paper appeared to have more than one test assessing executive function or verbal memory, the test finding the largest difference between ecstasy users and nonusers was included in the analysis. Correlations were individually performed. No corrections were made for multiple correlations.

These analyses are limited by small sample sizes, the inaccuracy of retrospective reporting of drug use, the varying contents of ecstasy pills, and because ecstasy exposure parameters are not independent. Furthermore, findings of significant group differences are more likely to be published and therefore be available for analysis. Volunteers using ecstasy more frequently also had a greater number of lifetime exposures to ecstasy and took higher average doses (in tablets) per use. Given the small sample sizes in these analyses, it is not clear whether decreased performance is more closely associated with frequent use, greater number of exposures, or higher dose. In theory, multiple regression could be used to address this issue. However, ecstasy exposure parameters are inconsistently reported in individual studies and very few studies present enough parameters to be used in such a multiple regression.

Selected results of executive function correlations are displayed in **Figures 5.2** and **5.3**. Executive function was significantly and negatively correlated with number of ecstasy exposures (occasions or tablets) ($r = -0.633$, $p = 0.049$, $n = 10$) and approached significance with time since last use ($r = 0.652$, $p = 0.057$, $n = 9$). The relationships between executive function and ecstasy exposure parameters are consistent with ecstasy use decreasing performance and recovery in performance occurring with abstinence. Verbal memory performance was not correlated with any measure of ecstasy exposure.

In addition, we conducted individual correlations between neurocognitive performance and volunteer gender distribution, age, and education. Neurocognitive performance was unrelated to the age, or education of either ecstasy users or nonusers. Performance on tests of executive function was positively correlated with proportion of female ecstasy users ($r = 0.625$, $p < 0.05$, $n = 11$). However, it was found that number of total lifetime exposures ($r = -0.72$, $p < 0.05$, $n = 11$) and average dose per use ($r = -0.837$, $p < 0.05$, $n = 7$) were negatively correlated with proportion of female ecstasy users. There was a trend for duration of use to be negatively correlated with proportion of women participating in a study as well ($r = -0.613$, $p = 0.06$, $n = 10$). This suggests that the relationship between gender and performance on these measures arose because women in these studies

Table 5.7: Immediate Verbal Memory in Ecstasy Users Compared to Nonusers.

Test	Users/ Non-Users N	Memory (Z-scores) Mean	SD	Days From Last Use Mean	SD	Number Of Uses Mean	SD	Reference
WMS Story Recall	15/15	-2.58	0.92	60	..	20	..	Rodgers 2000
RBMT Story Recall	25/19	-0.75	0.78	65.1	85.7	49.6	33.2	Morgan 1999
WMS Story Recall	24/24	-0.59	0.97	30	..	60	..	Bolla et al., 1998
WMS Verbal Memory Index	15/15	-1.82	1.00	60	..	20	..	Rodgers 2000
WMS Verbal Paired Assoc.	15/15	-0.44	1.12	60	..	20	..	Rodgers 2000
WMS Verbal Paired Assoc.	24/24	-0.38	1.71	30	..	60	..	Bolla et al., 1998
Coughlan List B	11/31	-0.44	0.96	> 2	..	41.9	49.3	Croft et al., 2000
VLMT	28/28	-0.88	0.85	41	71.1	93.4	119.9	Gouzoulis-Mayfrank et al., 2000
Word Recall	15/15	-0.95	0.71	7	0	..	..	Parrott & Lasky, 1998
Word Recall	10/10	-1.06	0.81	..	..	..	..	Parrott et al., 1998
Digit Span – Forward and Backwards combined	15/15	-0.24	0.95	60	..	20	..	Rodgers 2000
Digit Span - Forward	11/31	-0.72	0.89	> 2	..	41.9	49.3	Croft et al., 2000
Digit Span - Forward	24/24	-0.18	0.82	30	..	60	..	Bolla et al., 1998
Digit Span - Forward	28/28	-0.27	1.43	41	71.1	93.4	119.9	Gouzoulis-Mayfrank et al., 2000
Digit Span - Forward	10/10	-0.24	0.95	18	8	672	647	Semple et al., 1999

Abbreviations: RAVLT – Rey Auditory Verbal Learning Test. RBMT – Rivermead Behavioural Memory Test. WMS – Weschler Memory Scale, Revised. WRB – Walter Reed Army Institute of Research Performance Assessment Battery.

have taken less ecstasy than men have, and for shorter periods of time. Overall, these analyses suggest that the apparently decreased neurocognitive performance of ecstasy users is not explained by differences in the gender distribution, age, or education of volunteer groups.

Does ecstasy use cause this poor neurocognitive performance? The current data suggest the answer is "yes". A recent study found that 15 polydrug-using ecstasy users decreased in neurocognitive performance over the course of 12 months (Zakzanis and Young 2001). During these 12 months, volunteers used the drug in average of 2.4 times per month. Volunteers did not use ecstasy within two weeks of testing. Performance tended to decrease in all of the neurocognitive tasks used in the study, but the difference was only significant in tests of immediate and delayed verbal memory. Although this study lacked a nonuser comparison group, it suggests that something associated with the lifestyle of these volunteers causes decreased neurocognitive performance.

Table 5.8: Delayed Verbal Memory in Ecstasy Users Compared to Nonusers.

Test	Users/ Non-Users N	Memory (Z-scores) Mean	SD	Days From Last Use Mean	SD	Number Of Uses Mean	SD	Reference
WMS Story Recall	15/15	-2.12	1.32	60	..	20	..	Rodgers 2000
RBMT Story Recall	25/19	-0.82	0.91	65.1	85.7	49.6	33.2	Morgan 1999
WMS Story Recall	24/24	-0.67	0.97	30	..	60	..	Bolla et al., 1998
WMS Verbal Paired Assoc.	15/15	-0.89	1.03	60	..	20	..	Rodgers 2000
WMS Verbal Paired Assoc.	24/24	-0.33	1.67	30	..	60	..	Bolla et al., 1998
Coughlan List6 Word Recall	11/31	-0.63	1.69	> 2	..	41.9	49.3	Croft et al., 2000
RAVLT	24/24	-0.43	0.83	30	..	60	..	Bolla et al., 1998
VLMT	28/28	-0.41	1.70	41	71.1	93.4	119.9	Gouzoulis-Mayfrank et al., 2000
RAVLT	5/9	-2.31	1.89	138	..	218	..	Renemann et al., 2000
Word Recall	10/10	-1.13	0.88	..	..	..	..	Parrott et al., 1998
WRB Code Recall Day 1	22/23	-0.63	0.56	97.37	44.38	215	33	McCann et al., 1999
WRB Code Recall Day 2	22/23	-0.49	1.03	97.37	44.38	215	33	McCann et al., 1999
WRB Code Recall Day 3	22/23	-0.22	1.22	97.37	44.38	215	33	McCann et al., 1999

Abbreviations: RAVLT – Rey Auditory Verbal Learning Test. RBMT – Rivermead Behavioural Memory Test. WMS – Weschler Memory Scale, Revised. WRB – Walter Reed Army Institute of Research Performance Assessment Battery.

In addition, many studies have found that users with more ecstasy exposure perform worse than those with less exposure (Bolla et al. 1998; Dafters et al. 1999; Gouzoulis-Mayfrank et al. 2000; McCann et al. 1999b; Verkes et al. 2001). A certain skepticism concerning these correlations is warranted in some cases, such as when associations are sought between three different measurements of ecstasy exposure and performance on seven tasks and only one significant relationship is found without apparent correction for the twenty-one multiple comparisons (McCann et al. 1999b). More robust relationships between several measures of ecstasy exposure and neurocognitive performance are reported by Gouzoulis-Mayfrank et al. (2000). Also, Bolla et al. (1998) reported that greater previous exposure to ecstasy was associated with decreased immediate verbal memory and delayed visual memory, although decreases were apparently only statistically significant in users with "high" monthly ecstasy exposure (more than 4.4 pills per month). As described above, the trend-level correlation between decreased executive function and frequency of ecstasy use suggests repeated ecstasy exposure is associated with decreased neurocognitive performance. In contrast, the gender distribution, age, and education of volunteers appears unrelated to neurocognitive performance.

Table 5.9: Executive Functioning in Ecstasy Users Compared to Nonusers.

Test	Users/ Non-Users N	Executive Function (Z-scores) Mean	SD	Days From Last Use Mean	SD	Number Of Uses Mean	SD	Reference
Consonants 1 s.-Redun.	10/10	10.24	25.53	376.74	87.4	323.25	130.05	Wareing et al., 2000
Consonants 1 s.-Redun.	10/10	3.63	3.00	414.92	54.9	8.2	5.75	Wareing et al., 2000
Consonants 1 s.-Letters	10/10	-4.51	-1.40	376.74	87.4	323.25	130.05	Wareing et al., 2000
Consonants 1 s.-Letters	10/10	-3.78	3.06	414.92	54.9	8.2	5.75	Wareing et al., 2000
Consonants 1 s.-Vowels	10/10	-2.19	4.72	376.74	87.4	323.25	130.05	Wareing et al., 2000
Consonants 1 s.-Vowels	10/10	-2.13	1.53	414.92	54.9	8.2	5.75	Wareing et al., 2000
Consonants 2 s.-Redund.	10/10	-1.69	4.95	376.74	87.4	323.25	130.05	Wareing et al., 2000
Consonants 2 s.-Redund.	10/10	-0.61	1.58	414.92	54.9	8.2	5.75	Wareing et al., 2000
Consonants 2 s.-Letters	10/10	-12.91	3.63	376.74	87.4	323.25	130.05	Wareing et al., 2000
Consonants 2 s.-Letters	10/10	-6.45	8.94	414.92	54.9	8.2	5.75	Wareing et al., 2000
Consonants 2 s.-Vowels	10/10	2.78	-6.32	376.74	87.4	323.25	130.05	Wareing et al., 2000
Consonants 2 s.-Vowels	10/10	5.32	-3.48	414.92	54.9	8.2	5.75	Wareing et al., 2000
Consonants 4 s.-Redund.	10/10	0.85	1.83	414.92	54.9	8.2	5.75	Wareing et al., 2000
Consonants 4 s.-Redund.	10/10	1.45	5.64	376.74	87.4	323.25	130.05	Wareing et al., 2000
Consonants 4 s.-Vowels	10/10	-1.42	2.95	376.74	87.4	323.25	130.05	Wareing et al., 2000
Consonants 4 s.-Vowels	10/10	-2.99	1.90	414.92	54.9	8.2	5.75	Wareing et al., 2000
Digit Span Backwards	11/31	-0.59	1.18	41.8	49.3	2	-	Croft et al., 2000
Digit Span Backwards	28/28	-0.79	0.88	93.4	119.9	41	71.1	Gouzoulis-Mayfrank et al., 2000
Digit Span Backwards	10/10	-0.90	1.14	672	647	18	8	Semple et al., 1999
Match to Sample 1	22/23	0.31	1.09	215	33	97.37	44.38	McCann et al., 1999
Match to Sample 2	22/23	0.37	1.20	215	33	97.37	44.38	McCann et al., 1999
Match to Sample 3	22/23	-0.15	0.50	215	33	97.37	44.38	McCann et al., 1999
Spatial Span-Blocks	21/20	-0.85	0.00	73	68	15.7	9.5	Verkes et al., 2001
Spatial Span-Blocks	21/20	-0.69	0.92	230	170	9	7.5	Verkes et al., 2001
Spatial Span-Computer	15/15	-0.34	1.23	20	-	60	-	Rodgers, 2000
Spatial Span-Computer	16/16	0.13	0.76	35.6	17.5	20.4	33.6	Morgan, 1998 Study 1
Spatial Span-Computer	28/28	-0.21	0.37	93.4	119.9	41	71.1	Gouzoulis-Mayfrank et al., 2000

Table 5.9 continued: Executive Functioning in Ecstasy Users Compared to Nonusers.

Test	Users/ Non-Users N	Executive Function (Z-scores) Mean	SD	Days From Last Use Mean	SD	Number Of Uses Mean	SD	Reference
Spatial Span Errors-Computer	16/16	-0.23	0.83	35.6	17.5	20.4	33.6	Morgan, 1998 Study 1
Spatial Span Errors-Computer	10/10	-0.98	2.47	672	647	18	8	Semple et al., 1999
Spatial Span plus one-Blocks	21/20	-0.82	0.00	73	68	15.7	9.5	Verkes et al., 2001
Spatial Span plus one-Blocks	21/20	-0.64	0.91	230	170	9	7.5	Verkes et al., 2001
Spatial Span Secs.-Computer	10/10	-1.81	4.46	672	647	18	8	Semple et al., 1999
Spatial Span Usage Err-Computer	16/16	-0.50	2.15	35.6	17.5	20.4	33.6	Morgan, 1998 Study 1
Sternberg Figure Serial	21/20	-1.18	0.68	73	68	15.7	9.5	Verkes et al., 2001
Sternberg Figure Serial	21/20	-1.04	1.29	230	170	9	7.5	Verkes et al., 2001
Sternberg Figure Simult.	21/20	-1.28	-0.24	73	68	15.7	9.5	Verkes et al., 2001
Sternberg Figure Simult.	21/20	-0.93	1.17	230	170	9	7.5	Verkes et al., 2001
Sternberg Word Serial	21/20	-2.17	0.33	73	68	15.7	9.5	Verkes et al., 2001
Sternberg Word Serial	21/20	-0.75	1.50	230	170	9	7.5	Verkes et al., 2001
Sternberg Word Simult.	21/20	-0.90	0.59	73	68	15.7	9.5	Verkes et al., 2001
Sternberg Word Simult.	21/20	0.00	1.10	230	170	9	7.5	Verkes et al., 2001
Stroop-Errors	10/10	-1.00	2.57	672	647	18	8	Semple et al., 1999
Stroop-Sec	10/10	-0.46	1.36	672	647	18	8	Semple et al., 1999
TOL Excess Moves	16/16	-0.39	1.38	35.6	17.5	20.4	33.6	Morgan, 1998 Study 1
TOL Excess Moves-1	25/19	0.37	0.92	49.6	33.2	65.1	65.3	Morgan, 1998, Study 2
TOL Excess Moves-2	25/19	0.19	-1.11	49.6	33.2	65.1	85.7	Morgan, 1998, Study 2
TOL Initial Think	16/16	-0.08	0.69	35.6	17.5	20.4	33.6	Morgan, 1998 Study 1
TOL Initial Think-1	25/19	-0.69	0.87	49.6	33.2	65.1	85.7	Morgan, 1998, Study 2
TOL Initial Think-2	25/19	-0.67	0.85	49.6	33.2	65.1	85.7	Morgan, 1998, Study 2
TOL Proportion Perfect	16/16	-0.15	1.27	35.6	17.5	20.4	33.6	Morgan, 1998 Study 1
TOL Proportion Perfect-1	25/19	-0.55	0.95	49.6	33.2	65.1	85.7	Morgan, 1998, Study 2
TOL Proportion Perfect-2	25/19	-0.17	0.95	49.6	33.2	65.1	85.7	Morgan, 1998, Study 2
TOL Subsequ. Think	16/16	0.61	1.66	35.6	17.5	20.4	33.6	Morgan, 1998 Study 1
TOL Subsequ. Think-1	25/19	0.31	1.05	49.6	33.2	65.1	85.7	Morgan, 1998, Study 2
TOL Subsequ. Think-2	25/19	-0.11	0.91	49.6	33.2	65.1	85.7	Morgan, 1998, Study 2

Abbreviations: TOL – Tower of London. WMS – Weschler Memory Scale, Revised.

Table 5.9 continued: Executive Functioning in Ecstasy Users Compared to Nonusers.

Test	Users/ Non-Users N	Executive Function (Z-scores) Mean	SD	Days From Last Use Mean	SD	Number Of Uses Mean	SD	Reference
Trails B - Time	10/10	-0.40	1.13	672	647	18	8	Semple et al., 1999
WMS Mental Control	15/15	-0.10	0.63	20	-	60	-	Rodgers, 2000
Word Fluency - Phonologic	11/31	-0.39	0.81	41.9	49.3	2	-	Croft et al., 2000
Word Fluency - Phonologic	28/28	-0.43	0.57	93.4	119.9	41	71.1	Gouzoulis-Mayfrank et al., 2000
Word Fluency - Phonologic	10/10	0.48	1.63	672	647	18	8	Semple et al., 1999
Word Fluency - Semantic	11/31	-1.67	1.24	41.9	49.3	2	-	Croft et al., 2000
Word Fluency - Semantic	28/28	-0.51	1.18	93.4	119.9	41	71.1	Gouzoulis-Mayfrank et al., 2000
Word Fluency - Alternating	28/28	-0.35	0.71	93.4	119.9	41	71.1	Gouzoulis-Mayfrank et al., 2000

Abbreviations: TOL – Tower of London. WMS – Weschler Memory Scale, Revised.

Could the rave lifestyle cause the neurocognitive differences? Probably not. The neurocognitive performance of ecstasy users may be somewhat influenced by aspects of their lifestyle, such as repeated sleep and nutrient deprivation associated with attending late-night dance events. Nonetheless, the few scientific studies on these other possible factors (Cho et al. 2000; Dinges and Kribbs 1991; Kretsch et al. 1997) would not lead us to expect an effect comparable to what we see in studies of ecstasy users. Some studies have tried to control for the influence of lifestyle. Gouzoulis-Mayfrank et al. recruited volunteers (including the drug-free group) at dance events. This would tend to minimize differences in lifestyle, although it is still possible that volunteer groups had differences in the frequency with which they attended dance events. In the first published study that attempted to control for lifestyle (Verkes et al. 2001), researchers found that "moderate" ecstasy users (with 73 ± 68 reported exposures to ecstasy) had lower performance than nonusers attending a similar number of raves in the previous 12 months.

Could other drugs cause these neurocognitive differences? Other drugs may contribute to these differences, but they probably cannot fully account for them. Polydrug use is common in ecstasy users. In published studies, cannabis use has often been greater in ecstasy-using volunteers than in ecstasy-naïve volunteers. This is significant because chronic cannabis use can cause long-lasting residual decreases in neurocognitive performance (Pope and Yurgelun-Todd 1996). Three studies have compared users of both ecstasy and cannabis to users of cannabis alone. Selected results from these studies are shown in **Table 5.10**. Two of these studies have suggested that ecstasy is associated with lowered neurocognitive performance beyond that expected for cannabis (Gouzoulis-Mayfrank et al. 2000; Rodgers 2000). Rodgers et al. (2000) found that cannabis users and cannabis-using ecstasy users performed worse than volunteers who did not use drugs in two tests of delayed recall (Visual Paired Associates and

Figure 5.2: Relationship between Number of Exposures and Executive Function

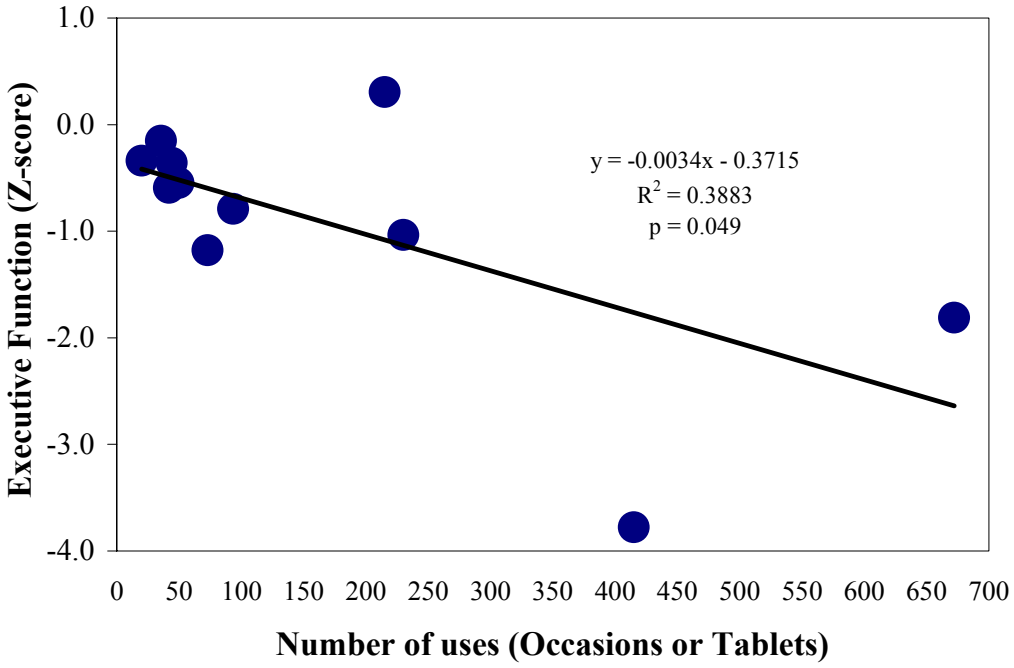


Figure 5.3: Relationship between Abstinence from Ecstasy and Executive Function

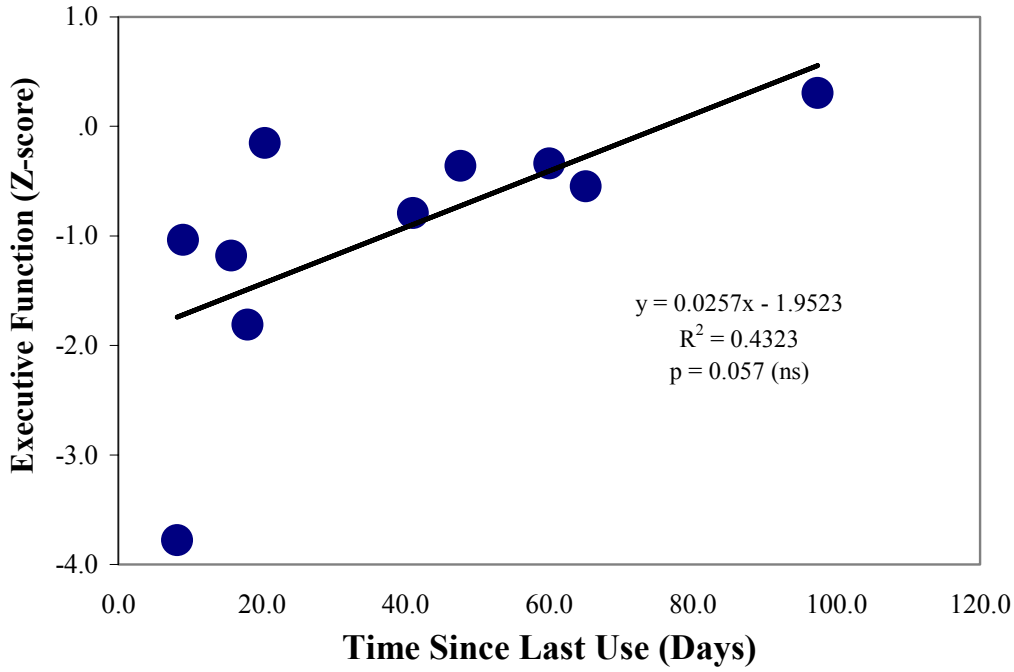


Table 5.10. Evidence for Contribution of Cannabis Use to Neurocognitive Performance in Ecstasy Users.

Measure	Sample Sizes for E&C / C / Nonusers	Users of Ecstasy and Cannabis (E&C) Z-scores		Users of Cannabis (C) Z-Scores		Reference
		Mean	SD	Mean	SD	
Delayed Memory Coughlan Words	11/18/31	-0.63	1.69	-0.87	1.88	Croft et al, 2000
Delayed Memory VLMT	28/28/28	-0.41	1.70	0.21	1.04	Gouzoulis-Mayfrank et al, 2000
Immediate Memory Coughlan Words	11/18/31	-0.44	0.96	-0.74	0.81	Croft et al, 2000
Immediate Memory VLMT	28/28/28	-0.88	0.85	-0.49	0.89	Gouzoulis-Mayfrank et al, 2000
Digit Span	15/15/15	-0.24	0.95	0.48	1.37	Rodgers, 2000
Digit Span Backwards	11/18/31	-0.59	1.18	-0.64	0.82	Croft et al., 2000
Digit Span Backwards	28/28/28	-0.79	0.88	-0.42	0.70	Gouzoulis-Mayfrank et al., 2000
Digit Span Forward	28/28/28	-0.27	1.43	0.03	1.17	Gouzoulis-Mayfrank et al, 2000
Digit Span Forward	11/18/31	-0.72	0.89	-0.50	0.83	Croft et al, 2000
Spatial Span Computer	28/28/28	-0.21	0.37	0.13	0.87	Gouzoulis-Mayfrank et al., 2000
Spatial Span Computer	15/15/15	-0.34	1.23	0.15	0.87	Rodgers, 2000
Word Fluency Phonologic	28/28/28	-0.43	0.57	-0.24	0.55	Gouzoulis-Mayfrank et al, 2000
Word Fluency Phonologic	11/18/31	-0.39	0.81	-0.08	0.96	Croft et al., 2000
Word Fluency Semantic	28/28/28	-0.51	1.18	-0.08	0.84	Gouzoulis-Mayfrank et al, 2000
Word Fluency Semantic	11/18/31	-1.67	1.24	-0.52	1.43	Croft et al., 2000

Abbreviations:VLMT = Verbal Learning Memory Test.
Measures were selected for inclusion if they assessed executive function or verbal memory and if similar measures had been used in other studies. In each case, neurocognitive scores have been normalized to those of nonusers.

Logical Memory). In a third study, another group (Croft et al. 2001) was unable to detect performance differences between cannabis users and users of both cannabis and ecstasy using a battery of neurocognitive tests. Furthermore, covariate analysis suggested that performance decreases were more closely related to cannabis than ecstasy use. In another study that attempted to control for the influence of other drugs, Morgan failed to detect differences between polydrug-using ecstasy users and polydrug users on tests of executive function (Morgan 1998) but detected lower verbal memory performance in the same group of ecstasy users compared to polydrug users (Morgan 1999). However, matching of drug use between comparison groups was imperfect in this study. Furthermore, Morgan found that immediate memory performance correlated as highly with cannabis use ($r = -0.48$, $p = 0.016$) as it did with ecstasy use ($r = -0.47$, $p = 0.019$). It is clear that future studies should control for use of cannabis and that the apparent magnitude of the ecstasy-associated neurocognitive performance decrease is likely exaggerated by cannabis use in studies. But other drug use probably does not fully explain the neurocognitive differences.

Are these changes due to serotonergic neurotoxicity? This is not yet known. The lower neurocognitive performance of ecstasy users may be due to either serotonergic neurotoxicity or some different ecstasy-related neurochemical alteration. Evidence that serotonin is involved is indirect. It has been demonstrated that acute serotonergic depletion (by dietary manipulation) can impair declarative verbal memory in healthy volunteers (Riedel et al. 1999). Three studies of ecstasy users have reported correlations between alterations in serotonergic measures and decreased neurocognitive performance (Bolla et al. 1998; Reneman et al. 2000a; Verkes et al. 2001), while one study failed to find a relationship between alterations in serotonergic measures and decreased performance on measures of cognitive function (McCann et al. 1999b). Bolla et al. reported that decreased CSF 5HIAA levels correlated with decreased visual memory performance in ecstasy users. Verkes et al. found that *d*-fenfluramine-induced cortisol release in ecstasy users significantly correlated with their memory span on the Corsi Block Tapping test. Reneman et al. reported that estimated cortical $5HT_{2A}$ receptor density correlated with impaired declarative memory in 5 ecstasy users. McCann et al found that ecstasy users had lower cerebrospinal 5HIAA than non-users and that ecstasy users exhibited deficits on tests of sustained attention, recall and logical reasoning. However, the researchers did not find a relationship between concentration of cerebrospinal 5HIAA and neurocognitive test performance. Taken together, these findings suggest a relationship between lower neurocognitive performance and ecstasy-induced serotonin depletions or neurotoxicity but with some qualifications. On the other hand, if MDMA-induced loss of serotonin or damage to serotonergic axons were sufficient to impair memory to the degree suggested by human studies, one would expect this effect to have been readily detected in prospective nonhuman animal studies. As discussed in the previous chapter, two studies (Broening et al. 2001; Marston et al. 1999) out of at least eleven (Frederick et al. 1998; Frederick et al. 1995; LeSage et al. 1993; McCreary et al. 1999; Ricaurte et al. 1993; Robinson et al. 1993; Seiden et al. 1993; Slikker et al. 1989; Spanos and Yamamoto 1989) have found evidence of lasting behavioral or memory impairment in MDMA-exposed animals.

Alternatively, some other effect of repeated ecstasy use may decrease neurocognitive performance. It is well established that chronic psychostimulant use lowers neurocognitive performance. For example, repeated cocaine use is associated with impaired neurocognitive functioning (Beatty et al. 1995; Bolla et al. 1999; O'Malley et al. 1992), although cocaine use *per se* does not necessarily produce neurocognitive deficits (Bolla et al. 1999). Cocaine is not a selective neurotoxin but, like MDMA, can cause both serotonergic (Jacobsen et al. 2000; Little et al. 1998) and cerebrovascular (Bartzokis et al. 1999; Herning et al. 1999) alterations. Selected measures from several studies assessing memory in cocaine users are summarized in **Table 5.11**. As can be seen, frequent cocaine use is associated with neurocognitive performance decreases similar in magnitude to those seen in some ecstasy users. In addition, normalizing scores tends to obscure the fact that ecstasy users in published studies generally performed substantially better than cocaine users and their matched nonusers when raw scores are compared.

Table 5.11. Memory Performance in Chronic Cocaine Users

Cocaine Using Population	Cocaine Use History	Drug Free Period	Memory Test	Z-score ± SD	Reference
19 chronic users	15.8 ± 10.3 g in preceding m; 6.3 ± 4.1 y total	181.7 ± 158.9 h	WMS Story Recall - Immediate	-0.49 ± 0.71	(Gillen et al. 1998)
23 in treatment users	at least 2 x/w for last y; 3.0 ± 3.6 g/w for 7.1 ± 4.7 y	21-40 d	WMS Story Recall - Immediate	-1.07 ± 0.77	(Beatty et al. 1995)
30 chronic users	at least 4 x/mg for 1 y; 2.3 ± 2.2 g/w, 4.5 ± 1.3 d/w for 6.7 ± 4.7 y	28 d	WMS Story Recall - Immediate	-0.03 ± 0.86	(Bolla et al. 1999)
60 incarcerated users	8.63 ± 4.36 g/w for 12.52 ± 4.36 y	35.85 ± 15.85 m	WMS Story Recall - Immediate	0.13 ± 1.1	(Selby and Azrin 1998)
19 chronic users	15.8 ± 10.3 g in preceding m; 6.3 ± 4.1 y total	181.7 ± 158.9 h	WMS Story Recall - Delayed	-0.52 ± 0.65	(Gillen et al. 1998)
23 in treatment users	at least 2 x/w for last y; 3.0 ± 3.6 g/w for 7.1 ± 4.7 y	21-40 d	WMS Story Recall - Delayed	-1.25 ± 0.84	(Beatty et al. 1995)
20 in treatment users	454 ± 65 g in 49.8 ± 38.0 m	23.6 ± 15 d	Story Recall - Delayed	1.21 ± 1.52	(O'Malley et al. 1992)
30 chronic users	at least 4 x/mg for 1 y; 2.3 ± 2.2 g/w, 4.5±1.3 d/w for 6.7±4.7 y	28 d	WMS Story Recall - Delayed	-0.06 ± 0.81	(Bolla et al. 1999)
60 incarcerated users	8.63 ± 4.36 g/w for 12.5 ± 4.36 y	35.85 ± 15.85 m	WMS Story Recall - Delayed	0.13 ± 0.99	(Selby and Azrin 1998)

Abbreviations: d = day; m = month; w = week; WMS – Weschler Memory Scale, Revised; y = year.

A number of studies have found that patients with depression have decreased performance in tests of executive function and verbal memory (Dupont et al. 1990; Fossati et al. 1999; Martin et al. 1991; Rush et al. 1983). It is possible that dysphoric mood, which borders on clinical depression in some repeated ecstasy users, lowers neurocognitive performance. Although these mood changes may contribute to lowered neurocognitive performance, they seem unlikely to fully explain performance reductions. In some studies, evidence of neurocognitive changes has been found in ecstasy users without apparent mood changes (McCann et al. 1999a; McCann et al. 1999b; McCann et

al. 1994; Verkes et al. 2001).

Does performance recover with abstinence from ecstasy? Only a few studies have looked for evidence of recovery. Morgan (1999) reported that a subset of three ecstasy users who had not taken ecstasy in over 6 months had significantly better immediate and delayed verbal memory than users with more recent use. In contrast, Wareing et al. (2000) were unable to find evidence of a significant abstinence-related improvement in working memory and executive function when 10 current ecstasy users were compared to 10 volunteers who reportedly had not used ecstasy in 6 months. It is therefore not clear from individual studies if there is recovery from this lower neurocognitive performance. Comparison of performance across studies yields mixed results. The trend-level correlation between improvement in executive function and time from last ecstasy use described above suggests that at least some recovery may occur with abstinence. On the other hand, we found no evidence of improvement in verbal memory with abstinence. In fact, studies in which volunteers had greater average time from last ecstasy use tended to perform worse on verbal memory tests (this was not significant due to the small sample size).

Is ecstasy use associated with alteration of a specific neurocognitive function? Parrott et al. (1998) suggest that changes in neurocognitive performance could be due to either a change in cognitive strategy or impaired abilities. Because there is no evidence of improved ability in ecstasy users, it currently seems unlikely that a change in cognitive strategy has occurred. Parrott (2000) presents two theories that might explain decreased neurocognitive performance in ecstasy users. First, Parrott hypothesizes that changes in the functioning of specific brain regions might explain specific neurocognitive alterations. For example, loss of serotonin in the frontal cortex might lead to impulsivity, while hippocampal changes might decrease memory performance. Parrott points out that this hypothesis fails to account for the many areas of functioning that appear unaltered in ecstasy users, despite theoretically widespread serotonergic changes. However, as discussed above, there seems to be no *a priori* reason to assume that performance changes are due to serotonergic changes. Second, Parrott hypothesizes that inhibition of cortical functioning may explain the specific pattern of changes in ecstasy users. Because the prefrontal cortex is important for executive function, this second possibility is consistent with other reports in which researchers hypothesized specific changes in working memory and/or executive function (Dafters et al. 1999; Gouzoulis-Mayfrank et al. 2000; Wareing et al. 2000).

Executive function is typically associated with complex cognition, such as novel problem solving and the ability to modify behavior in response to environmental changes. Executive function relies heavily on working memory, the ability to hold and manipulate information in short time memory. Impairments in executive function or working memory would be expected to alter functioning in a range of neurocognitive tasks. Ecstasy-induced changes in working memory are consistent with the nonhuman animal literature. The first published evidence of drug-free, long-term alteration in behavior after MDMA exposure was detected in performance of a delayed non-match to place memory task (Marston et al. 1999).

Of the tasks categorized as measuring executive function, statistically significant differences between ecstasy users and non-users were detected in 13 of 57 measures (22.8%) in 11 different studies. The trend-level correlations between executive function and both number of ecstasy exposures and time since last ecstasy use suggest that ecstasy has a modest effect on some aspects of executive function. Nonetheless, it is not yet possible to pick out a particular area of executive function that is affected. The percent of statistically significant tests in this area is lower than for tests of verbal memory (55.8%, 24/43 in 13 studies). Thus, one might argue that some aspect of executive function involving working memory may be particularly affected. Evidence for changes in verbal recall and recognition is stronger than evidence for changes in visual memory (significant group differences seen in 16% (4/25) of measures in 7 studies). Overall, there does not yet seem to be sufficient evidence to implicate one particular area of functioning.

Possible Significance of Neurocognitive Differences and MDMA Neurotoxicity

How severe are these neurocognitive decreases? They do not indicate impairment in day-to-day activities. The differences occur in neurocognitive tests in which young, healthy people perform well. Thus, these differences are generally small in magnitude despite their statistical significance. In fact, neither the investigators nor the MDMA-using volunteers themselves appear to be aware of any cognitive impairment in volunteers in most studies (McCann et al. 1999b; Rodgers 2000).

However, there is a method of interpreting neurocognitive performance that would classify the ecstasy-using volunteers in some publications as having clinically significant impairments in performance. In neurocognitive assessment, it is common practice to interpret test scores following at least 1.3 SD units below the normative mean as "borderline" and those 2.0 SD units below the mean as "impaired" (Lezak 1995). If one were to use similar standards for studies comparing ecstasy users to non-users, then several studies could be considered to have detected "impairment". This interpretation is limited by the fact that it assumes that neurocognitive performance by the nonusers represents the full range of normal scores, which is often not true.

The earliest study (Krystal et al. 1992) suggesting clinically significant impairment compared the performance of 9 ecstasy users who had been pre-selected for low CSF 5HIAA (R. Doblin, personal communication) to published norms for the revised Weschler Memory Scale. Krystal et al. reported that five of nine volunteers had scores on the initial or delayed paragraph tests of the Weschler Memory Scale that were at least 1 SD unit less than normative values. Studies using published normative scores rather than groups of nonusers for comparison are susceptible to biases since the comparison cannot control for any unusual testing conditions. For example, the results reported by Krystal et al. were possibly influenced by the intravenous l-tryptophan infusion given approximately 3 hr earlier to many of the volunteers. In addition, some of the volunteers had reportedly traveled from other states the day of testing or the previous day. Furthermore, there was a high prevalence of personal and/or family history of psychopathology in the volunteers. Subsequent reports have used matched comparison

groups with less psychopathology and found more modest differences between users and nonusers. For example, Bolla et al. (1998) did not detect significant differences between ecstasy users with 60 (25-300) exposures and nonusers using the same memory test employed by Krystal's group.

Wareing et al. (2000) detected large differences between ecstasy users and nonusers in a task involving working memory. When volunteers were asked to generate consonants in a random order at a rate of one per second, the ten ecstasy users performed dramatically worse than the ten nonusers (3.78 ± 3.06 SD units decrease in the number of consonants produced, $P < 0.01$). This difference is much larger than that seen in other studies using tasks involving working memory (e.g., -0.79 ± 0.88 SD units in the backwards Digit Span task in the Gouzoulis-Mayfrank study). It appears possible that short-term residual effects contributed to this impairment. Ecstasy users in the Wareing study had last used ecstasy only 8.20 ± 5.75 days ago. Volunteers had also used more frequently than in most other studies (8.43 ± 3.33 times per month). It is also possible that tasks with external time constraints (i.e., are not self-paced) are best at distinguishing between users and nonusers.

Rodgers (2000) compared 15 users of ecstasy and cannabis to 15 cannabis users and 15 drug-free individuals. Neurocognitive testing was performed on a day when volunteers reported that they had not used any drugs, but time since last drug use was not recorded. Both drug-using groups had lower scores on immediate and delayed recall for the details of a story than drug-free volunteers. However, only the ecstasy users performed worse than drug-free volunteers on delayed verbal and visual paired associates. Although normalized performance differences were modest in delayed visual paired associates, the small distribution of scores for drug-free volunteers created a large difference when scores in delayed visual paired associates are normalized. Scores for ecstasy users were -2.73 ± 2.92 standard deviation units, while cannabis users scored an average of -0.50 ± 1.65 standard deviation units. Furthermore, when all tests of immediate visual memory were combined to form an index score for visual memory, Rodgers did not detect any differences in immediate visual memory between ecstasy users and the other two groups. The difference in visual paired associates was larger than seen in other studies using similar or identical tests. For example, Bolla et al. (1998) found that performance of ecstasy users with an average of 60 previous exposures, most recently an average of 30 days previously, had performance on delayed visual paired associates that was not significantly different (-0.33 ± 1.67 standard deviation units) from that of drug-free volunteers. In fact, statistically significant decreases in visual memory performance were only detected in 16% (4/25) of measures in the 7 different studies in which visual memory was assessed. Because the difference seen by Rodgers is significantly larger than seen in other studies using the same tests, it appears possible that volunteers had used ecstasy very recently and short-term withdrawal effects of ecstasy were being detected. Alternatively, it is possible that there was some particular condition associated with ecstasy exposure in the volunteers in Rodgers's study that led to unusually large toxicity.

Finally, Reneman et al. (2000b) reported dramatic differences in delayed verbal memory between 5 ecstasy users who had used an average of 218 (50-500) tablets and 9 nonuser volunteers. Time from last ecstasy use was an average of 138 (60-360) days. Frequency of use was not reported. In this study, delayed verbal recall was -2.31 ± 1.89 SD units in comparison to nonusers. Interestingly, the first author stated in a personal communication that no differences were seen between these ecstasy users and nonusers in other measures from the Rey Auditory Verbal Learning Test, the Rivermead Behavioural Memory Test, the revised Weschler Memory Scale (Dr. Liesbeth Reneman, personal communication to R. Doblin). Given the many other studies that have measured delayed verbal memory and found more modest differences, it seems likely that the magnitude of the differences in this study is largely due to the small sample size of this preliminary report. This would be consistent with the lack of significant effects in the same volunteers using closely related measures of memory.

Overall these few measures finding large differences between ecstasy users and nonusers appear to be exceptions. The differences between ecstasy users and nonusers are clinically very small and could be considered insignificant if we were certain that recovery occurred.

Will ecstasy neurotoxicity become more obvious as users age? This is difficult to predict. From a neurochemical perspective, age-related decrease in SERT density appears modest — estimated at 4.3% per decade in one recent study (van Dyck et al. 2000) — while 5HT receptors undergo more complex age-related changes (reviewed in Meltzer et al. 1998). One would hope that these changes will not cause ecstasy users to exceed a hypothetical threshold for developing symptoms of neurotoxicity. However, we simply do not understand 5HT or serotonin-related disorders sufficiently to make predictions with any confidence. For example, late onset affective disorders are probably influenced by many nonserotonergic factors, such as social isolation and cerebrovascular disease.

What types of problems could ecstasy neurotoxicity cause? No one knows. Some have speculated that ecstasy neurotoxicity may lead to dysregulation of a range of areas, with psychopathology developing based on whichever systems are most vulnerable to functional impairment in a given individual. There is currently no direct evidence that addresses this speculation. This speculation is based on theories of what role the serotonergic system plays in the brain. The serotonergic system has been thought of as a modulatory system, interacting with both behavioral inhibition and facilitation systems (Depue and Spoont 1986; Spoont 1992). Increasing 5HT is frequently seen as inhibiting behavioral activation, while decreasing 5HT is seen as facilitating responsiveness to external stimuli.

Another approach to predicting problems that could result from serotonergic neurotoxicity is to examine the various psychiatric disorders that are treated with SSRIs and other serotonergic drugs. Disorders that are treated with serotonergic drugs include depression, obsessive-compulsive disorder, and anxiety disorders (such as panic disorder, generalized anxiety disorder, and social phobia). Taken together, these disorders affect

more than 10% of the general population. Because serotonergic drugs are useful in these disorders and serotonergic abnormalities can be seen in many patients with these disorders, some have speculated that serotonergic neurotoxicity may increase risk of affective disorder. This makes sense based on our limited understanding of serotonin, but has not been demonstrated. Furthermore, given the modulatory role of 5HT, one cannot assume that all disorders treatable with serotonergic drugs are caused by serotonergic malfunction.

It is worth noting that neurotoxic phenethylamines have been self-administered by humans for over 60 years. In this time, no evidence has been published suggesting that methamphetamine or amphetamine increase risk of Parkinson's disease, despite damaging dopaminergic axons. In contrast, the link between Parkinson's disease and MPTP, a meperidine analogue and dopaminergic neurotoxin, was rapidly discovered (Davis et al. 1979; Langston et al. 1983). Similarly, concerns about the selective serotonergic neurotoxicity induced by ecstasy and other drugs are not fueled by a toxic syndrome identified in users. Instead, they are motivated by the intuition that the potentially dramatic decreases in indices of serotonergic functioning must have some adverse behavioral consequences.

Risks of Neurotoxicity in Clinical MDMA Studies

In assessing risks of MDMA neurotoxicity, it is necessary to separately consider the risks of serotonergic changes and those of neurocognitive performance changes. This is because these changes may have different mechanisms and may occur after different ecstasy exposures. These two areas of risk are discussed below. It is concluded that the risks of neurotoxicity in volunteers exposed to 125 mg MDMA in a clinical setting are minimal. While risks of neurocognitive performance changes after one or two doses of up to 2.5 mg/kg MDMA (175 mg in a 70 kg person) appear to remain very small, risks of serotonergic neurotoxicity after doses above 125 mg MDMA are difficult to assess. Furthermore it is important to acknowledge that the risks of MDMA neurotoxicity are controversial and other knowledgeable scientists believe that risks are more than minimal (Gijsman et al. 1999; McCann and Ricaurte 2001).

Possible serotonergic neurotoxicity. There is extensive evidence that illicit ecstasy users often develop changes in neurofunctioning. Correlations have been reported between ecstasy exposure and measures such as CSF 5HIAA levels, SERT density, myo-inositol increases, $5HT_{2A}$ binding, and EEG alterations. In some cases, there are questions as to whether these changes can be considered evidence of serotonergic neurotoxicity rather than responses to the pharmacological effects of MDMA. However, as discussed in the section on interpreting studies, long-lasting decreases in SERT density and CSF 5HIAA levels of ecstasy users can be considered as evidence of neurotoxicity. These studies therefore indicate that commonly consumed doses of illicit ecstasy can produce serotonergic neurotoxicity. Animal studies suggest that there is a threshold exposure required for serotonergic neurotoxicity (O'Shea et al. 1998). Doses below this threshold do not produce long-term serotonergic changes in animals. Therefore, the most salient question is what dose of MDMA produces neurotoxicity in humans. As discussed

in the chapter on animal neurotoxicity studies, there are not sufficient data to estimate the neurotoxic MDMA dose in humans with any confidence. However, theoretical and empirical evidence indicate 125 mg MDMA is probably not neurotoxic.

Interspecies pharmacokinetic comparisons suggest that neurotoxic doses in rats produce MDMA blood concentrations that are significantly higher than those produced by 125 mg MDMA in a human. Vollenweider et al. (2001) compare published pharmacokinetic data for humans and rats and conclude that human exposure to MDMA after 125 mg, po, (1.78 mg/kg for a 70 kg human) is significantly less than the lowest known consistently neurotoxic MDMA dose in Sprague-Dawley rats, 20 mg/kg, sc, (Battaglia et al. 1988b; Commins et al. 1987b). At these doses, human MDMA plasma AUC are approximately 30% of the rat AUC. Similarly, human Cmax are approximately 10% of rat Cmax. While this comparison is reassuring, it is limited because the threshold for neurotoxicity is not well established in rats. The threshold for neurotoxicity in this rat strain appears to be above 10 mg/kg (Battaglia et al. 1988b) and below 20 mg/kg (Commins et al. 1987b). Therefore human MDMA exposure (measured as AUC) after 125 mg is likely between 30% and 60% of the exposure required for neurotoxicity in rats. It is not known whether rats and humans have different vulnerability to the same MDMA exposure. Furthermore, human pharmacokinetic data suggest that small increases in dose above 125 mg may lead to large increases in exposure to MDMA. Thus, while 125 mg MDMA is not expected to produce neurotoxicity based on these comparisons, the neurotoxic dose in humans may be only modestly higher. Interspecies scaling is further discussed in the chapter on animal neurotoxicity.

The calculated safety of 125 mg MDMA is supported by preliminary empirical evidence. Vollenweider et al. (2001; personal communication) measured SERT density using PET before and after clinical MDMA exposure. They were unable to detect any lasting effect of 1.5 or 1.7 mg/kg MDMA in six MDMA-naive healthy volunteers. Given the small sample size and unclear sensitivity of this measure, it is possible that a modest change in SERT density could have gone undetected. It is a fact of science that small changes may go undetected in individual studies. Therefore, risks of neurotoxicity must be discussed with all volunteers in MDMA studies, even though animal studies and data from ecstasy users give no indication that such small changes would have any detectable consequence.

Studies of illicit users provide some insight into the number of ecstasy exposures required to produce detectable serotonergic alterations. However, this type of analysis is limited because it must assume that all illicit ecstasy exposures are neurotoxic, which is probably not true. As discussed below, measures of serotonergic alterations may have a complex relationship with actual damage.

Reported correlations in many studies suggest a linear relationship between ecstasy exposure and measured alterations. For example, estimated SERT density in ecstasy users in one study (McCann et al. 1998) was linearly correlated with number of ecstasy exposures ($r = -0.50$, $p = 0.005$). If this linear relationship in users with 70 or more exposures could be generalized to individuals with fewer exposures, then the effects of one or two exposures in a clinical setting should be too small to measure. The average

decrease in estimated SERT density in these volunteers was approximately 1 SD unit and only one ecstasy user was clearly outside the range of nonuser values. In the conference comments compiled by Turner and Parrott, Gamma extrapolates from the correlation in the McCann PET study (Turner and Parrott 2000). Given the modest effects of more than 70 ecstasy exposures, Gamma calculates that the effects of a single ecstasy exposure should cause a decrease of only 0.1%, which is within normal SERT density variance. However, the apparent linear correlation seen in these papers may be an artifact of the limited exposure ranges of the studied users. Even if there is a linear exposure-toxicity relationship over a given exposure range, it cannot be assumed that this relationship remains linear over the entire range of possible exposures.

The interpolated curve in **Figure 5.1** provides some limited evidence that CSF 5HIAA decreases may be logarithmically related to number of illicit ecstasy exposures. A logarithmic dose-response function would imply that illicit users could experience large alterations in neurofunctioning early in their ecstasy use history. The absolute magnitude of further changes from subsequent exposures would become progressively smaller and could appear linear over limited exposure ranges. This possibility appears consistent with the reported resistance of some serotonergic axons to ecstasy-induced neurotoxicity in the nonhuman animal studies (O'Hearn et al. 1988). Of course, this would not mean that there is an upper limit to possible ecstasy-induced toxicity since the behavioral or cognitive consequences of ecstasy exposure may have complex relationships with these neurofunctional measures.

If the interpolated curve in **Figure 5.1** is accurate, 2 ecstasy exposures would be predicted to decrease CSF 5HIAA by 2.6 ng/ml. Because the standard deviation for CSF 5HIAA is around 7 ng/ml, such a decrease would be unlikely to reduce this metabolite to a level that could be considered abnormal. This calculation is a simplification given that serotonergic neurotoxicity is dose-dependent and unlikely to occur after lower doses.

There are intrinsic limitations to our ability to quantify serotonergic neurotoxicity. We can measure indices of serotonergic functioning. Changes in these indices of serotonergic functioning reflect, to some unknown degree, the permanent redistribution of axons. The long-term consequences of this axonal redistribution are unknown. Theoretical arguments not withstanding, there is no evidence that the consequences of this axonal redistribution are severe. Drugs with capacity to produce similar changes have been used for over 60 years without evidence of clinical impairment due to serotonergic neurotoxicity. However, without large population studies, some changes may not have been detected.

Possible changes in neurocognitive performance. Increasing evidence suggests that repeated ecstasy exposure can sometimes cause neurocognitive changes. These changes are clinically small and could be considered insignificant if it was clear that they were short-term and reversible. Because evidence of reversibility is inconsistent and data are few, risks of neurocognitive changes should be taken seriously when designing and assessing possible clinical studies. Exposure to MDMA in previous clinical studies has not been found to cause lasting neurocognitive changes. However, chronic exposure to

seemingly similar doses in illicit contexts appears to sometimes cause such changes. It is not known if these changes are due to occasional high doses, the frequency of drug exposure, or conditions of illicit use. Therefore, clinical exposure to MDMA should be kept at the minimum necessary to achieve study goals.

Not every ecstasy exposure causes lasting neurocognitive changes. No lasting changes in neurocognitive performance have been reported in clinical MDMA studies. Dr. Charles Grob et al. administered doses of up to 2.5 mg/kg MDMA in a clinical setting, testing neurocognitive performance before and approximately two weeks after drug exposure. As illustrated in **Table 2.5**, no significant changes occurred in the 14 volunteers assessed. Dr. Franz Vollenweider et al. also report that neurocognitive performance in their volunteers was unchanged by one or two exposures of up to 1.7 mg/kg MDMA in a clinical setting (Vollenweider et al. 2001). Testing by Grob et al. appears to have been adequately powered to detect even small changes. Vollenweider et al. have not yet published or made available details of their analyses. When published, data from Vollenweider et al. will be particularly interesting because many of their volunteers were previously MDMA-naive. These reports suggest that there are minimal risks of neurocognitive changes after one or two exposures to MDMA in a clinical setting.

In contrast to the findings of these clinical studies, studies of illicit ecstasy users have sometimes found evidence of decreased performance in neurocognitive tasks. These studies of illicit ecstasy users are important to consider when designing clinical trials because they provide evidence of what types of ecstasy exposure could lead to toxicity. Taken together, studies of illicit ecstasy users support the conclusion that MDMA exposure *per se* does not necessarily cause lasting neurocognitive performance changes. But, there is evidence that, under certain unknown-but-common conditions, as few as 20 exposures to illicit ecstasy may lead to decreased neurocognitive performance (e.g., Rodgers 2000). The conclusion that not all ecstasy exposures cause lasting neurocognitive changes is consistent with nonhuman MDMA neurotoxicity research. Despite extensive serotonergic damage, animals in these studies have generally not shown evidence of lasting behavioral changes or neurocognitive impairments. As discussed in the previous chapter, two studies (Broening et al. 2001; Marston et al. 1999) out of at least eleven (Frederick et al. 1998; Frederick et al. 1995; LeSage et al. 1993; McCreary et al. 1999; Ricaurte et al. 1993; Robinson et al. 1993; Seiden et al. 1993; Slikker et al. 1989; Spanos and Yamamoto 1989) have found evidence of lasting behavioral or memory impairment in MDMA-exposed animals.

The most relevant studies of illicit ecstasy users are prospective studies and those retrospective studies that adequately controlled for use of other drugs. Gouzoulis-Mayfrank et al. (2000) found that users of ecstasy and cannabis performed worse than a comparison group matched for the use of cannabis in a variety of tasks. In this study, ecstasy users had taken 1.4 ± 0.9 pills 2.4 ± 1.6 times per month for 27 ± 18 months. Thus, these individuals had used 93.4 ± 119.9 tablets, or 3.36 pills per month. Rodgers (2000) found that 15 cannabis-using ecstasy users who had used ecstasy an average of 20 times over 5 years performed worse than 15 cannabis users in tests of delayed verbal and visual memory. In an important prospective study, Zakzanis et al. (2001) found that 15

polydrug-using ecstasy users decreased in performance of a memory task (and tended to decrease in other tasks) over 12 months. During that time, the volunteers used an average of 1.75 pills an average of 2.4 times per month. Volunteers were free from ecstasy use for at least two weeks before each testing session. Thus, as few as 29 exposures to 50 ecstasy pills may decrease neurocognitive performance. Considered in another way, volunteers in this study ingested 4.2 pills per month. In contrast, Croft et al. (2001) were unable to detect an effect of ecstasy exposure in users of ecstasy and cannabis who had taken 41.5 ± 49.3 ecstasy pills when they were compared to matched cannabis users. In all these cases, it is unclear whether the decrease in performance is a cumulative residual effect or a frank neurotoxic effect.

The contrasting findings of these studies suggest that only some conditions or patterns of ecstasy exposure lead to neurocognitive changes. It is not known what conditions or patterns of ecstasy exposure these are. Toxic ecstasy exposures may be ones that are above a certain dose, are frequent, lead to hyperthermia, occur with certain other drugs, or have some combination of these conditions. Each of these possible factors will now be considered.

The doses consumed by ecstasy users are difficult to estimate. Volunteer reports of previous ecstasy use may be inaccurate; the potency of ecstasy pills varies; and few studies have quantitatively analyzed ecstasy pills. These uncertainties should be kept in mind during the following discussion. In the report by Saunders and Doblin (1993), the average potency of MDMA-containing pills was 87.4 mg MDMA, which is slightly higher than the average of 76 mg MDMA per pill in the study by Sherlock et al. (1999). If pills containing inactive amounts of MDMA (less than 20 mg) are excluded, the mean potency across these two studies is 90.8 ± 35.0 mg MDMA. This suggests that the average dose may have been 127 ± 95.2 mg MDMA in the study by Gouzoulis-Mayfrank et al. and 159 ± 61.3 mg MDMA in that of Zakzanis et al. (the standard deviation is wider and more accurate in the Gouzoulis-Mayfrank study because they provided standard deviations for measures of ecstasy exposure). These estimated doses are also very similar to doses used in clinical MDMA studies (and apparently not associated with neurocognitive changes). This suggests that either neurocognitive changes in illicit users are due to the occasional high dose or that some factor aside from dose is a risk factor for neurocognitive changes.

Frequent ecstasy exposure may be a risk factor for lasting neurocognitive changes. In both the Gouzoulis-Mayfrank and Zakzanis studies, volunteers used ecstasy 2.4 times per month (Gouzoulis-Mayfrank provided a standard deviation of 1.6 times per month). In most clinical MDMA studies, volunteers appear to have received MDMA at intervals of 14 days or more.

It is possible to hypothesize reasons that there could be increased toxicity with frequent ecstasy exposure. It must be emphasized that these hypothetical reasons are speculative. Doses of MDMA that are not neurotoxic have been shown to cause cerebral oxidative stress in animal studies (Schmidt and Taylor 1988). Thus, recent MDMA exposure could theoretically deplete endogenous defenses against oxidative stress, leaving the individual

vulnerable to subsequent toxicity from MDMA. Among other considerations, MDMA can oxidatively inactivate tryptophan hydroxylase, possibly lowering serotonin levels (Schmidt and Taylor 1988; Stone et al. 1989a; Stone et al. 1989b). Serotonergic depletion (by dietary manipulation) can impair declarative verbal memory in healthy volunteers (Riedel et al. 1999). Finally, Chang et al. (2000) found that two exposure to MDMA in a clinical setting decreased cerebral blood flow at 10 to 21 days after the second exposure. Although these decreases do not appear to be permanent (Chang et al. 2000; Gamma et al. 2001), one might speculate that further MDMA exposure during this period of decreased cerebral blood flow might carry increased risks of toxicity.

In addition to patterns of ecstasy exposure, conditions commonly present during illicit ecstasy exposure may be risk factors for neurocognitive changes. In rodents, hyperthermia potentiates MDMA neurotoxicity (Broening et al. 1995; Colado and Green 1995; Colado et al. 1993; Malberg and Seiden 1998). Hyperthermia associated with exercise or warm or humid conditions may potentiate toxicity in illicit users as well. Concurrent use of hallucinogens also potentiates neurotoxicity in rodents (Gudelsky et al. 1994) and is common in illicit ecstasy users. If serotonergic neurotoxicity in rodents can be related to neurocognitive changes in humans, these factors would increase risk of neurocognitive changes.

Based on the available literature, it seems likely that any long-term neurocognitive changes that occur as the result of clinical MDMA exposure will be subtle. In the only published prospective study of illicit ecstasy users, delayed memory of ideas from a story was decreased by 0.63 ± 2 SD units after approximately 29 ecstasy exposures over 12 mo (Zakzanis and Young 2001). Any neurocognitive changes, if they occur after one or two clinical MDMA exposures, seem likely to be much less than this amount. Assuming that each ecstasy exposure in the Zakzanis study contributed equally to these changes, a single MDMA exposure could be predicted to decrease performance in a similar task by about 0.02 SD units. It should again be noted that it is possible that some previously undetected toxicity will manifest itself as users age, since long-term follow-up studies have not been published.

Monitoring for Chronic Neurotoxicity in Clinical MDMA Studies

While current data suggest that MDMA can be safely administered in a clinical setting to healthy volunteers without lasting toxicity, neurotoxicity is still possible in some clinical studies. Measurable serotonergic neurotoxicity cannot be currently ruled out in studies that employ doses above 125 mg MDMA. Measurable neurocognitive changes remain possible in studies employing individual doses above 2.5 mg/kg MDMA. Subtle changes, too small to detect in small clinical trials, also cannot be excluded after any dose. In addition, current data only address the safety of studies that include healthy individuals receiving one or two doses. Given these data limitations, researchers may wish to monitor for neurotoxicity in some studies. Monitoring for neurotoxicicity may be informative in studies of the possible therapeutic effects of MDMA in patients. Monitoring for neurotoxicicity is unlikely to be useful in clinical studies employing

ecstasy users as volunteers. This is because previous and ongoing ecstasy use is likely to obscure the possible chronic effects of clinical MDMA exposure (if any occurred).

There are a number of putative measures of neurotoxicity, including neurocognitive, serotonergic, and neurofunctional measures. Although lumbar puncture (to measure CSF 5-HIAA) could be considered, it may not be informative in ongoing ecstasy users and may be unduely burdensome for patient populations. Other putively serotonergic measures are difficult to interpret. Therefore, neurocognitive performance measures may be the best method for detecting toxicity. Decreases in performance would represent an unambiguous measure of toxicity, in contrast to serotonergic and neurofunctional measures. Furthermore, studies of illicit ecstasy users suggest that neurocognitive performance measures are sensitive to the effects of relatively few toxic ecstasy exposures.

Because no specific deficits have been demonstrated, a standard neurocognitive test battery seems useful. Declarative verbal memory is an area of functioning for which there is the most extensive evidence of ecstasy-related changes and may be measured with the Ray Auditory Verbal Learning Test or similar tests. There is also evidence that executive function and working memory may be affected in ecstasy users and that deficits will be most apparent when there is high cognitive demand (such as when a high rate of responses is required). The Wisconsin Card Sorting Task is one of the most widely used measures of executive functioning and decreased performance on it reportedly correlated with ecstasy exposure in one study (Dafters et al. 1999).