MDMA Literature Review Update: March 2004-January 2005

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Introduction

This document will review and discuss research on or about methylenedioxymethamphetamine (MDMA) published between March 2004 and January 2005, and is a yearly update to the review first conducted in 2001 (Baggott et al. 2001). Two clinical trials (Pacifici et al. 2004; Farre et al. 2004) and a few studies in Ecstasy users (Halpern et al. 2004; McCardle et al. 2004) appearing subsequent to March 2004 appear in the previous update to the literature review (Jerome 2004). Except for these studies in humans, most research published after March 2004 but before or during January 2005 is addressed in this review.

The review is intended to reach an understanding of the current knowledge concerning the risks and benefits of MDMA. This means that the focus of the review is on human research, with a secondary focus on specific studies in non-human animals. This review considers all clinical trials of MDMA in humans, all studies of ecstasy users, with "ecstasy" referring to material represented as MDMA, and in vitro and non-human animal studies with findings that may be relevant to the risks and benefits of MDMA. This review will not cover studies examining the demographics of ecstasy users in a given region, drug discrimination studies in non-human animals, or other behavioral pharmacology studies. However, some of these studies may be referred to when their findings increase understanding of studies included within the review.

I located research reports through periodic searches of the PubMed database and through contacts with researchers. The review was performed with a cut-off date of January 2004, with exceptions made only for any clinical trial of MDMA or any study in ecstasy users reporting significant results. Research reports appearing after the cut-off date will be addressed in later reviews. Abstracts and full text reports of nearly all of the papers cited within this review can be found in MAPS' Psychedelic Bibliography.

To date, no studies in humans have altered our original assessment of the risks and benefits for participants in human trials of MDMA**.** Nearly all study findings reported in recent publications either replicate previously reported findings or are similar in nature to previously reported findings. Hence recent MDMA research does not raise any new questions or concerns about the safety of human trials with MDMA. If they continue to be replicated, some recent in vitro and non-human animal study findings may lead to revised interpretations of earlier research into MDMA neurotoxicity, but making such revisions now would be premature. Specifically, a number of research studies question the validity of markers of brain chemistry treated as indicative of neurotoxicity, and a few studies suggest that high doses of MDMA combined with hyperthermia may produce more extensive MDMA neurotoxicity. However, as noted, both of these recent developments occurred in studies in non-human animals, and do not necessarily affect interpretations of studies in humans.

Only two clinical trials of MDMA were published after March 2004, and one of these trials was a re-examination of data from earlier studies. However, no serious adverse events occurred during either study. An ongoing study of MDMA-assisted psychotherapy

in people with posttraumatic stress disorder (PTSD) has also been underway since April, 2004, and MDMA has not produced any serious adverse events.

Research investigating potential links between specific drugs and a specific birth defect failed to find a specific link between ecstasy and this heart defect. Though study findings detected a risk associated with sympathomimetic drugs, the researchers could not detect risks associated with specific drugs, including ecstasy. Hence this report does not increase or decrease estimated risk of reproductive toxicity from exposure to MDMA. However, women who are pregnant or lactating are excluded from all clinical trials of MDMA to date.

Studies in ecstasy users continue to examine the effects of repeated use on mood, psychological well-being, cognitive function, and brain function. Thirteen research studies in ecstasy users have been published since March 2004, and researchers made one research presentation. Study findings concerning long-term effects on mood and psychological well-being continue to find a relationship between use of many drugs and decline in psychological well-being, and a few studies also found that intensity of ecstasy use is associated with using other drugs with more intensity. Studies of cognitive function in ecstasy users also continued to find relationships between repeated use of ecstasy and impairments in memory and executive function, with findings similar in nature to previously reported findings. Lastly, a series of studies examining brain chemistry and function have found some changes in patterns of brain function, but did not find significant indications of brain injury. As has been the case with previously reported findings from imaging studies, findings from recently published studies in this area reported mixed results. Imaging study findings continue to question strong support for a link between MDMA and changes in psychological function while still supporting a link between MDMA and impaired cognitive function. None of the studies presented findings that would lead to increases or decreases in estimated risk to participants in clinical trials of MDMA, though it appears that links between regular ecstasy use and negative mood or psychological problems are complex and less strong than previously believed.

Recent studies conducted in vitro or in non-human animals explored the presence, nature and potential causes of MDMA neurotoxicity, the effects of environment and individual differences on MDMA neurotoxicity, the effects of MDMA on thermoregulation, potential developmental toxicity and effects on the liver and immune system. Researchers comparing MDMA with drugs known to reduce brain serotonin or damage serotonin neurons found that MDMA did not produce the same effects as these drugs. For instance, it appears that reduced brain serotonin was not always correlated with signs of neuronal injury. If researchers replicate these findings, they may be cause for reconsidering all previous studies employing these measures as indices of neurotoxicity. Other researchers tested hypotheses about the source or sources of MDMA neurotoxicity at the cellular level, or examined the combined effects of a behavior, such as strenuous exercise or exposure to loud noise, in combination with MDMA. A number of researchers investigated mechanisms of MDMA-induced hyperthermia in rodents, examining the roles played stimulation of the sympathetic nervous system, specific neurotransmitter systems, and cellular metabolism in producing MDMA-associated hyperthermia.

Researchers administered MDMA to neonatal and young rats to detect developmental toxicity, producing inconsistent study findings, but offering enough evidence for continuing to exclude pregnant or lactating women from clinical trials of MDMA. Researchers continued to investigate the effects of MDMA on liver cells and the immune system, and how MDMA affects the function of the metabolic enzyme CYP2D6. Study results were similar to previously reported findings in these areas, and include detection of signs of oxidative stress in liver cells and changes in immune response after MDMA. Very few of the in vitro and non-human animal studies published during this period describe new phenomena or report new findings, and the few studies that do so, such as those finding differences in effects of MDMA and another known neurotoxin, do not at present change the estimated risks of clinical trials with MDMA.

Clinical trials

Introduction

MDMA has been administered to over 240 people in Phase 1 clinical trials conducted throughout the world (see Baggott et al. 2001; Jerome and Baggott 2002; Jerome 2004 for detailed reviews). Clinical trials examined the subjective, physiological, neuroendocrine and immunological effects of MDMA (for example Cami et al. 2000; Forsling et al. 2001; Grob et al. 1996; Harris et al. 2002; Lamers et al. 2003; Lester et al. 2000; Liechti et al. 2001; Mas et al. 1999; Pacifici et al. 2004; Tancer and Johanson 2003), and at least two teams of researchers have examined MDMA metabolism (see for example de la Torre et al. 2000; Farre et al. 2004; Samyn et al. 2002). As of January, 2005, seven research teams have performed clinical trials of MDMA in humans. This includes researchers in the Netherlands (e.g. Lamers et al. 2003), Spain (e.g. Farre et al. 2004; Pacifici et al. 2004), Switzerland (e.g. Gamma et al. 2000; Liechti et al. 2001), the United Kingdom (e.g. Forsling et al. 2001) and the United States (e.g. Grob et al. 1996; Harris et al. 2002; Tancer and Johanson 2003). MDMA was well-tolerated in all studies, with no occurrence of serious adverse events during these studies.

Five research participants have completed a randomized, double-blind, placebo-controlled trial of 125 mg MDMA administered in each of two drug-assisted psychotherapy sessions in people with posttraumatic stress disorder (PTSD), and a seventh participant has undergone the first of two experimental sessions (Mithoefer 2005, personal communication) without any occurrences of serious adverse events.

Previous to the start of this study, a team of researchers in Spain began performing a dose-response study of MDMA in women with PTSD arising from sexual assault. The study was halted by a local drug law administration (the Madrid Anti-Drug Authority), apparently for political reasons.

A randomized, dose-response study of MDMA in people with anxiety arising from diagnosis with advanced stage cancer and a life expectancy of less than 12 months received approval from both relevant IRBs, and the FDA also granted permission for the study. Screening and enrollment of participants will begin once the principal investigator receives a Schedule 1 handling license from the DEA.

Clinical trials have been conducted with doses of MDMA ranging from 0.25 mg/kg, or about 17.5 mg (Grob et al. Unpublished) to 2.5 mg/kg, or about 175 mg (Grob et al. Unpublished), with the most frequently used doses ranging from 75 to 145 mg. More detailed discussion of study findings can be found in the IB and updates of the IB (Baggott et al. 2001; Jerome and Baggott 2003; Jerome 2004). As noted in the IB and in previous updates to the IB, these studies have produced a consistent physiological and psychological profile of MDMA. In controlled settings, MDMA elevates blood pressure and heart rate and produces a slight increase in body temperature. Participants in controlled studies of MDMA reported experiencing stimulant-like and hallucinogen-like effects, but their reports on other self-reported effects indicated that MDMA possesses a

unique pharmacological profile distinct from that of the stimulants or hallucinogens (Liechti et al. 2001). Informal reports of subjective effects and responses to specific items on some measures (see Vollenweider et al. 1998; Tancer and Johanson 2003; Tancer and Johanson 2001) have detected the increases in sociability, closeness to others and empathy referred to in anecdotal reports, retrospective studies and uncontrolled trials (see discussion in Baggott et al. 2001). However, an attempt at formal measurement of these effects failed to detect them (Harris et al. 2002). As discussed in the IB and previous updates to the IB, MDMA elevated stress hormones and increased levels of the anti-diuretic hormone arginine vasopressin (Baggott et al. 2001; Jerome and Baggott 2003; Jerome 2004). Researchers in Spain have found that MDMA produces transient immunological changes. These changes include decreased numbers of CD4 cells, increased numbers of NK cells, increased levels of immunosuppressant and anti-inflammatory cytokines, and decreased levels of immunostimulating and pro-inflammatory cytokines (see Pacifici et al. 2004; Pacifici et al. 2002).

As of January 2005, there have been two published clinical trials of MDMA (Gamma et al. 2004; Pizarro et al. 2004), and one conference presentation of data from a clinical trial (Tancer and Johanson 2004). One paper compared positron emission imaging data with electroencephalographic data simultaneously recorded in a largely drug-naïve sample that received 1.7 mg/kg MDMA (Gamma et al. 2004), and the other examined MDMA pharmacokinetics in a sample of men who had reported previous experience with ecstasy (Pizarro et al. 2004). The conference presentation examined the effects of daily fluoxetine (Prozac) administration on the subjective effects of MDMA (Tancer and Johanson 2004).

Gamma et al. 2004

Gamma and colleagues compared data from [$H_2{}^{15}0$]-PET imaging with simultaneously recorded data from low resolution brain electromagnetic tomography (LORETA), with recordings made after placebo and after 1.7 mg/kg (approximately 119 mg) MDMA in a sample of six women and ten men (Gamma et al. 2004). This research report is based on data that has already appeared in previous reports (Frei et al. 2001; Gamma et al. 2000), where the researchers presented information on subjective and physiological effects of MDMA in this sample. MDMA was tolerated by this largely drug-naïve sample, and no serious adverse events occurred.

In this study, the researchers measured brain activity while participants performed the Continuous Performance Task, which required them to respond upon the appearance of a specific pair of letters, and during a control task, which required viewing non-target characters only. Simultaneous measurements occurred during two one-minute PET scans, with epochs of EEG lasting from about 30 seconds to one minute. Correlations between raw PET and EEG made during the control task detected positive correlations between beta band activity and increased cerebral blood flow (CBF) in temporal and frontal cortices after placebo, and positive correlations between Beta2 band activity and CBF in the occipital and parietal cortices after MDMA. When MDMA and placebo PET scans were compared across control task and Continuous Performance Task, Gamma and

colleagues found that MDMA was associated with global decreases in CBF during both tasks. However, cross-drug comparisons of EEG data found more limited decreases (for instance, decreased alpha and theta bands). The researchers found no significant correlations between raw PET and EEG taken during cognitive task performance, whether performed after placebo or MDMA. More generally, the researchers failed to find significant relationships between changes CBF seen in the PET scans and changes in EEG activity, even when length of EEG unit recordings were increased to make them more similar to PET imaging. This report might help neuroscientists to arrive at an understanding of the relationship between EEG and imaging data, and the limitations of such comparisons. As expected, the researchers detected positive correlations between beta activity and CBF, assumed to be an indirect measure of excitatory activity. In contrast, the researchers were surprised when they detected a positive relationship between delta activity and increased CBF, since delta activity is assumed to be an indicator of inhibitory activity. Gamma and colleagues offer several explanations for this finding. They note that previous investigations of EEG and PET occurred in patient populations, whereas this study examined a non-patient sample. They also consider the possibility that inhibitory activity might be associated with increased CBF.

Pizarro et al. 2004

Pizarro and colleagues gave 100 mg MDMA to seven men reporting past experience with ecstasy. In this report, the researchers described findings on MDMA pharmacokinetics in blood and urine, and did not provide information on subjective, physiological or neuroendocrine effects of MDMA. Pizarro and colleagues successfully detected both R-(-) and S-(+) enantiomers of MDMA and the MDMA metabolites HMMA and HHMA (DHMA) in blood and urine over a two-day period. No serious adverse events occurred during this study. The researchers reported novel findings concerning enantioselective metabolism of MDMA, but none of these findings alter the current estimated risks or benefits in participating in a human trial of MDMA.

In agreement with earlier reports by the same team (e.g. de la Torre et al. 2000; Lanz et al. 1997; Pizarro et al. 2002), Pizarro and colleagues reported that in humans, HMMA was a major MDMA metabolite, and MDA a minor metabolite. Pizarro and colleagues reported the half-life of MDMA to be 11 hours, a somewhat higher figure than the 7 to 9 hour half-life reported in earlier studies (de la Torre et al. 2000; Mas et al. 1999). The R-(-) enantiomer of HMMA had a half-life of 42 hours. It is unclear what significance these pharmacokinetic findings have in terms of understanding the subjective, physiological or neuroendocrine effects of MDMA, especially given the small sample size and gender restrictions of this sample.

Study findings are similar to previous reports of enantioselective metabolism (preferential metabolism of one enantiomer) of MDMA, with S-(+)-MDMA metabolized more rapidly than R-(-)-MDMA. This study also reported that HMMA was not enantioselectively metabolized, a finding that is also in agreement with earlier reports by the same research team (Pizarro et al. 2003). Metabolism of the metabolite HHMA, described here for the first time, was enantioselective, with R-(-)-HHMA metabolized more rapidly than S-(+)-

HHMA, but the ratio is less enantioselective than predicted. The authors hypothesize that this difference in degree of enantioselective metabolism is an indicator that MDMA inhibits function of the enzyme CYP2D6. These results support conclusions voiced in a recent commentary and review by one member of the same research team that genetic variation in human CYP2D6 function has little relevance in explaining variance in response to MDMA (de la Torre and Farre 2004). De la Torre and Farre note that larger doses of MDMA inhibit CYP2D6 in people with intact enzyme, rendering them similar to those with reduced CYP2D6 activity levels.

In addition to the two clinical trials described above, a review written by one of the leading experts in human MDMA pharmacology and pharmacokinetics expressed skepticism and caution in relation to generalizing findings from neurotoxicity studies in non-human animals to humans (De la Torre and Farre 2004). In this review, de la Torre concluded that non-linear pharmacokinetics make it difficult to rely on interspecies scaling to arrive at human-equivalent doses on the basis of studies in non-human animals, and that many non-human animal studies use doses of MDMA that are far greater than commonly used doses in humans. In this and another review, De la Torre also presents evidence indicating that MDMA is a potent inhibitor of the metabolic enzyme CYP2D6 (De la Torre and Farre 2004; De la Torre et al. 2004). Previously, some researchers had argued that genetic variation in CYP2D6 function could pose a risk for people taking MDMA (See for example Schifano et al. 2003; Tucker et al. 1994). However, if most active doses of MDMA inhibit CYP2D6 function, then variations in enzyme function may be less significant than previously imagined. In vitro studies support this conclusion (Heydari et al. 2004; Wu et al. 1997), and researchers failed to detect a relationship between one or more dysfunctional CYP2D6 gene and fatality (O'Donohoe et al. 1998; Gilhooly and Daly 2002).

Tancer and Johanson 2004

Tancer and Johanson presented data on the effects of fluoxetine pretreatment on the subjective effects of MDMA in eight ecstasy-user volunteers at the 2004 College on Problems of Drug Dependence (CPDD) conference. The study employed a crossover design, wherein people received placebo for a week, followed by two weeks of fluoxetine. MDMA and two other test drugs were administered first during placebo administration and then during fluoxetine administration, with fluoxetine administered one hour prior to test drug administration. MDMA (1.5 mg/kg, or about 105 mg) was compared with the stimulant d-amphetamine (10 mg) and the serotonin releaser and 5HT2C antagonist m-chlorophenylpiperazine (mCPP) (0.5 mg/kg, or about 35 mg). Participants completed the Profile of Mood States (POMS), a measure of state mood, researcher-created visual analog scales, the Hallucinogen Rating Scale (HRS) and the Addiction Research Center Inventory (ARCI), another measure of alterations in consciousness, every hour after drug administration. Fluoxetine pre-treatment attenuated most of the scale scores elevated after MDMA (specifics not supplied in abstract), while having little effect on scales increased by amphetamine. Fluoxetine reduced some of the scale scores elevated by mCPP, such as Arousal, Elation and Vigor, but increased scores on other scales, such as Bad Drug Effects and HRS Cognition and Somasthesia (bodily

sensations, physical effects). Data on the impact of fluoxetine pre-treatment on the physiological effects of MDMA were not reported in this poster. No information is presented on side effects or occurrence of serious adverse events during this study, but it seems likely that none occurred.

These findings are in agreement with earlier studies of interactions between MDMA and selective serotonin uptake inhibitors, or SSRIs (Liechti et al. 2000; Liechti and Vollenweider 2000; Pacifici et al. 2004). Previous reports employed citalopram (Celexa) (Liechti et al. 2000; Liechti and Vollenweider 2000) and paroxetine (Paxil) (Pacifici et al. 20004). Liechti and colleagues found that citalopram pre-treatment attenuated or eliminated most of the subjective effects of MDMA, including positive mood, anxiety over loss of control, perceptual alterations, elevated blood pressure, and heart rate. The only effect that citalopram did not attenuate or reduce was the slight elevation in body temperature seen after MDMA. Pacifici and colleagues found that paroxetine pretreatment attenuated or nearly eliminated the immunological effects of MDMA. Taken together, all three research studies suggest that serotonin release plays a significant role in producing the acute pharmacological effects of MDMA, including subjective effects. However, it is also possible that these drugs attenuate the effects of MDMA by interfering with drug metabolism rather than by preventing MDMA from releasing serotonin. These studies also suggest that SSRIs can be safely co-administered along with MDMA, but that doing so reduces most of MDMA's acute pharmacological effects.

Bateman et al. 2004 - Case Series

Human reproductive toxicity of MDMA and other drugs was assessed in a case-control study of 296 women in two cities in the North of England (Bateman et al. 2004). Some of these researchers had previously reported a possible link between ecstasy use and birth defects (McElhattan et al. 1999). In the current study, the researchers matched women who had given birth to children with ventricular septal defect, a specific heart defect, with women who gave birth to healthy infants. Bateman and colleagues reported that exposure to ecstasy was too low in these samples to establish risk, with only three people reporting ecstasy use, and all reported ecstasy use occurring before pregnancy. However, the authors detected a link between using cold or cough remedies containing sympathomimetic drugs or non-steroidal anti-inflammatory medications (NSAIDs) and increased risk of ventricular septal defect. Since MDMA is also sympathomimetic, it is possible that MDMA poses such a risk as well, but the authors also suggest that viral infections might pose a risk as well, since cold and cough remedies are usually taken in response to a viral infection. Exposure to ecstasy was lower in this sample than the authors had expected, possibly because the mean age in this sample was older than 25, and also possibly because women restricted their substance use after learning they were pregnant.

Studies in Ecstasy users

Introduction

The majority of human studies pertaining to MDMA or ecstasy continue to be of people reporting repeated, and often regular, ecstasy use, henceforth referred to as "ecstasy users." This literature is extensively reviewed in the IB and in all previous updates of the IB (Baggott et al. 2001; Jerome and Baggott 2003; Jerome 2004), and in other reviews (Cole and Sumnall 2003A; Green et al. 2003; Parrott 2004), including a meta-analysis of ten studies of memory in ecstasy users (Verbaten 2003). A few researchers have examined the acute effects of ecstasy in naturalistic settings (e.g. Brookhuis et al. 2004; Curran et al. 2004), and some researchers have studied sub-acute effects of MDMA (Curran et al. 2004; O'Regan et al. 2004). However, most research in this area consists of comparisons between samples of ecstasy users and people who report no or very little lifetime use of ecstasy. These studies have assessed brain structure and function, mood or psychological well-being, cognitive function and a number of other variables, such as neuroendocrine profile. Most studies seek to detect possible indicators of MDMA neurotoxicity in humans and any relationships between at least one indirect indicator of reduced serotonin function and changes in mood or cognitive function.

As stated in the IB and in all subsequent updates, studies in ecstasy users can be treated as a conservative estimate of the upper limits of risk involved in human clinical trials of MDMA. It is expected that participants enrolled in clinical trials will experience far less risks than people who repeatedly use illicit ecstasy of unknown purity and strength in uncontrolled conditions. Nevertheless, this review will examine and use studies in ecstasy users in estimating risks and benefits of participating in clinical trials of MDMA.

Nearly all studies of ecstasy users are either retrospective or longitudinal, with the majority being retrospective (see Baggott et al. 2001; Jerome and Baggott 2003; Jerome 2004). Longitudinal studies have assessed people who chose to self-administer ecstasy prior to study enrollment (Daumann et al. 2004A; DAumann et al. 2004E; Zakzanis and Young 2001) or who choose to self-administer ecstasy during the course of the study (Lieb et al. 2002). To date, there remains only one genuinely prospective study of the long-term effects of MDMA in humans (Ludewig et al. 2003; Vollenweider et al. 2000). Data from this study has been presented at conferences, and there are plans for the publication of at least one report from this study (Gamma, personal communication). The researchers failed to detect reduced serotonin transporter sites or impaired cognitive function three weeks after administering 1.5 to 1.7 mg/kg MDMA.

The difficulties inherent in the majority of studies of ecstasy users have already been discussed in the IB and previous updates to the IB, and elsewhere (Baggott et al. 2001; Cole and Sumnall 2003; Green 2003; Jerome and Baggott 2003; Jerome 2004) and so will not be discussed in greater detail. Because retrospective studies make comparisons between volunteers who chose to self-administer ecstasy and volunteers who chose not do so, they are unable to eliminate the possible impact of pre-existing differences

between these groups, including differences that may have led to the decision to self-administer ecstasy repeatedly. As well, a majority of these studies use small sample sizes, and up until very recently, samples were often poorly matched on self-reported use of other substances. Recent studies have used better strategies, such as employing both polydrug using and drug-naïve controls (Thomasius et al. 2003), or seeking out both ecstasy users and controls reporting very moderate use of other substances (Halpern et al. 2004). A recent investigation employing hair analysis in a sample of people who believed they had only taken ecstasy (Kalasinsky et al. 2004) suggests that even in these cases, people might have unwittingly exposed themselves to other compounds, such as MDA and amphetamines. Kalasinsky and colleagues examined hair samples from 21 Toronto-area ecstasy users. They detected MDMA in most hair samples, but they also detected the related compound MDA in the hair of 19 of 21 participants (90%) and amphetamine or methamphetamine in 12 participants (57%). These findings are significant because some researchers have hypothesized that MDA and amphetamine pose an equal or greater risk of neurotoxicity than MDMA. Because of the aforementioned methodological flaws and because a large number of studies only include individuals who repeatedly use ecstasy in uncontrolled settings with a lifetime exposure far exceeding levels in clinical trials, studies in ecstasy users do not provide a good model for predicting effects in clinical trials of MDMA.

Research published subsequent to March 2004 continues to support our initial risk/benefit analysis, and does not increase or decrease estimated risks of a few exposures to MDMA. Recent studies of psychological well-being in ecstasy users lend even more support to the existence of an association between polysubstance use, especially when intense, and self-reported psychological problems, and not a unique or specific link between ecstasy use and psychological difficulties. However, data in this area was inconclusive at the time of the IB, and so this does not reflect a great change in estimated risk. Likewise, recent studies continue to find impairment in memory and executive function (planning and decision making) reported in previous studies. Earlier and more recent studies continue to detect impairment in the same areas of cognitive function, including verbal and visual memory and various facets of executive function. The only potentially novel findings reviewed here are of a relationship between ecstasy use and polysubstance use, and detection of a positive association between intensity of ecstasy use and intensity of substance use (Butler and Montgomery 2004; Milani et al. 2004; Sumnall et al. 2004A). These findings are significant because they suggest that when researchers did not control for polysubstance use, at least some of the effects they attributed to ecstasy use may have been at least partly due to use of use of other substances. Beyond such potential qualifications in interpreting previous studies, reports appearing after the most recent update of the literature review do not alter our analysis of the risks and benefits of participating in clinical trials of MDMA.

Studies in Naturalistic Settings and Retrospective Studies

Investigators have used retrospective studies (e.g. Cohen 1995; Peroutka et al. 1988; Liester et al. 1992; Solowij and Hall 1992) to investigate the acute subjective effects of ecstasy. Some researchers have turned to naturalistic or on-site investigations (e.g. Curran

et al. 2004; van Wijngaart et al. 2001), or they have assessed effects of self-administered ecstasy in the laboratory (Brookhuis et al. 2004). Findings from naturalistic and retrospective studies have generally matched findings from clinical trials, with differences discussed in the initial literature review (Baggott et al. 2001). Some researchers have studied specific effects or behaviors, such as ecstasy effects on skills related to driving motor vehicles (Brookhuis et al. 2004). In this study, discussed in the last most recent update to the IB, the researchers found impaired performance an hour after self-administration of ecstasy, and even greater impairment three to five hours later after additional consumption of ecstasy and other substances.

One naturalistic study was published subsequent to the last previous update of the literature review (Hoshi et al. 2004), and researchers in England presented data from a study in a naturalistic setting at the Proceedings of the Physiological Society (Wolff et al. 2004). Study findings in both cases are novel but not strongly related to the risks or benefits of MDMA in humans. In addition to these naturalistic studies, a retrospective study of ecstasy users in Brazil that was published in 2003 has been located and reviewed here (de Almeida et al. 2003).

Researchers assessed changes in ecstasy users' accuracy in recognizing facial expressions immediately after drug self-administration and four days afterwards (Hoshi et al. 2004). In this naturalistic study, participants matched a series of faces, presented via computer, with the appropriate emotion label (anger, disgust, fear, happiness, sadness or surprise). Hoshi and colleagues tested participants at a nightclub and again four days later. Participants were 16 people reporting use of ecstasy at the nightclub (on "Day 0") and 21 people who reported they had not used ecstasy that evening. The researchers also assessed mood, self-reported subjective effects, and pulse rate. Since seven of the 21 controls had taken ecstasy previously, this study is not a comparison of ecstasy users with non-users. Hoshi and colleagues found that people who had just taken ecstasy were more accurate than controls in detecting facial expressions of fear on Day 0, and less accurate than controls in recognizing expressions of fear on Day 4. Ecstasy intoxication was associated with less self-reported aggression and sedation, while ecstasy was sub-acutely associated with increased self-reported aggression, but the authors failed to detect any changes in depressive symptoms, as measured via Beck Depression Inventory (BDI) on either testing day. As expected from clinical trials, ecstasy intoxication was associated with elevated pulse rate. Self-reported subjective effects of ecstasy, such as increased energy and teeth-grinding, were similar to those reported in clinical trials and retrospective studies, and there was a trend for ecstasy users to report greater openness to others than controls on Day 0, and similar levels of openness to others on Day 4. Self-reported subjective effects, changes in mood and physiological effects were similar to those reported in other studies, and sub-acute effects seen on Day 4 were also similar to findings in clinical trials and retrospective studies (e.g. Liechti et al. 2001; Solowij and Hall 1992; Vollenweider et al. 1998). Findings concerning changes in sensitivity to spotting fearful expressions in others await future investigation. It is possible that shifts in attention to facial expressions are one of the factors involved in producing the entactogenic effects of MDMA, such an increase in closeness to others. On the basis of laboratory research altering tryptophan levels, the authors propose that decreased ability

to spot expressions of fear four days later might be indicative of lower brain serotonin levels, but this finding is also preliminary. Though this research team often re-assesses participants seven days after drug use (see Curran and Travill 1998), they did not report assessment of this sample seven days later. It is notable that ten of 16 ecstasy users in this study reported some use of cocaine on Day 0, while only one control reported cocaine use, raising the possibility that at least some of the effects seen on either day are due to combined use of cocaine and ecstasy. It should also be noted that the researchers did not test tablets, urine or hair for presence of MDMA on Day 0. However, the effects reported by participants suggest that most of them had taken MDMA or a related entactogen.

Another team of researchers in England assessed pulse rate, blood pressure, plasma levels of cortisol, arginine vasopressin and oxytocin, plasma sodium levels and urinary osmolality (number of particles in urine, an indirect measure of electrolyte content) in 51 people attending a nightclub (Wolff et al. 2004, data presented at the Proceedings of the Physiological Society). The researchers measured pulse rate immediately prior to visiting the club and an unspecified number of hours later, after clubbing. Twenty-one out of 31 participants with a positive urinary drug screen tested positive for MDMA. Only one participant reported using both alcohol and ecstasy. Like Hoshi et al, Wolff and colleagues reported elevated pulse rate in ecstasy users (though the abstract does not directly compare ecstasy user pulse rate with pulse rate people who had not used ecstasy). The researchers did not present all data on blood pressure in this abstract, but stated that all of the five participants who had blood pressure considered hypertensive had taken MDMA during the evening. Though Wolff and colleagues reported on various changes in plasma neuroendocrine hormone concentrations, the only statistically significant finding was for higher plasma oxytocin in people who had taken MDMA when compared with people who had not taken MDMA. The other non-significant findings were of elevated cortisol in all participants that was greater in people who had taken MDMA, and elevated vasopressin in people who had taken MDMA. Plasma sodium was significantly reduced immediately after MDMA, but not after using alcohol or other substances. Urinary osmolality was higher in people who had taken MDMA when compared with controls, but this difference was not statistically significant. Somewhat surprisingly, plasma sodium levels in this sample were not correlated with vasopressin levels. Wolff and colleagues compare sodium levels from the naturalistic study with levels detected in a human trial of 40 mg MDMA (Henry et al. 1999; Forsling et al. 2001). The researchers consider the data presented here and in their previous studies as a partial explanation for hyponatremia seen after ecstasy use in uncontrolled settings. Study findings do not suggest that the previous controlled study underestimated or overestimated risk of hyponatremia after MDMA. It should be noted that when hyponatremia appears after ecstasy use in uncontrolled settings, this rare but serious complication probably arises from the combined effects of excessive water consumption, vigorous exercise without electrolyte replacement, and direct pharmacological effects of MDMA or related compounds. Participants in research studies will not be exercising vigorously, and researchers investigating MDMA-assisted therapy further reduce risk of hyponatremia by restricting liquid consumption and providing participants with electrolyte-containing beverages. In addition to providing information related to risk of hyponatremia, these findings hint at a possible role for oxytocin in some of the physiological and subjective

effects of MDMA. However, the researchers did not assess relationships between drug effects and plasma oxytocin levels.

Researchers in Brazil interviewed 52 regular (habitual) ecstasy users living in Sao Paolo (de Almeida et al. 2003). This study collected information about drug use parameters and habits, self-reported reasons for using ecstasy, attitudes toward ecstasy use, and desirability of the acute effects of ecstasy. Like samples of European and North American ecstasy users, Brazilian ecstasy users tended to restrict use to weekends, with the majority of participants taking ecstasy one or more times a month but no more than once a week. Most participants used the drug for dancing or socializing. Though de Almeida and colleagues did not make direct comparisons between their sample and samples of ecstasy users outside Brazil, it seems that Brazilian ecstasy users might be slightly older on average and more likely to have completed a college degree than ecstasy users in Europe or North America. Thirty-five (67.3%) participants reported that ecstasy effects were mostly positive, while 17 participants stated that effects were both positive and negative. Positive effects were similar to those reported in clinical trials and retrospective studies, and included euphoria (happiness, being at ease), increased energy, feeling tender, and increased closeness to others. Negative effects were also similar to side effects reported in retrospective studies of ecstasy users and controlled trials of MDMA, and included dry mouth, perceived elevation in heart rate and insomnia. Hence it appears that Brazilian ecstasy users take ecstasy in settings similar to those favored by European or North American ecstasy users, and that they report experiencing similar positive and negative drug effects.

Data on physiological effects gathered in both of the naturalistic studies described above are similar to findings in clinical trials and retrospective reports, and include elevated pulse and blood pressure. Subjective effects and changes in mood assessed by Hoshi and colleagues are also similar to effects seen in previous naturalistic studies performed by the same group of researchers (Curran et al. 2004) and others (Brookhuis et al. 2004), and seen in clinical trials (for example Cami et al. 2000; Grob et al. 1996; Harris et al. 2002; Lamers et al. 2003; Liechti et al. 2001; Tancer and Johanson 2003). Each naturalistic study presented some new data on the effects of ecstasy. However, degree of accuracy in detecting fearful expressions does not directly affect estimated risks or benefits of MDMA, and changes in plasma oxytocin in ecstasy users do not significantly increase estimated risk of hyponatremia for people taking part in clinical trials of MDMA. Both reports may stimulate further basic research concerning MDMA effects on social cognition (thinking about actual or imagined others) and on neuroendocrine effects of MDMA. A retrospective study of ecstasy users in Brazil suggests that people in disparate cultures report that ecstasy produces similar positive and negative effects.

Mood and Psychological Well-Being

Findings supporting a link between ecstasy use and decline in psychological well-being, changes in mood or impulsivity were inconclusive when first reviewed in 2001 (Baggott et al. 2001). At that time, some studies reporting significant findings (e.g. Parrott et al. 2000) while others did not (e.g. Verkes et al. 2000). While there continue to be studies

that find differences between ecstasy users and controls in these areas, an increasing number of them report that these differences, when apparent, are not uniquely related to ecstasy use. As early as 2001, Daumann and colleagues found that self-reported psychological problems were more closely associated with cannabis use than ecstasy use (Daumann et al. 2001). Findings from a more recent longitudinal study by the same research team support this conclusion (Daumann et al. 2004). One study reported that former ecstasy users, but not moderate or heavy current users, scored higher on measures of depression (de Win et al. 2004), while another study found that while both current and former ecstasy users had higher depression scores than non-drug users, only current ecstasy users had higher depression scores than polydrug user controls (Roiser and Sahakian 2003). A third study detected higher anxiety scores in both current and former users, but only when compared with non-drug users, and not when compared with polydrug users (Thomasius et al. 2003). Recent studies also continued to find that changes in mood and psychological well-being were more strongly related to substance use generally than to ecstasy use (Bond et al. 2004; Daumann et al. 2004; Dafters et al. 2004). The bulk of recent research findings suggest a complex relationship between ecstasy use, drug use and changes in psychological well-being.

To date, four studies published between March 2004 and January 2005 examined mood, impulsivity or psychological well-being in ecstasy users (Butler and Montgomery 2004; Milani et al. 2004; Singer et al. 2004; Sumnall et al. 2004A). These studies tended to report significant differences in mood between people reporting ecstasy use and people not reporting any lifetime use. However, in every study, it appeared that presence and intensity of polysubstance use was equally or more strongly associated with decline in psychological well-being or increased impulsivity than ecstasy use. Two studies suggest that heavy ecstasy users are more affected than light users (Butler and Montgomery 2004; Milani et al. 2004). It should be noted that two studies found an association between intensity of ecstasy use and intensity of polysubstance use. None of the studies provide support for an increased estimate of risk of decline in psychological well-being after a limited number of exposures to MDMA, and all studies offer at least preliminary evidence that earlier findings of an association between ecstasy use and psychological problems are at least partially due to the failure to match groups for presence and intensity of substance use.

While three of four studies compared ecstasy users with non-ecstasy users, none of the studies employed matched samples or selection of samples on the basis of drug use characteristics, and in all cases participants were divided on the basis of responses to drug use questionnaires. Study participants include university undergraduates (Butler and Montgomery 2004), dance event attendees or club-goers (Singer et al. 2004; Sumnall et al. 2004A), and young residents of selected English and Italian cities (Milani et al. 2004). In most cases, participants were below the age of 30. Three of four studies focused on mood or psychological problems (Milani et al. 2004; Singer et al. 2004; Sumnall et al. 2004A), while the fourth examined impulsivity (Butler and Montgomery 2004).

Anxiety

A study comparing 42 young ecstasy users (9 tablets per year, lifetime consumption not reported) and 58 non-ecstasy recruited from the same neighborhoods in Cleveland, Ohio, failed to find increased anxiety scores on the Brief Symptom Inventory (BSI), a measure similar to, but shorter than, the more commonly used Symptom Checklist 90 (SCL90) (Singer et al. 2004). In a study of 768 young people residing in London, Manchester (UK), Rome and Padua, Milani and colleagues also failed to find higher self-reported anxiety in either light or heavy ecstasy users when compared with non-ecstasy users (Milani et al. 2004). These researchers divided their sample into six groups on the basis of drug use, and made comparisons across all groups. Light ecstasy users in this study reported a lifetime consumption of 7.26 ± 6.79 occasions, with an average dose per occasion of 1.3 ± 0.9 tablets, while heavy users reported a lifetime consumption of 321 ± 368 occasions, and an average dose of 3.2 ± 1.5 tablets. Milani and colleagues detected an interaction between gender and drug use, wherein women reporting use of alcohol and tobacco reported more anxiety, depression and somatization (feelings of physical discomfort or illness) than men with the same drug use histories. Lastly, Sumnall and colleagues reported that lifetime and weekly ecstasy use was associated with higher scores on one measure of anxiety (the Beck Anxiety Inventory, or BAI), in a sample of 100 dance event attendees probably residing in or near Liverpool, England, but not with higher scores on another anxiety measure (Sumnall et al. 2004A). However, these researchers also found that higher BAI scores were also associated with greater lifetime amphetamine use, units of alcohol consumed per week and frequency of use for a number of substances.

Depression and Symptoms of Depression

Three studies assessed self-reported depressed mood or depression symptoms (Milani et al. 2004; Singer et al. 2004; Sumnall et al. 2004A), and of those three, only one (Sumnall et al. 2004A) detected a significant association between ecstasy use and increased depressive symptoms. In their assessment of young residents of London, Manchester, Rome and Padua, Milani et al. failed to find associations between ecstasy use and increased levels of depressive symptoms. Both light (lifetime consumption of 7.26 ± 6.79 occasions) and heavy (lifetime consumption on 321 ± 368 occasions) ecstasy users did not have higher depression scores than people reporting no drug use, little drug use, or polydrug use without ecstasy use (Milani et al. 2004). However, as was the case with anxiety in this study, the researchers detected an interaction between gender and drug use history, with women who reported cannabis use or light ecstasy use having higher depression scores than men with the same drug use histories. In their comparison of young Cleveland-area residents, Singer and colleagues found only a trend for ecstasy users to have higher depression scores than non-ecstasy users, and these findings only became apparent when the researchers employed a more lenient test of significance. These researchers found that ecstasy users in their sample were more likely than non-users to report experiencing childhood physical abuse or neglect and emotional neglect, raising the possibility that pre-existing factors related to increased rates of childhood abuse might also have affected both depressive symptoms and patterns of drug use.

Lastly, Sumnall and colleagues found that frequency of ecstasy use, but not lifetime ecstasy use, was associated with higher depression scores in their sample of 100 Liverpool-area dance event attendees. People in this sample who drank more units of alcohol per week and who drank more frequently also reported more symptoms of depression.

Aggression and Hostility

Two studies that assessed aggression or hostility in ecstasy users failed to detect increased levels of either psychological problem (Milani et al. 2004; Singer et al. 2004). Milani and colleagues assessed hostility with a modified version of the SCL90R, and Singer and colleagues assessed hostility with the BSI. As well, ecstasy users assessed by Singer and colleagues did not report experiencing any increased aggression or delinquency-related problems when compared with non-ecstasy users. Previous research has sometimes found increased aggression or aggressive response to threat in ecstasy users (see Gerra et al. 2001), while other researchers have found that anger is more accessible for both polydrug users and ecstasy users when compared with population norms (Bond et al. 2004). Recent study findings do not provide evidence for a link between ecstasy use and increased anger, aggression or hostility.

Impulsivity and Risk-Taking

Only one study published subsequent to the last update of the IB directly assessed impulsivity in ecstasy users (Butler and Montgomery 2004). In this study, 254 undergraduates completed a questionnaire on drug use history and patterns of use, and the Impulsivity, Venturesomeness and Empathy scale (IVE), a measure of self-reported trait impulsivity, and 249 of the 254 participants completed the Bets16, a behavioral measure of risk taking and impulsivity designed by the researchers. Participants completing the Bets16 evaluated pairs of bets consisting of one small but guaranteed win and one larger but less certain win. 28 undergraduates in this sample reported using ecstasy on fewer than 20 occasions, 18 reported use on more than 20 occasions, 116 did not report using any illicit substance, 55 reported cannabis use only, and 37 reported polydrug use without use of ecstasy. Butler and associates found that all participants who had used at least two illicit substances had higher Impulsiveness and Venturesomeness scores. Polydrug users, light ecstasy users and heavy ecstasy users also had higher novelty-seeking scores on the Tridimensional Personality Questionnaire (TPQ) than non-drug and cannabis users. Heavy ecstasy users were significantly more likely to favor high-risk bets than non-drug users, but moderate ecstasy users were no more likely than other groups to favor risky bets. Taken together, these findings suggest that, as one might expect, people reporting polydrug use are more impulsive (acting without thinking) and venturesome (consciously or intentionally taking risks) than people who do not report polydrug use. Butler and colleagues also detected an association between heavy ecstasy use and increased risk-taking. It should be noted that heavy ecstasy users in this sample also reported using higher maximum and average doses of ecstasy per use (1 versus 1.55 tablet average dose, and 1 versus 3.3 tablets maximum dose). A number of recent studies found an association between intensity of and scope and intensity of polydrug use (Milani et al. 2004; Scholey

et al. 2004; Sumnall et al. 2004A). Hence it is possible that lifetime ecstasy dose, size of dose per use, or use of other drugs may all contribute to increased risk-taking in heavy ecstasy users.

Though not referred to as a measure of impulsivity per se, Singer and colleagues reported that ecstasy users were more likely than non-ecstasy users to engage in risky sexual behavior, such as unprotected sex (Singer et al. 2004). Ecstasy users in this sample of Cleveland-area residents also differed from non-users in several respects, including use of other drugs, more self-reported life problems, and history of childhood abuse, raising the possibility that risky sexual behavior might be related to these factors as well as or in addition to ecstasy use. However, increased likelihood of sexual risk-taking may be similar in nature to the increased likelihood of making or preferring risky bets seen in undergraduates reporting heavy ecstasy use. When examined together, both studies suggest an association between ecstasy use and impulsivity, but neither strongly suggests a unique relationship.

Other Psychological Problems and Traits

In their study of 100 Liverpool-area dance event or nightclub attendees, Sumnall and colleagues assessed symptoms of obsessive-compulsive disorder (OCD) with the Padua Inventory-Revised (PI-R) and dissociative symptoms with the Dissociative Experience Scale (DES). Obsessive compulsive symptoms include frequent washing or checking, and dissociative experiences refer to cases where people feel as if they or their surroundings are not real. Ecstasy use was unrelated to reporting obsessive-compulsive symptoms, but frequency of ecstasy and amphetamine use were both associated with increased levels of dissociation. Sumnall and colleagues note that these experiences of dissociation did not trouble study participants, possibly because scale items resemble drug effects (Sumnall et al. 2004A).

Self-reported life problems, including those related to health, friends, family, school, and vocation (current or planned career or work) were assessed by Singer and colleagues (2004). The 42 ecstasy users taking part in this study (no lifetime consumption listed; 9.9 tablets in last year) indicated higher rates of problems with family, peers, vocation, social life and leisure, but they did not report having more health-related problems or increased delinquency-related problems. Ecstasy users were also more likely than non-users to have taken at least one other substance. Taken together, these findings suggest that at least in some cases, regular ecstasy use is associated with and perhaps preceded by relational and other life problems. Study findings do not conclusively demonstrate a unique link between ecstasy use and these life problems.

Concluding Remarks

Similar to previous research examining mood, personality and psychological well-being in ecstasy users and controls, research findings published between March 2004 and January 2005 remain inconclusive but tend to offer only tenuous support for a unique link between ecstasy use and decline in psychological well-being. Recent research found links

between ecstasy use and anxiety and depression, but these studies did not an association between ecstasy use and aggression or hostility. One study found that heavy ecstasy use was associated with greater risk-taking, but that polydrug use, with or without ecstasy use, was associated with self-reported impulsivity and novelty-seeking. Since all of the studies used retrospective designs, none of the findings demonstrate a causal relationship between repeated ecstasy use and psychological problems, and at least some data, like that of Singer and colleagues (2004) concerning increased childhood abuse or neglect in ecstasy users, suggest that at least some differences may be due partially or wholly to pre-existing factors.

Relationships between polydrug use and ecstasy use were found in all four studies. Three of four studies indicated that ecstasy users were more likely than non-users to have used other substances besides ecstasy, and three of four studies also found that intensity of ecstasy use was associated with intensity of substance use. This means that samples of ecstasy users are more likely to contain polysubstance users than samples of non-users. An association between presence and intensity of polysubstance use and presence and intensity of ecstasy use also suggests that findings from previous studies comparing ecstasy users and non-ecstasy using controls could have been affected by other variables, such as factors leading to or supporting polydrug use.

Findings from recent research studies are generally similar to previously reported findings and continue to find that relationships between use of any one drug and a specific psychological trait, problem or behavior are complex. Very few findings implicate a unique association between ecstasy use and increased depression, anxiety, hostility, impulsivity or other psychological problems. If anything, these and other recent reports suggest that the original analysis offered in the IB overestimated the risk of decline in psychological well-being for repeated ecstasy users and for participants in clinical trials of MDMA. Relying on both earlier and more recent research studies, it appears that participants in human trials of MDMA face a very minimal risk of decline in psychological well-being.

Cognition

Researchers have investigated cognitive function in ecstasy users for over fifteen years (see Baggott et al. 2001; Krystal et al. 1992). Most studies report finding subtle but detectable impairments in executive function and memory in ecstasy users, though these results are not found in all studies. Findings concerning cognitive function in ecstasy users have been reviewed in the IB and in each successive update of the IB (Baggott et al. 2001; Jerome and Baggott 2003: Jerome 2004), and are addressed in other publications as well (Cole and Sumnall 2003A). A number of recent studies suggest that moderate ecstasy use is not associated with impaired cognition (Gouzoulis-Mayfrank 2003; Halpern et al. 2004), and two studies found that former ecstasy users fared worse than current users on some measures of memory (Curran et al. 2003; Thomasius et al. 2003). Recent research continues to detect impaired memory and executive function in ecstasy users, but does support an estimation of more than minimal impairment in cognitive function for participants in clinical trials of MDMA.

Five studies of cognitive function in ecstasy users and controls were published between March 2004 and January 2005 (Daumann et al. 2004B; Daumann et al. 2004E; Daumann et al. 2004D; Fisk et al. 2004; Wareing et al. 2004A), and one study examining the contribution of various drugs to cognitive function within a sample of polydrug users (Verdejo-Garcia et al. 2004). Comparisons of ecstasy users and controls were performed by two research teams, one in Germany (Daumann et al. 2004A; Daumann et al. 2004B; Daumann et al. 2004C) and one in England (Fisk et al. 2004; Wareing et al. 2004A). All of these studies compared the performance of ecstasy users and controls on memory tasks (Daumann et al. 2004A; Daumann et al. 2004B; Daumann et al. 2004C; Fisk et al. 2004; Wareing et al. 2004A), and two studies compared ecstasy users and controls on measures of executive function (Fisk et al. 2004; Wareing et al. 2004A). The researchers examining a sample of people enrolled in a substance abuse program measured executive function, but not memory (Verdejo-Garcia et al. 2004). Additionally, the study of self-reported and behavioral impulsivity described earlier (Butler and Montgomery 2004) could be considered a measure of executive function.

The focus in all research reviewed here was on assessing specific cognitive domains rather than on comprehensive assessments of cognitive function. Studies compared ecstasy users with cannabis users (Daumann et al. 2004B; Daumann et al. 2004C), and light polydrug users (Daumann et al. 2004D; Fisk et al. 2004; Wareing et al. 2004A), and made comparisons between continuing versus abstinent ecstasy users roughly eighteen months after a first assessment (Daumann et al. 2004D). None of the studies employed non-drug using controls. Four of five studies used retrospective designs (Daumann et al. 2004B; Daumann et al. 2004C; Fisk et al. 2004; Wareing et al. 2004A), and one was longitudinal (Daumann et al. 2004D). Though one study made comparisons over time, none of the studies measured cognitive function before and after onset of ecstasy use, rendering all studies retrospective at this level of comparison.

As has been the case with previous reviews, findings from these studies are sometimes contradictory, but suggest that ecstasy users have subtle impairments in some areas of executive function and memory, while but not in other areas of memory or executive function. Ecstasy users had lower scores in specific areas of executive function (Fisk et al. 2004; Verdejo-Garcia et al. 2004). Likewise, ecstasy users had lower scores on specific measures of memory, such as measures of visual memory (Daumann et al. 2004B) or computation span (Fisk et al. 2004; Wareing et al. 2004A), but showed little to no differences on measures of working memory (Daumann et al. 2004B; Daumann et al. 2004D; Fisk et al. 2004; Wareing et al. 2004A). A longitudinal study failed to find an association between continued ecstasy use and impaired memory (Daumann et al. 2004D) despite detecting an association between continued use and changes in brain function. The within-subjects study found an association between intensity of ecstasy use and impaired working memory (Verdejo-Garcia et al. 2004), while intensity of cocaine and cannabis use were associated with impaired executive function. None of the studies produced findings that would lead to a change in the estimated risk for study participants in trials of MDMA offered in the IB (Baggott et al. 2001). As was true of research

examined in previous reviews, findings supporting impaired memory in ecstasy users continue to be stronger than findings of impaired executive function.

Executive Function

Executive function refers to skills related to planning and decision-making, and is sometimes associated with impulsivity-related behaviors such as response inhibition. Approximately a quarter to a half of studies of executive function in ecstasy users have detected some impairment in this area (Baggott et al. 2001; Jerome and Baggott 2002; Jerome 2004). It is possible that mixed results in this area continues to encourage further investigations into executive function. An increasing number of researchers have concluded that executive function is not unitary and may consist of at least three to four separate domains (Alting von Geusau et al. 2004; Fisk et al. 2004; Verdejo-Garcia et al. 2004). These domains are shifting or set shift, updating and inhibition. Some researchers, such as Verdejo-Garcia and colleagues, also consider working memory to be a component of executive function.

Both 42 current ecstasy users and 17 former ecstasy users who reported being abstinent for at least six months had lower scores than 31 moderate polydrug users on computation span, a measure of number-related memory and executive function, though all three groups scored similarly on a measure of verbal recall and executive function (Wareing et al. 2004A). Current users in this study reported a lifetime consumption of 553 ± 681 tablets and had last taken ecstasy 21 ± 25 days before testing, and former ecstasy users reported a lifetime consumption of 385 ± 362 tablets, and had last taken ecstasy 781 ± 616 days previous to testing. Analyses controlling for amount of alcohol, cannabis and tobacco used in the last three months continued to find lower computation span scores in current and former ecstasy users. In a study employing the same "span" tasks and a random generation task (Fisk et al. 2004), the same team of researchers reported that 44 ecstasy users with a lifetime consumption of 377 ± 343 tablets and who were abstinent from ecstasy for 76 ± 192 days had lower computation span scores than 59 moderate polydrug users. However, Fisk and colleagues failed to find any differences between ecstasy users and moderate polydrug users on the random item generation task, a commonly used measure of executive function.

Behaving impulsively can be considered a component of planning or decision-making, and so may be viewed as an aspect of executive function, possibly related to "inhibition" in the model proposed by Miyake (as cited in Fisk et al. 2004). As described in "Mood and Psychological Well-Being", undergraduates who used ecstasy on 20 or more occasions were more likely to favor risky bets than undergraduates reporting no illicit substance use (Butler and Montgomery 2004). Moderate ecstasy users, who had used ecstasy on fewer than 20 occasions, were not more likely to make risky bets than non-drug users. It is notable that all polydrug using individuals in this sample had higher scores on a measure of impulsivity and venturesomeness, but that only heavy ecstasy were more likely to favor risky bets. Study findings suggest an association between intensity of ecstasy use and impulsivity, but they also raise issues as to whether reduced

inhibition in ecstasy users may be partially related to polydrug use or pre-existing factors leading to polydrug use.

Researchers in Spain investigated possible relationships between intensity of substance use and performance on tests of executive function and working memory (conceived of as a component of executive function) in 38 detoxified polydrug users enrolled in a rehabilitation program (Verdejo-Garcia et al. 2004). Intensity of use was defined as average dose per use multiplied by frequency of use per month multiplied by chronicity of use. The researchers did not provide figures for lifetime ecstasy consumption, but stated that average dose per use times frequency of use was 6.47 tablets, and duration of use was 0.8 years. The researchers failed to find any associations between intensity of ecstasy use and measures of cognitive flexibility or response inhibition, but found a relationship between intensity of ecstasy use and working memory, described below. Generalizing from these study findings should be approached with caution, as the sample was restricted to people being treated for substance abuse or dependence, and the researchers did not make any cross-group comparisons.

Studies that found ecstasy users had difficulties with one or more component of executive function did not agree on the identity of the component. In a study reviewed in the most recent update to the IB, Alting von Geusau and colleagues found that men reporting ecstasy use were impaired on "cognitive flexibility" or shifting when compared with men who did not use ecstasy (Alting von Geusau et al. 2004), though female ecstasy users were no more likely than female controls to exhibit this difficulty. These findings are somewhat similar to those reached in a study that did not divide executive function into components (Halpern et al. 2004), except that Halpern and colleagues found that both men and women who used ecstasy, particularly those reporting use on 50 or more occasions, had lower scores on a measure of shifting. By contrast, Fisk and colleagues found ecstasy users were impaired on information updating (Fisk et al. 2004). Finally, Verdejo-Garcia and colleagues found an association between intensity of ecstasy use and impaired working memory (Verdejo-Garcia et al. 2004). Except for the use of Digit Span for working memory, these studies did not share any measures in common, so it is possible that differences in measures might lie behind differences in results. Additionally, and as noted above, one of the studies did not compare across groups but instead made within-group comparisons. These findings may also reflect the need for a clearer working definition of executive function.

When examined alongside previous research, the most recent publications continue to present findings supporting a complex relationship between repeated ecstasy use and impairments in executive function. There is enough evidence supporting such a relationship, yet some studies fail to detect it, and others suggest that only certain aspects of executive function are affected by ecstasy use while others are not.

Working Memory

For the most part, research reviewed in the IB and both previous updates to the IB have found lower scores on measures of working memory in at least some groups of ecstasy

users (Baggott et al. 2001; Jerome and Baggott 2002; Jerome 2003). However, some later publications have qualified these findings (see for instance Alting von Geusau et al. 2004; Curran et al. 2003; Halpern et al. 2004). It is notable that in the last update to the IB, researchers using Digit Span as a test of working memory were less likely to detect differences between ecstasy users and controls than researchers using other measures, such as the N-back task or Spatial Span (see Daumann et al. 2003; Hanson and Luciana 2004; Wareing et al. 2004B). Despite these clarifications, the majority of previous studies detected at least some impaired working memory in repeated ecstasy users.

Three studies examined working memory in ecstasy users (Daumann et al. 2004D; Verdejo-Garcia et al. 2004; Wareing et al. 2004A), with only two making between-group comparisons. Two of three studies did not find impaired working memory in ecstasy users, while the third reported an association between intensity of ecstasy use and impaired working memory.

Daumann and colleagues assessed working memory in a longitudinal study of ecstasy users, comparing people who reported continued ecstasy use eighteen months after baseline with people who reported abstinence from ecstasy after baseline (Daumann et al. 2004D). At baseline, both groups performed similarly on the N-back task, a measure requiring participants to delay response to a target until after observing a specified number ("N") of successive non-target presentations. Eighteen months after baseline, the nine current or "continuing" ecstasy users, who reported a lifetime consumption of 185 ± 202 tablets, did not make significantly more errors than the eight former ecstasy users (lifetime consumption = 243.8 ± 271.9 tablets). Current users responded more slowly than abstinent users on the most difficult level of the task, but they responded more quickly on less difficult levels. In another study using Digit Span and Word Span, Wareing and colleagues failed to detect significant differences in working memory task performance in 42 current ecstasy users, 17 former ecstasy users, and 31 moderate polydrug users (Wareing et al. 2004A). Yet in their study of 38 substance-free polydrug users enrolled in a drug rehabilitation clinic, Verdejo-Garcia et al. detected an association between intensity of ecstasy use and lower Digit Span scores (Verdejo-Garcia et al. 2004). Considered in the light of recent findings of an association between intensity of ecstasy use and intensity of polydrug use (e.g. Milani et al. 2004; Sumnall et al. 2004A), the association that Verdejo-Garcia and colleagues detected may be due at least in part either to intensity of polysubstance use or to pre-existing factors leading to intense substance use. When taken together, findings from these three studies offer only weak support for a relationship between repeated ecstasy use and impaired working memory.

Verbal and Visual Memory

Researchers have assessed memory in ecstasy users perhaps since the first studies in ecstasy users appeared (e.g. Gouzoulis-Mayfrank et al. 2000; Krystal et al. 1992; Morgan 1999; Rodgers et al. 2000), with lower scores detected in ecstasy users when compared with controls. One of the few longitudinal studies of ecstasy users found lower scores a year after continued ecstasy use (Zakzanis and Young 2001), with prose recall especially affected. A discussion of these studies and their limitations can be found in the IB

(Baggott et al. 2001) and elsewhere, as noted earlier in this review. Researchers continue to find that ecstasy users perform less well on measures of memory than non-drug using or cannabis-using controls. Other studies have found that cannabis use is more closely associated with reductions in memory than ecstasy use (Dafters et al. 2004; Simon et al. 2002), while still others have found lower verbal memory scores in former, and not current, ecstasy users (Curran et al. 2003; Thomasius et al. 2003). A quantitative analysis of ten research studies detected an effect of ecstasy use on immediate recall, and an effect of cannabis use on delayed recall (Verbaten 2003). While the two studies that employed the strictest matching of ecstasy users and controls on use of other drugs failed to detect impaired verbal memory in current users (Thomasius et al. 2003; Halpern et al. 2004), a number of studies have found this difference, suggesting that repeated use of ecstasy is associated with impaired memory, especially impaired verbal recall.

Two studies published between March 2004 and January 2005 assessed memory in ecstasy users and controls (Daumann et al. 2004B: Daumann et al. 2004C). Both were performed by the same team of researchers in Germany. One study assessed both verbal and visual memory (Daumann et al. 2000D), and the longitudinal study described earlier assessed memory with a requiring participants to learn associations between images (faces) and verbal information (professions) (Daumann et al. 2004). Both the English and Spanish research teams assessed working memory only (Fisk et al. 2004; Verdejo-Garcia et al. 2004; Wareing et al. 2004A).

Researchers compared learning and memory task performance in 12 ecstasy users and 12 controls matched on cannabis use (Daumann et al. 2004B). Daumann and colleagues found that ecstasy users reporting a lifetime consumption of 201.7 ± 224.2 tablets who had used ecstasy 51.6 ± 56.4 days previous to testing performed similarly to 12 cannabis user controls (Daumann et al. 2004B). In another study comparing ecstasy users with cannabis users (Daumann et al. 2004C), 13 Ecstasy users reporting a lifetime consumption of 324.5 ± 416.63 tablets and 13 cannabis user controls performed the LGT-3, a measure of memory consisting of two verbal memory tasks (pair association recall, prose recall) and two visual memory tasks (visual association task, spatial association). Ecstasy users in this study scored significantly lower than cannabis user controls on immediate recall on the visual association task, and showed a trend for scoring lower on the three memory tasks. The same study found that lifetime ecstasy consumption and average ecstasy dose per use were inversely associated with visual association scores, implying a link between intensity of ecstasy use and impaired visual memory. These findings are somewhat surprising, since in most previous studies, repeated use of ecstasy is more strongly linked with impaired verbal recall than visual recall (Baggott et al. 2001). However, some studies published after the initial review have found that ecstasy users score lower on measures of visual recall (e.g. Fox et al. 2002; Gouzoulis-Mayfrank et al. 2003). These studies continue to support the possibility that repeated exposure to ecstasy poses a risk to verbal or visual memory.

Other Functional Domains

Only one of six studies published subsequent to the most recent update of the IB examined a cognitive functional domain beyond executive function and memory (Verdejo-Garcia et al. 2004). Verdejo-Garcia and colleagues administered a test of analogical reasoning to people in a substance abuse clinic (Verdejo-Garcia et al. 2004). They found that intensity of ecstasy use was associated with impaired analogical reasoning, which involves recognizing similarities between one problem or relationship and applying that information to the analogous problem. Some researchers have reported detecting impaired reasoning in ecstasy users (McCann et al. 1999; Gouzoulis-Mayfrank et al. 2000), while others have failed to find general differences in reasoning (Bhattachary and Powell 2001; Dafters et al. 2003), and many studies match participants on the basis of estimated verbal IQ (see for example Curran et al. 2003; Morgan 1999; Reneman et al. 2001B). It is also notable that rather than sampling from dance event attendees, undergraduates, or through word of mouth, Verdejo-Garcia and colleagues assessed people enrolled in a drug abuse treatment clinic. Hence it is possible that findings from this sample may not be readily generalizable to ecstasy users not diagnosed with a substance abuse problem. There are very few findings assessing reasoning apart from IQ in ecstasy users. Given the paucity of study findings relating specifically to reasoning in ecstasy users and the unusual nature of this sample, it is difficult to interpret these findings.

Concluding Remarks

Recent research on cognitive function in ecstasy users continued to find selective impairments in memory and executive function. Recent study findings offer a stronger case for impairment in verbal and visual recall than for impairment in working memory or executive function, but impairments in all three areas are detected in at least one study. Because most researchers employed retrospective study designs, these findings do not offer clear support for a causal relationship between ecstasy use and impaired memory or executive function. However, it is notable that studies that matched controls and ecstasy users on use of other substances still found that ecstasy users have lower scores on measures of memory. These findings are not cause for revising the estimated risk of impaired memory from exposure to MDMA in controlled settings. As noted in previous reviews, evidence supports the existence of such a risk, but an examination of study findings also suggests that this risk is minimal for people enrolled in clinical trials of MDMA.

Functional and Structural Imaging Studies

After noting that signs of damage to brain serotonin axons appeared in animals after repeated doses of MDMA, researchers have used various imaging methods to detect similar effects in humans. Early studies used PET to measure number of serotonin transporter sites with radioactive compounds, or ligands (McCann et al. 1998; Reneman et al. 2001A; Semple et al. 1999). Other studies have sought to detect signs of brain injury or stress by measuring levels of compounds associated with neuronal injury

(Chang et al. 1999; Obergriesser et al. 2001; Reneman et al. 2002B), or by measuring numbers of 5HT2A receptors with radioactively labeled 5HT2A receptor antagonists (Reneman et al. 2002A). Previous imaging studies have been reviewed in the IB and in subsequent updates to the IB (Baggott et al. 2001; Jerome and Baggott 2003; Jerome 2004). To date, PET imaging studies have consistently found fewer serotonin transporter sites in the brains of current ecstasy users, while results of studies measuring substances associated with brain injury have been less consistent (Chang et al. 1999; Obergriesser et al. 2001; Reneman et al. 2001C; Reneman et al. 2002B). Earlier reviews have also noted a dissociation between findings from imaging studies and findings from studies of cognitive function in ecstasy users. For instance, Reneman and colleagues found lower levels of serotonin transporter in women reporting ecstasy use, and not in men, while this research team never detected gender differences in cognitive function (compare Reneman et al. 2001A and Reneman et al. 2001B). Imaging studies reviewed in the two most recent updates to the IB indicate a risk of reduced serotonin system function after repeated ecstasy use that appears to resolve after prolonged abstinence (Buchert et al. 2003; Reneman et al. 2001A).

Four imaging studies comparing the brains of ecstasy users with those of controls have been published between March 2004 and January 2005. These include two structural imaging studies (Buchert et al. 2004; Daumann et al. 2004C), and two functional magnetic resonance imaging (fMRI) studies, both performed by the same research team (Daumann et al. 2004B; Daumann et al. 2004D). Imaging and assessments of cognitive function were made in all four samples, though one research team chose to publish study results for the two assessments separately (Buchert et al. 2003; Buchert et al. 2004; Thomasius et al. 2003). One structural imaging study used voxel-based PET (Buchert et al. 2004) and the other used MRS, and each of the fMRI studies measured brain function during different types of cognitive task. For the most part, findings reported in these studies were similar to at least some previously reported findings.

Buchert and colleagues used voxel-based imaging to re-examine PET scans made with the radioligand McN5652 in 30 current ecstasy users, 29 former ecstasy users, 29 polydrug user controls and 29 non-drug user controls (Buchert et al. 2004). Previously, they had published an analysis of these scans using regions of interest (Buchert et al. 2003). Current ecstasy users in this study reported a lifetime consumption of 831 ± 1269 tablets, and were abstinent from ecstasy for 25 ± 15 days, former users reported a lifetime consumption of 793 ± 677 tablets and were abstinent for 520 ± 486 days, and samples were matched for gender and use of other substances. As was true of their initial analysis (Buchert et al. 2003), the researchers found that current ecstasy users, but not former ecstasy users, had lower numbers of serotonin transporter sites. The re-analysis detected lower serotonin transporter sites in a wider number of areas than detected in the previous study, including cingulate as well as caudate and thalamus. When scans of current users were compared with scans of same-gender polydrug users, the researchers discovered that female ecstasy users had fewer serotonin transporter sites than polydrug using women, while male ecstasy users had very few areas of lower serotonin transporter sites when compared with polydrug using men. The researchers also found that ecstasy using women had wider areas with lower numbers of serotonin transporter sites when compared with

polydrug using women. These findings are similar to results of a PET imaging study that used a different ligand, Beta-CIT (Reneman et al. 2001A). Buchert and colleagues' voxel-based analysis found slightly lower numbers of serotonin transporter sites in ecstasy users than their initial analysis (Buchert et al. 2003), but they still found comparably small changes in serotonin transporter sites when compared with the first study performed with the ligand McN5652 (McCann et al. 1998). It is notable that to date, very few studies of cognitive function in ecstasy users have detected any interactions between gender and ecstasy use, and when detected, the findings are of greater impairments in men, and not women (Alting von Geusau et al. 2004; Bolla et al. 1998). There are probably many reasons for this dissociation brain serotonin transporter levels and cognitive functioning in ecstasy users, including imprecision in both types of measurement. However, such a mismatch between the two types of finding indicates that assessments of memory or executive function cannot serve as an indirect measure of brain serotonin transporter sites.

Daumann and colleagues measured levels of compounds associated with neurons in order to detect neuronal injury in the brains of 13 ecstasy users (lifetime consumption of 324.54 ± 416.63 tablets) and 13 cannabis user controls with proton magnetic resonance spectroscopy (^{1}H-MRS) (Daumann et al. 2004C). Participants in this study also completed measures of verbal and visual memory, as described above in "Cognitive Function." Daumann and colleagues expected to find lower ratios of one compound (N-acetylaspartate, or NA) to another (creatine, or Cr) in the hippocampus. The researchers detected a trend for ecstasy users to have lower left hippocampal NA/Cr ratios, but this trend did not reach significance. Levels of NA and choline (Cho) in frontal brain areas were found to be similar across the two groups. Lifetime consumption of ecstasy and average dose per use were associated with impaired immediate visual recall (see "Verbal and Visual Memory.") However, the researchers failed to detect an association between any of the ecstasy use parameters they measured and reduced hippocampal NA/Cr ratios. Because ecstasy users in this study were more likely than controls to report amphetamine use, it is possible that lower NA/Cr ratios are associated with use of this psychostimulant. An earlier publication using MRS failed to find any signs of decreased NA in the brains of ecstasy users (Chang et al.1999) while another study detected decreased frontal cortex NA in ecstasy users (Reneman et al. 2002B). Variance in intensity of ecstasy use and use of other substances across samples, as well general variance, might at least partially explain mixed results from MRS studies. The findings of Daumann and colleagues support a dissociation between results of their imaging study and assessment of cognitive function, so that measures of NA/Cr ratios cannot serve as indirect evidence of functional impairment.

As well as conducting the MRS study described above, Daumann and colleagues also performed two functional imaging studies (Daumann et al. 2004B; Daumann et al. 2004D). In one study, brain function was compared in ecstasy users and cannabis user controls while they performed a learning and memory task, and a control task that did not involve learning or memory (Daumann et al. 2004B). Twelve ecstasy users reporting a lifetime consumption of 201.7 ± 224.2 tablets had lower left hippocampal activity when retrieving associations than cannabis users, and they did not show the bilateral increase in

hippocampal activity usually seen during retrieval (Daumann et al. 2004B). However, there were no detectable associations between parameters of ecstasy use, such as lifetime consumption or average dose per use, and differences in brain activity. These findings share similarities with the MRS findings described earlier, as both affect the left hippocampus and neither is associated with impaired cognitive function. In another functional imaging study, Daumann and colleagues measured the brains of ecstasy users and controls while they performed the N-back task described above in "Working Memory." When performing the most difficult block of the n-back task, nine current users reporting abstinent from ecstasy for 42.5 ± 32.8 days prior to testing showed a greater increase in parietal brain activity when compared with 8 former ecstasy users who reported being abstinent over a year ago (Daumann et al. 2004D). Average dose per use across the entire period of this longitudinal study was associated with greater parietal activity during the N-back task in current ecstasy users. Daumann and colleagues interpreted their findings as demonstrating that ecstasy use produced long-term effects that grew with continued use and that did not vanish after cessation of use. However, these long-term effects were not linked to changes in working memory. It is interesting that both functional imaging studies failed to find associations between changes in brain activity and scores on memory or working memory tasks. These findings suggest that cognitive function cannot be treated as an indirect measure of differences in patterns of brain function. Since functional imaging studies were not performed alongside scans of serotonin transporter sites, it is difficult to establish whether differences in brain activity are associated with reduced serotonin system function.

Concluding Remarks

It appears that studies comparing current ecstasy users with former users and polydrug users continue to find a slight but significant decrease in numbers of serotonin transporter sites in current, but not former, ecstasy users. To date, two studies performed on different samples and using different ligands reported that women who used ecstasy had a greater reduction in serotonin transporter sites than men (Buchert et al. 2004; Reneman et al. 2001A). The most recent investigation of compounds associated with neuronal injury (Daumann et al. 2004C) adds to the collection of inconclusive findings in this area. One functional imaging study detected differences in the brain activity of ecstasy users and cannabis users during working memory tasks, but found very few differences in task performance (Daumann et al. 2004B). Likewise, the other functional imaging study found differences in brain activity in current and former ecstasy users (Daumann et al. 2004D) without finding impaired task performance in current users. Taken together, these study findings continue to suggest that repeated use of ecstasy may produce changes in the serotonin system or in brain function. However, the apparent dissociation between imaging data and scores on measures of memory raise questions about the relationship between these measures. Participants in all of the imaging studies reported extensive use of ecstasy. Moderate users do not seem to exhibit the same changes in serotonin system function (Reneman et al. 2001A) or in cognitive function (Gouzoulis-Mayfrank et al. 2003; Halpern et al. 2004). This suggests that the risk of incurring either damage to serotonin axons or changes in brain function is minimal after a small number of exposures to MDMA during a clinical trial.

Overall Conclusion

A thorough examination of studies in ecstasy users published in the last year did not provide any cause for revising the estimated risks or benefits to study participants in clinical trials of MDMA. As was the case of research reviewed in 2003, recent studies offer stronger support for impaired memory than for decline in psychological well-being in ecstasy users. Imaging studies continue to offer inconclusive support for long-term effects of ecstasy on brain structure and function, with data from PET scans detecting reduced numbers of serotonin transporter sites, particularly in women, and functional imaging studies finding differences in brain activity that are not reflective of changed task performance. None of the recently reported study findings increase the degree of estimated risk, and findings failing to support a unique link between ecstasy use and decline in psychological well-being do not significantly reduce this risk. Since lifetime ecstasy consumption in these studies far exceeds the number of doses employed in clinical trials and since at least two recent studies indicate that moderate ecstasy users do not show reduced serotonin transporters or impaired memory or executive function (Gouzoulis-Mayfrank et al. 2004; Halpern et al. 2004; Reneman et al. 2001B), study participants in trials of MDMA should experience minimal risk of these changes. It remains true that study participants in clinical trials of MDMA may face several risks related to psychological well-being, cognitive function and brain function, but that these risks remain minimal.

In vitro and non-human animal studies

Introduction

Neuroscientists interested in studying pharmacological and toxicological effects of MDMA continue to conduct studies in rats, mice, and monkeys, and in vitro studies with human, rodent, and other cell lines. The bulk of the research published between March 2004 and January 2005 focused on MDMA neurotoxicity, metabolism, and pharmacology, while other studies focused on specific issues relating to MDMA pharmacology or toxicity.

This review will only examine research containing information that can be used in making estimates of the potential risks or benefits of MDMA to humans, and hence will not examine drug discrimination or the majority of behavioral pharmacology studies. However, the review will address some of this research if and when findings shed light on relevant research. People wishing to learn more about studies excluded from this review can learn more about drug discrimination and behavioral research in non-human animals in reviews published in 2003 (Cole and Sumnall 2003B; Green et al. 2003).

Most of the studies published subsequent to the most recent update of the IB sought to clarify or further examine an issue, problem or hypothesis proposed in previously published reports. These include studies examining neurotoxicity and hyperthermia. In a few cases, researchers tried to replicate features of the dance event or nightclub environment to see whether these features altered the degree of MDMA neurotoxicity or hyperthermia. Though results from several recent publications question the accuracy of brain serotonin and other measures as indicators of MDMA neurotoxicity, none of these studies produced findings that significantly increase or decrease the estimated risk to human study participants in trials of MDMA. There are now even more studies clearly suggesting that variations in the enzyme CYP2D6 are of little significance in estimating risk of adverse events with MDMA, but evidence supporting this case has already been discussed in the IB itself and in all successive updates (see Baggott et al. 2001; Jerome and Baggott 2003; Jerome 2004). Studies in non-human animals continue to find reduced brain serotonin and other changes associated with damage to serotonin axons, and some studies detected effects on muscle tissue, the immune system and the liver. High ambient and body temperature continues to play a role in MDMA toxicity in non-human animal models. A number of studies sought to understand mechanisms of MDMA-induced hyperthermia, and include a review seeking to incorporate findings on hyperthermia from separate research programs in non-human animals. Studies published between March 2004 and January 2005 offer a number of interesting findings concerning MDMA, but do not alter the degree of estimated risks and benefits faced by humans exposed to MDMA in clinical trials.

Neurotoxicity

A majority of the published studies of MDMA in non-human animals are concerned with MDMA neurotoxicity. The history of MDMA neurotoxicity research in non-human animals has been addressed in the IB and in updates to the IB (Baggott et al. 2001; Jerome and Baggott 2003; Jerome 2004), and elsewhere (Cole and Sumnall 2003; Green et al. 2003). Researchers continue to study mechanisms of MDMA neurotoxicity, environmental factors that might exacerbate or attenuate it, and means of abating it. Some reports offer support for one of several models of MDMA neurotoxicity, with a number of studies seeking to establish the source of oxidative stress seen after MDMA.

Several recently published studies call into question the use of reduced brain serotonin as an indicator of MDMA neurotoxicity, while others failed to find signs of neurotoxicity after lower doses of MDMA. If researchers continue to discover difficulties with using reduced brain serotonin as a measure of MDMA neurotoxicity, this may lead to questioning the significance of a large body of research findings in non-human animals. However, at present, findings questioning the accuracy of lower brain serotonin as a marker of MDMA neurotoxicity have not yet been replicated, and it is notable that other researchers have detected damage to serotonin neurons without relying on measures of brain serotonin, serotonin transporter sites, or levels of glial activation (Callahan et al. 2001). While future study findings may lead to a reconsideration of the significance of previous studies in non-human animals, reconsideration of this research would be premature at present.

A study in mice measured striatal dopamine and serotonin levels after central or peripheral administration of MDMA or the reactive MDMA metabolite HHMA (DHMA) (Escobedo et al. 2004). When assessed seven days after drug administration, the researchers found that peripherally administered MDMA reduced striatal dopamine and metabolites, but only at the highest dose of centrally administered MDMA, a dose far in excess of brain MDMA levels measured after peripheral administration. Peripherally injected HHMA failed to reduce striatal dopamine seven days later, but intrastriatal administrations at the highest dose tested reduced striatal dopamine. HHMA was detected in plasma, but not in brain, after MDMA administration. The researchers' failure to detect HHMA in brain indicator that HHMA does not cross the blood-brain barrier and does not arise during metabolism in the brain. Study findings fail to indict HHMA as a direct producer of MDMA neurotoxicity in mice, but suggest that HHMA may be metabolized into other compounds that are responsible for mouse MDMA neurotoxicity. Since mice are the only species so far to show dopamine neurotoxicity after MDMA, studies in mice are probably not relevant to estimates of human MDMA neurotoxicity, but the findings may be helpful in considering studies in other species that seek to separate the effects of MDMA from effects produced by its metabolites.

Three studies in rats generated interesting findings concerning the presence and significance of presumed indicators of MDMA neurotoxicity (Orio et al. 2004; Sanchez et al. 2004; Wang et al. 2004). Two of these studies were performed in the Dark Agouti rat strain (Orio et al. 2004; Sanchez et al. 2004), a rat strain believed to be more sensitive

to the effects of MDMA than other strains, while the other study was performed in the more typical Sprague-Dawley strain. Orio and colleagues administered a single i.p. injection of 12.5 mg/kg MDMA to Dark Agouti rats housed in a comfortably warm (22 C. or 72 F) or a cool (4 C, or 39 F) environment, and then assessed microglial activation and levels of glial fibrillary acidic protein (GFAP) in frontal cortex and hypothalamus one to 24 hours later, and 7 days later (Orio et al. 2004), with increased GFAP levels considered indicative of neurotoxicity. The researchers found increased microglial activation in rats kept at warm and cold ambient temperatures, and failed to detect any changes in GFAP in rats from either condition. The cold environment prevented hyperthermia, but did not prevent microglial activation, or increased levels of proinflammatory cytokine IL-Beta. Orio and colleagues also determined that increased IL-Beta was not associated with hyperthermia, since an IL-Beta antagonist failed to attenuate MDMA-induced hyperthermia, and the serotonin uptake inhibitor fluoxetine reduced IL-Beta levels without reducing hyperthermia. The authors concluded that 12.5 mg/kg MDMA produced a stress response in the brain, but that it did not produce neurotoxicity. In a study in Sprague-Dawley rats, Wang and colleagues also assessed GFAP levels after administering three doses of 7.5 mg/kg MDMA in a six-hour period (Wang et al. 2004). Rather than use interspecies scaling, Wang and colleagues selected their dose with "effects scaling," which estimates dose equivalence on the basis of producing similar pharmacological effects, such as drug recognition in drug discrimination studies. Rats had lower brain serotonin levels after MDMA, but they did not have fewer brain serotonin transporter sites or increased GFAP levels. By contrast, the known serotonin neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) decreased numbers of serotonin transporter sites and increased GFAP levels. Assuming that GFAP levels and number of serotonin transporter sites, are accurate measures of serotonin neurotoxicity, these findings suggest that reduced serotonin levels may be less indicative of neurotoxicity, and that at least at the doses used in this study, MDMA does not produce effects similar to those of a known serotonin neurotoxin. Lastly, Sanchez and colleagues assessed serotonin and dopamine levels in Dark Agouti rats given three doses of up to 6 mg/kg MDMA in a six-hour period to simulate human "binge" dosing, and compared effects of 4 mg/kg given in a 19 C (66 F) and a 30 C (86 F) environment. MDMA dose-dependently reduced brain serotonin and increased body temperature, but failed to reduce brain dopamine at either dose regimen or when given at either ambient temperature. This research was spurred on in part by findings of MDMA-induced dopamine toxicity in non-human primates that were later retracted when the researchers learned that they had administered the wrong drug (Ricaurte et al. 2002; Ricaurte et al. 2003).

If the results described above are replicated in future studies, they may indicate that lower brain serotonin may not be a sufficient indicator of damage to serotonin axons. If this is the case, then less weight may be given to studies that determined MDMA neurotoxicity solely on the basis of lower brain serotonin levels. Since each study assessed specific brain areas, such as cortex (Orio et al. 2004; Sanchez et al. 2004; Wang et al. 2004), hypothalamus (Orio et al. 2004) or hippocampus (Sanchez et al. 2004; Wang et al. 2004), it is possible that researchers would have detected fewer serotonin transporter sites or increased GFAP in other brain areas. It is also notable that researchers have used other methods of detecting MDMA neurotoxicity, such as reduced anterograde axonal transport

after repeated doses of MDMA (Callahan et al. 2001), so recent research cannot be used to dismiss all MDMA neurotoxicity studies. Since one of the studies listed above found dose-dependent reductions in brain serotonin (Sanchez et al. 2004), and since the study of anterograde axonal transport relied on considerably higher doses of MDMA, it might also be the case that higher dose regimens could have affected serotonin transporter and GFAP levels. Nevertheless, these provocative findings should stimulate further research into evaluating the accuracy and sensitivity of MDMA neurotoxicity measures. Secondly, these studies suggest that MDMA effects on brain serotonin are dose-dependent. Controversy over interspecies scaling (de la Torre and Farre 2004) may lead to more research employing "effects scaling" or other means of calculating equivalent drug doses across species.

Researchers investigating the relationship between bioenergetic stress and MDMA neurotoxicity in neonatal and adult rats assessed glycogenolysis (glycogen consumption) in the left caudal quarter of rat brains one hour after a single s.c. administration of 20 mg/kg MDMA (Darvesh and Gudelsky 2004). The authors found that MDMA given at 17 º C (approximately 63 º F) was not associated with increased glycogenolysis in 21-day old or 70-day old rats, and that MDMA administered at 24 º C (approximately 75 º F) increased glycogenolysis in both groups of rats. However, when striatal serotonin was assessed seven days after MDMA administration, Darvesh and Gudelsky only detected reduced brain serotonin in adult rats kept at both 17 and 24 º C, suggesting that glycogenolysis was not associated with MDMA-induced reductions in brain serotonin. If brain glycogenolysis is a good measure of bioenergetic stress, then these findings in turn suggest that bioenergetic stress does not play a prominent role in MDMA neurotoxicity. The possibility that reduced brain serotonin may not be a good indicator of damage to serotonin axons further clouds an interpretation of these findings, since in this case, glycogenolysis, but not reduced brain serotonin, may be a better marker of MDMA neurotoxicity.

Garcia-Osta and colleagues compared the effects of 10 mg/kg MDMA with 5 mg/kg para-chlorophenylalanine (PCPA), a compound that interferes with serotonin synthesis and depletes brain serotonin, in rats killed from 2 to 48 hours post-drug (Garcia-Osta et al. 2004). The researchers measured serotonin in frontal cortex and hippocampus, tryptophan hydroxylase (TPH) gene expression (TPH is an enzyme that transforms tryptophan into serotonin), and levels of a protein associated with neuronal activity. MDMA and PCPA both increased this protein and decreased TPH gene activity early after drug administration, but MDMA was associated with increased TPH activity 48 hours post-drug, while PCPA reduced TPH gene expression 48 hours later. MDMA produced a transient decline in frontal cortex serotonin that began returning to normal 48 hours later, and a lasting reduction in hippocampal serotonin that was present 48 hours later. By contrast, PCPA-associated reductions in frontal cortex and hippocampal serotonin levels were still detectable 48 hours later. These study results are in agreement with earlier reports of MDMA interfering with serotonin synthesis (e.g. Che et al. 1995; Johnson et al. 1992), but also suggest that MDMA does not permanently reduce TPH activity, and that reductions in TPH gene expression does not necessarily indicate MDMA neurotoxicity.

Sveen and colleagues incubated neonatal rat hippocampal slices for a week with 50 or 100 mcM MDMA (about 0.996 or 1.93 mg), and examined slices with the Fluoro-Jade stain (Sveen et al. 2004). They failed to find any signs of neurotoxicity. The lack of evidence for MDMA-associated neuronal damage implies that serotonin projections into the hippocampus or MDMA metabolites are needed to produce neurotoxicity . However, neonatal rats may be less sensitive to the neurotoxic effects of MDMA than adults (Broening et al. 1995; Broening et al. 2001; Colado et al. 1997, but see Meyer and Ali 2002). One previous in-vivo study using Fluoro-Jade staining in rat forebrain detected signs of neuronal loss after higher doses of MDMA in combination with hyperthermia (Schmued et al. 2002) while another study examining mouse striatum detected signs of oxidative stress, such as indications of DNA fragmentation, but no signs of cell death (Fornai et al. 2004).

Winsauer and colleagues compared the effects of intra-peritoneal (i.p.) and subcutaneous (s.c.) MDMA on brain neurotransmitter levels in rats (Winsauer et al. 2004), with i.p. dosing considered more similar to oral dosing, the most common route of administration in humans. Rats received twice-daily doses of saline or 10 mg/kg MDMA for four consecutive days, and the researchers measured monoamine levels in brain areas from rats killed 3, or 13 to 14 days after the last saline or MDMA administration. Both i.p. and s.c. MDMA reduced serotonin in most brain areas three days post-MDMA, but brain serotonin levels were normal in most brain areas 14 days later, though levels of the serotonin metabolite 5-HIAA was reduced in some brain areas. Neither i.p. nor s.c. MDMA altered brain norepinephrine levels, and s.c. MDMA failed to change dopamine or epinephrine levels either three or 14 days later. Winsauer and colleagues detected increased midbrain dopamine levels three days after i.p. MDMA, decreased hippocampal dopamine 13 to 14 days later, lower midbrain dopamine levels three days post-drug and lower hypothalamic dopamine 13 to 14 days later (Winsauer et al. 2004). It is notable that this is the first study of brain epinephrine levels after MDMA. Because changes in brain neurotransmitter may be secondary to changes in other neurotransmitter systems or may reflect changes in numbers active receptors for a neurotransmitter, the significance of these findings is not clear, and the authors do not make claims concerning the cause or causes of altered neurotransmitter levels. To date, no one has reported any other indicators of damage to other neurotransmitter systems after MDMA.

Two in vitro studies examined the role played by dopamine in MDMA serotonin neurotoxicity, either in connection with MDMA metabolites (Jones et al. 2004) or in production of oxidative stress through generation of hydrogen peroxide (Hrometz et al. 2004). In the first study, the researchers assessed inhibition of the serotonin transporter and reactive oxygen species levels (the direct cause of oxidative stress) in human cell cultures made to express the human serotonin or dopamine transporter and exposed to one of four compounds; MDMA, MDA, or two thioether compounds proposed as potential MDMA metabolites. Jones and colleagues found that all four compounds inhibited the serotonin transporter and produced reactive oxygen species in cells. The thioether compounds produced more reactive oxygen species than MDMA or MDA. Jones and colleagues also found that all four compounds, especially the thioether

compounds, ushered dopamine into cells expressing the serotonin transporter. In the other study, Hrometz and colleagues measured hydrogen peroxide in serotonin-transporter expressing human cells after exposure to dopamine, MDMA, and the two combined (Hrometz et al. 2004). The combination of MDMA and dopamine was associated with a far greater increase in hydrogen peroxide than either compound individually, and MDMA alone did not increase hydrogen peroxide. The researchers used the SSRI fluoxetine and the MAO-B inhibitor L-deprenyl to investigate whether presence of the serotonin transporter or an aspect of dopamine metabolism were involved in hydrogen peroxide production. They found that both fluoxetine and L-deprenyl reduced, but did not eliminate, hydrogen peroxide production after the combination of MDMA and dopamine. None of the combinations described above reduced cell viability. Both the work of Jones and colleagues and that of Hrometz and colleagues imply that dopamine plays a role in MDMA-associated oxidative stress.

A study in mice found that a protein called metallothionein-1 (MT-1) protected the rodents from MDMA neurotoxicity (Xie et al. 2004). Gene expression associated with this protein increased after administration of 47 mg/kg MDMA, and MT-1 "knockout" mice had lower brain dopamine after receiving four doses of 30 mg/kg MDMA than "wild-type" mice. When MT-1 "knockout" mice were given supplemental MT-1 before MDMA, their brain dopamine levels were more similar to those of wild-type mice. Xie and colleagues also found that methamphetamine and MPTP increased MT-1 gene expression, suggesting that this protein is called on in response to various compounds with dopamine neurotoxicity. This study suggests that at least in mice, some neuroprotective genes are activated in response to repeated doses of MDMA.

Previous investigations of the effects of environmental factors on MDMA neurotoxicity centered mainly around studies of ambient temperature (Malberg et al. 1996; Malberg et al. 1998; Malpass et al. 1999), and one study examined the effects of loud noise and MDMA on cardiac tissue (Gesi et al. 2002). Three studies published between March 2004 and January 2005 attempted to model specific features of common settings of MDMA use to study their effects on neurotoxicity (Darvesh and Gudelsky 2004; Gesi et al. 2004) or muscle tissue (Duarte et al. 2004). The first study will be discussed here, and the second will be addressed below in "Toxicity."

Darvesh and Gudelsky found that rats receiving a single s.c. injection of 20 mg/kg MDMA at 24° C (75 ° F) had lower striatal serotonin levels than rats that received the same dose of MDMA in a 17° C (63° F) environment, with brain serotonin measured 1 hour post-drug (Darvesh and Gudelsky 2004). It should be noted young rats did not exhibit reduced brain serotonin after receiving MDMA under either ambient temperature. Gesi and colleagues studied potential interactions between MDMA and continuously broadcast loud noise on striatal dopamine, tyrosine hydroxylase (an enzyme involved in dopamine synthesis), and GFAP in brain tissue from mice killed seven days after exposure. The researchers found that combining four injections of 7.5 mg/kg MDMA with six hours of white noise produced a greater decrease in striatal dopamine and a greater increase in GFAP than MDMA alone. The combination of prolonged loud noise and MDMA increased locomotion in an open field two hours and seven days post-

treatment, possibly indicating that MDMA and noise-exposed mice were less anxious than saline-treated mice. This research team has previously examined the effects of MDMA and loud noise on heart tissue (Gesi et al. 2002A; Gesi et al. 2002B). Humans who use ecstasy at dance events and nightclubs differ from mice in that they choose to expose themselves to loud noise and likely find the experience enjoyable, while the mice may find loud noise to be stressful. In this case, it is possible that stress, and not loud noise, may increase MDMA neurotoxicity (Johnson et al. 2002, but see Johnson et al. 2004). Future rodent models of dance events may wish to train animals to associate a reward with loud noise before combining noise with MDMA. If these study results are replicated, then they might suggest another way in which people consuming ecstasy at a party or dance event may face a greater risk than people in clinical trials of MDMA.

None of the MDMA neurotoxicity studies reviewed here present findings that require a change in the estimated risks and benefits to participants in trials of MDMA. If further research continues to find that brain serotonin and serotonin transporter levels are not reliable indicators of serotonin axon toxicity, as reported by Wang and colleagues, then a large body of research will need to be reconsidered. However, more research following along these lines is needed before concluding that earlier research overestimated MDMA neurotoxicity, and it should be noted that methods other than those described above have also detected indicators of damage or dysfunction in serotonin axons (Callahan et al. 2001). It is already recognized that higher ambient and body temperature play a role in MDMA neurotoxicity, and so recent findings do not lead to changes in the current understanding of MDMA neurotoxicity or effects on the serotonin system. As discussed in "Clinical Trials," MDMA produces a slight increase in body temperature when given in controlled settings, and clinical trials are conducted in comfortably warm environments, so that people will be very unlikely to experience the high ambient or body temperatures that exacerbate MDMA neurotoxicity in rodents. Findings of potentiated neurotoxicity when MDMA was combined with loud noise, and findings of increased hyperthermia and muscle damage when MDMA was combined with vigorous exercise, described below in "Toxicity") all point to the significance of setting in accentuating or attenuating drug-related risks. After considering current research findings, the risk of MDMA neurotoxicity in humans enrolled in clinical trials remains low.

Long-term behavioral effects of MDMA

Studies in non-human animals have examined anxiety, social interaction, and cognitive function subsequent to various doses of MDMA. Many, but not all, previous reports used MDMA in doses intended to reduce brain serotonin (for example Clemens et al. 2004; McGregor et al. 2003A; McGregor et al. 2003B; Sumnall et al. 2004B; Thompson et al. 2004). Some researchers used non-human animal studies to detect long-term effects potentially linked with ecstasy use, such as anxiety or impaired cognitive function. To date, the body of research into the long-term behavioral effects has been contradictory (Baggott et al. 2001; Green and McGregor. 2002; Jerome and Baggott 2003; Jerome 2004).

Seven studies addressing one or more behavioral effects of MDMA have been published since the last update of the literature review (Jerome 2004). Five studies assessed anxiety (Gesi et al. 2004; Ho et al. 2004; Piper and Meyer 2004; Sumnall et al. 2004B), and two examined changes in cognitive function (Piper and Meyer 2004; Winsauer et al. 2004). The researchers performed most of these studies in adult rats, though one study used "periadolescent" rats (Piper and Meyer 2004), and another used mice (Gesi et al. 2004). While fewer reports examining potential long-term effects of MDMA on anxiety in rats appeared this year than in past years, the issue remains of interest to some researchers (Ho et al. 2004; Piper and Meyer 2004; Sumnall et al. 2004B), as do the issues of sensitization to other drugs (Sumnall et al. 2004B). An investigation of the combined effects of loud noise and MDMA also assessed open field activity, usually treated as a measure of anxiety, seven days post-treatment (Gesi et al. 2004).

Ho and colleagues assessed the acute and long-term effects of a single 7.5 or 15 mg/kg dose of MDMA on Sprague-Dawley rats previously rated as low or high on trait anxiety on the basis of performance on the elevated plus-maze, with anxiety assessed again via plus-maze, open field and active avoidance. When tested 9 to 15 days later, MDMA did not increase or decrease anxiety, and the researchers did not detect an interaction between MDMA and trait anxiety. However, 7.5 mg/kg MDMA increased active avoidance in high-anxious rats and decreased it in low-anxious rats fifteen days post-MDMA, a finding that may indicate that MDMA accentuated differences in trait anxiety, or that MDMA facilitated fear-based learning in high-anxious rats and impaired fear-based learning in low-anxious rats. The researchers also detected a non-significant increase in immobility in the "forced swim" test, considered a model of "depression-like" behavior in rodents. Ho and colleagues' findings do not support the hypothesized interaction between MDMA and trait anxiety in rats proposed by Green and McGregor (2002), but it should be noted that Ho and colleagues performed a within-strain comparison rather than making cross-strain comparisons. It is interesting that a presumably non-neurotoxic dose of MDMA (7.5 mg/kg) accentuated differences in active avoidance seen in "low anxious" and "high anxious" rats, a sign that this change did not arise as a result of damage to serotonin axons. In another study examining the long-term effects of MDMA on plus-maze performance (Sumnall et al. 2004B), rats pre-treated with four injections of 10 mg/kg MDMA every 2 hours performed similarly to vehicle-treated rats. The researchers also found that MDMA and vehicle treated rats behaved similarly on the plus maze after heroin, ethanol, cocaine and MDMA, though there were differences relating to single behaviors, such as cocaine producing fewer head dips in MDMA-pretreated rats than in vehicle-pretreated rats, suggesting lower levels of anxiety. Though the MDMA dose regimen used by Sumnall et al. generally failed to alter the anxiety-related effects of other drugs, it did lower hippocampal serotonin levels and estimated numbers of serotonin transporter sites, suggesting that changed serotonin levels do not necessarily lead to changes in behavioral sensitivity to other drug.

Two studies, one in rats and one in mice, found reduced anxiety after MDMA (Gesi et al. 2004; Piper and Meyer 2004). Piper and Meyer gave "periadolescent" two doses of 10 mg/kg MDMA every five days, administering the second dose 4 hours after the first dose (Piper and Meyer 2004). Rats received approximately seven pairs of doses for a month-

long period. Approximately nine days after the final dose, these rats spent more time in the open arms of the elevated plus maze, a sign of reduced anxiety, without increased activity in the open field. Piper and Meyer detected reduction in estimated serotonin transporter binding in MDMA-treated rats that was slightly less extreme than seen after more traditional dose regimens used in MDMA neurotoxicity research, and an association between hippocampal serotonin transporter sites and reduced anxiety. In a study in mice discussed above in "Neurotoxicity", Gesi and colleagues found that mice that received a demonstrably neurotoxic regimen of MDMA in combination with prolonged loud noise demonstrated increased open field activity seven days post-drug (Gesi et al. 2004). When it was not administered along with loud noise, the same dose of MDMA altered striatal dopamine and increased markers of MDMA neurotoxicity, but did not affect open field activity.

Researchers have examined the long-term effects of MDMA on learning and memory in non-human animals, often with mixed results (see discussion in IB; Frederick et al. 1995; Frederick et al.1997; Seiden et al. 1993; Taffe et al. 2002; Taffe et al. 2003; Williams et al. 2003). Two recent publications tackled long-term effects of MDMA on learning and memory (Piper and Meyer 2004; Winsauer et al. 2004). In one study, Piper and Meyer detected impaired object recognition in "periadolescent" rats given two injections 10 mg/kg MDMA four hours apart every five days for a month (Piper and Meyer 2004). Though rats in this study had moderately reduced levels of serotonin transporter, the researchers failed to detect any relationship between numbers of serotonin transporter sites and impaired object recognition. These findings are similar to dissociations between indirect measures of serotonin transporter sites and memory and executive function in ecstasy users (see Buchert et al. 2003; Curran et al. 2003; Gijsman et al. 2002; Thomasius et al. 2003; Verkes et al. 2000).

Researchers examined the acute effects of the muscarinic antagonist (anti-cholinergic) scopolamine on learning and memory in rats before and after administering a regimen of 10 mg/kg MDMA given twice-daily on four consecutive days (Winsauer et al. 2004). As expected, scopolamine impaired acquisition and recall, and both MDMA regimens impaired acquisition and performance on the days MDMA was administered. Winsauer and colleagues found that the second MDMA regimen affected acquisition and recall to a greater degree than the first regimen. As described above in "Neurotoxicity", Winsauer and colleagues found that both i.p. and s.c. MDMA reduced serotonin in brain regions, including hippocampus, hypothalamus and (after s.c. dosing) cortex. However, contrary to expectations, the researchers found that MDMA attenuated scopolamine-induced impairment in acquisition and memory. The researchers offer several hypotheses for these findings, including the attenuation of serotonin-regulated inhibition of acetylcholine release, and the possibility that the combined effects of reduced serotonin and acetylcholine are different from reductions in each system alone. Though it is not clear that findings from a scopolamine challenge can be generalized to reduced cholinergic function in humans, as seen with Alzheimer's disease, these findings do not support claims that reductions in brain serotonin will further exacerbate impaired learning or memory seen with cholinergic deficits.

None of the studies referred to above employed comparable methods, and each focused on different long-term effects, but their findings suggest that the relationship between MDMA neurotoxicity and subsequent behavioral changes is complex and that presence or absence of changes in anxiety or learning after MDMA are not necessarily linked to damage to brain neurotransmitter systems. Similar dissociations can be seen when examining findings in ecstasy users. Findings from these studies in non-human animals do not argue for increasing or decreasing estimated risk to humans participating in clinical trials of MDMA.

Self-Administration

Previous research has found that rodents and non-human primates will self-administer MDMA and appear to find it rewarding (Fantegrossi et al. 2002; Fantegrossi et al. 2004; Robledo et al. 2004; Schenk et al. 2003; Wakonigg et al. 2004A). Two studies published between March, 2004 and January, 2005 examined MDMA self-administration (Daniela et al. 2004; Robledo et al. 2004B). In one study (Robledo et al. 2004B), both normal mice and knockout mice lacking the mu opioid receptor developed conditioned place preference, the tendency to prefer being in a location associated with a drug, in response to injections of 10 mg/kg MDMA. Unsurprisingly, these findings suggest that the rewarding properties of MDMA are more closely linked with dopamine than with opioids. Dopamine also played a role in the rewarding properties of MDMA for rats (Daniela et al. 2004). Rats stopped working for injections of 0.25 mg/kg MDMA when pre-treated with a D1 receptor antagonist, while rats worked even harder to receive 2 mg/kg MDMA when it was combined with the D1 antagonist, suggesting a role for D1 receptors in the rewarding properties of MDMA. These MDMA self-administration studies do not increase or decrease estimated abuse potential in humans. Up to approximately 6% of ecstasy users in a representative sample of Munich residents reporting ecstasy use were diagnosed with ecstasy abuse or dependence (Lieb et al. 2002), though higher figures have been reported in non-representative samples (Cottler et al. 2001; Topp et al. 2002). MDMA-naïve participants did not report wishing to use MDMA outside the laboratory (Liechti et al. 2001).

Thermoregulation

Because hyperthermia is one of the most common serious adverse effects of ecstasy use in uncontrolled settings and because it might make MDMA neurotoxicity worse, researchers continue to perform studies on MDMA and hyperthermia in rodents and in vitro (Baggott et al. 2001; Jerome and Baggott 203; Jerome 2004). Ten studies published or located between March 2004 and January 2005 examined the thermoregulatory effects of MDMA. Researchers carried out studies in rodents (Bexis et al. 2004; Blessing et al. 2003; Duarte et al. 2004; Herin et al. 2004; Ho et al. 2004; Ootsuka et al. 2004; Rusyniak et al. 2004; Sprague et al. 2004), pigs (Rosa-Neto et al. 2004) and in vitro (Rusyniak et al. 2004). Most studies examined at least one model of MDMA-induced hyperthermia, while two studies in rodents examined the effects of environmental variables on MDMA-induced hyperthermia. In addition, a team of researchers offered their synthesis of the

literature, focusing on the role of the sympathetic nervous system in MDMA-induced hyperthermia (Mills et al. 2004).

Researchers have investigated cutaneous vasoconstriction (constricted blood vessels near the skin), the sympathetic nervous system, specific neurotransmitter systems, mitochondrial metabolism, and hypothalamic activity as potential causes or factors involved in MDMA-induced hyperthermia. Blessing and colleagues reported that MDMA (6 mg/kg in rabbits, 10 mg/kg in rats) induced cutaneous vasoconstriction and hyperthermia, and that the atypical antipsychotic drugs clozapine and olanzapine reduced both effects (Blessing et al. 2003). An examination of sympathetic system activity in anesthetized rats and rabbits found that MDMA-induced hyperthermia was linked with the stimulation of spinal 5HT2A receptors (Ootsuka et al. 2004). Herin and associates reported that pretreatment with the serotonin 5HT2A receptor antagonist M100907 reduced hyperthermia in rats given 8 or 12 mg/kg S-(+)-MDMA (Herin et al. 2004), and the same 5HT2A antagonist reversed hyperthermia when given an hour after 12 mg/kg S-(+)-MDMA. Bexis and colleagues reported that administering the GABA(B) receptor agonist baclofen, but not the GABA(A) agonist muscimol, reduced hyperthermia in rats given 15 mg/kg MDMA (Bexis et al. 2004). Surprisingly, baclofen attenuated elevated body temperature without reducing MDMA-induced hyperactivity or elevated heart rate. Examining another transmitter system, Sprague and colleagues found the alpha1 receptor antagonist prazosin and the beta(3) antagonist SR59230A reduced MDMA-induced hyperthermia in rats (Sprague et al. 2004). As expected, researchers investigating the effects of MDMA on mitochondrial metabolism (discussed below) found that 40 mg/kg MDMA induced hyperthermia (Rusyniak et al. 2004), but found only slight indicators of mitochondrial dysregulation. An in vitro study performed by these researchers found that only extremely high concentrations of MDMA produced signs of dysregulation in mitochondrial metabolism. By contrast, the same research team reported that mice lacking a gene for uncoupling protein 3 (UCP3) were resistant to MDMA-induced hyperthermia (Mills et al. 2003). Sprague and colleagues also found that thyroid hormones are involved in methamphetamine-induced hyperthermia as well (Sprague et al. 2004), suggesting a common pathway for both types of drug-induced hyperthermia. Researchers in Denmark used PET imaging to scan the brains of anesthetized pigs given 1 mg/kg MDMA (Rosa-Neto et al. 2004), and found that increased cerebral blood flow in the hypothalamus was correlated with increased body temperature in individual pigs. It is notable that pig body temperature increased by 2 or 3 degrees C, whereas clinical trials using doses equal to or higher than 1 mg/kg produce only slight increases in human body temperature (see discussion below).

Researchers also examined potential impact of environmental factors and individual characteristics in MDMA-induced hyperthermia. Duarte and colleagues measured body temperature via radiotelemetry in a study of the effects of MDMA and strenuous exercise on rhabdomyolysis (muscle and organ damage) in mice (Duarte et al. 2004, see discussion below in “Toxicity”). Exercise increased body temperature, and 10 mg/kg MDMA, with or without exercise, further increased body temperature. In their study of more and less anxious rats described above in “Neurotoxicity”, Ho and colleagues reported that 15 mg/kg MDMA, but not 7.5 mg/kg, increased body temperature in both

less and more anxious rats, even though less anxious rats had higher baseline body temperatures (Ho et al. 2004).

Several investigations of MDMA neurotoxicity continue to support a role for high ambient temperature in MDMA-induced hyperthermia in rodents (Orio et al. 2004; Sanchez et al. 2004), and other researchers have studied the impact that other environmental factors, such as social interaction (Brown and Kiyatkin 2004) or ambient temperature (Darvesh and Gudelsky 2004) have on MDMA-induced hyperthermia. These studies found an association between higher ambient temperatures and higher body temperatures in rats given MDMA, and the relationship was detected in Dark Agouti and Long-Evans rats (Brown et al. 2004; Sanchez et al. 2004). One study found that interacting with an ovarectomized female rat also increased MDMA-induced hyperthermia, but not to the same degree as high ambient temperature (Brown and Kiyatkin 2004). These findings raise the possibility that people taking ecstasy in warm environments may be more likely to experience hyperthermia than people taking part in clinical trials of MDMA, placing them at greater risk for any potential MDMA neurotoxicity as well.

One of the research teams responsible for a number of rodent hyperthermia studies (e.g. Mills et al. 2003; Ootsuka et al. 2004; Sprague et al. 2004) reviewed research on MDMA-induced hyperthermia and offered a synthesis of the literature (Mills et al. 2004). Mills and colleagues posited that the sympathetic nervous system is involved in more than one mechanism behind MDMA-induced hyperthermia, with these mechanism including dysregulation of mitochondrial metabolism, vasoconstriction, and changes in monoamine release that affect brain areas that regulate thermoregulation. Mill and colleagues suggest that MDMA-induced hyperthermia can be reduced by using alpha or beta norepinephrine receptor antagonists.

Though each study used different measures and methods, an examination of these findings suggests that MDMA elevates body temperature through several potentially independent mechanisms. It is not clear how many of these mechanisms are present in humans, though Mills and colleagues note that unlike rodents, human adults do not possess brown adipose tissue, a source of non-shivering heat produced by uncoupling of mitochondrial metabolism. At doses used in controlled settings, MDMA produces a slight and sometimes undetectable increase in body temperature (see for instance Harris et al. 2002; Liechti and Vollenweider 2001; Mas et al. 1999; Tancer and Johanson 2003). Humans may be less sensitive than rodents are to the thermoregulatory effects of MDMA, or it may be that the effects will only be seen after the higher doses often used in rodent studies. Furthermore, clinical trials are not conducted in uncomfortably hot laboratories, and participants are not encouraged to exercise vigorously during clinical trials of MDMA. Participants undergoing MDMA-assisted psychotherapy will for the most part be sitting, reclining or lying down and will be involved in introspection. Hence risk of experiencing hyperthermia during a clinical trial of MDMA appears to be minimal.

Developmental Toxicity

Research on the potential developmental toxicity of MDMA has produced a number of contradictory findings (see Broening et al. 1995; Broening et al. 2001; Koprich et al. 2003; Meyer et al. 2002; Williams et al. 2003). In general, researchers found an absence of developmental effects when they gave MDMA prior to postnatal day 1, but effects appeared when they gave MDMA on or after postnatal day 10 (PND10), a period believed to be analogous to the third trimester of pregnancy in humans. Rats exposed to high and frequent doses of MDMA during this period exhibited impaired performance on measures of spatial memory (Williams et al. 2003). Some researchers have proposed that MDMA does not produce developmental effects before PND10 because it does not induce hyperthermia during this period (Aguirre et al. 1995, but see Meyer and Ali 2002). A few researchers also found increased anxiety and reduced social interactions after rats were given MDMA during a period after postnatal day 28, referred to by some as rat "periadolescence" (Bull et al. 2003; Bull et al. 2004; Fone et al. 2002).

Three studies published between March, 2004 and January, 2005 investigated the developmental effects of MDMA, two in neonatal rats and one in slightly older rats. In one study, neonatal rats received 10 mg/kg MDMA twice-daily (the second dose four hours after the first) on postnatal days 1 to 4 (Meyer et al. 2004), and in the other study, rats received 5, 10 or 10 mg/kg twice-daily MDMA from PND11 to PND20 (Vorhees et al. 2004). Meyer and colleagues found that MDMA exposure immediately after birth reduced hippocampal serotonin when assessed 25 days later, but did not reduce forebrain serotonin levels. The researchers reported that MDMA increased signs of apoptotic (programmed cell death) activity a day afterwards. Numbers of hippocampal serotonin transporter sites were reduced after MDMA 25 and 60 days post-drug, while forebrain serotonin transporter sites were reduced 60 days later. When Meyer and colleagues examined brain serotonin transporter binding 9 months post-drug, fewer serotonin transporter sites were detected in some areas (visual and somatosensory cortex, caudate-putamen, and nucleus accumbens), but not in others. Inducing hyperthermia through a warm incubator had little effect on brain serotonin, serotonin transporter sites, or signs of apoptosis. Meyer and colleagues did not assess learning, memory or locomotion. Earlier studies failed to find changes in brain serotonin after giving MDMA to similarly aged rats (Broening et al. 1995; Broening et al. 2001), possibly as a result of using different dose regimens. In the second study, Vorhees and colleagues administered MDMA from PND11 and PND20. They found an association between MDMA and impaired performance on the Morris water maze, but not in the Barnes maze, a dry version of the water maze wherein a goal box is hidden in one of several holes in a brightly lit, and thus aversive, arena (Vorhees et al. 2004). However, water maze performance in MDMA-treated rats was only impaired when this task was presented before the Barnes maze. Vorhees and colleagues did not assess brain serotonin or serotonin transporter sites, though an earlier report by the same researchers detected lower levels of serotonin and dopamine in MDMA-treated rats (Koprich et al. 2003B). In contrast with this study, previous research detected an unqualified impairment in spatial memory after the same dosing schedule (Williams et al. 2003).

These and other studies in rats suggest that MDMA administered in the third trimester of pregnancy could alter developing brain organization, and that intense prenatal exposure could subsequently impair memory or learning. Several studies point to one or more "critical periods" of increased developmental toxicity, with most studies centering on the third trimester of pregnancy, though at least one study found developmental toxicity in rats born to mothers exposed to MDMA (Koprich et al. 2003B, but see Kelly et al. 2002). As described in "Clinical Trials," a case-control comparison examining a specific heart defect failed to establish a link between ecstasy use and the defect, largely owing to the extremely low level of ecstasy exposure seen in their sample (Bateman et al. 2004). It is possible that it is difficult to establish developmental toxicity in humans because most women discontinue or curtail use upon learning they are pregnant (Ho et al. 2001). Since at least some studies detect developmental toxicity after MDMA, women who are pregnant or lactating should continue to be excluded from clinical trials of MDMA.

As described above in "Neurotoxicity," Darvesh and Gudelsky compared the effects of a single s.c. 20 mg/kg dose of MDMA on striatal serotonin 7 days post-drug in 21-day old and 70-day old rats (Darvesh and Gudelsky 2004). The authors detected reduced striatal serotonin only in adult rats. Somewhat surprisingly, these findings suggest that MDMA administered during a period occurring slightly later than the period described above failed to affect brain serotonin. However, the authors did not assess learning, memory or any other long-term effects in these rats.

Piper and Meyer administered MDMA during the period between PND35 and PND60, using a schedule of two 10 mg/kg doses spaced four hours apart, and with injections given every five days across this month-long period (Piper and Meyer 2004). As discussed above in "Long Term Behavioral Effects," rats receiving MDMA displayed less anxiety and impaired object recognition afterwards, effects not always seen in adult rats. However, few studies in adult rats have employed the dose regimen that Piper and Meyer used, so it is possible that these effects might be the result of dose regimen, and not developmental stage. As noted above, only reduced anxiety was related to changes in brain serotonin. Study findings suggest that long-term effects of MDMA may be age-dependent, but are preliminary at present. These findings raise the possibility that adults and adolescents may face risks of slightly different long-term effects, but do not increase or decrease the potential risk estimates for people taking part in clinical trials of MDMA.

Toxicity

Three studies of MDMA toxicity have been published between March, 2004 and January 2005. In one study, a team of researchers in Portugal first described in "Thermoregulation" examined the possible effects of strenuous exercise and MDMA on soleus (calf) muscle tissue by giving mice a 10 mg/kg injection of MDMA, placing them in a treadwheel set to spin at 75% of the maximum running speed for a mouse, or combining the two treatments (Duarte et al. 2004). When given alone, both strenuous exercise and MDMA were associated with signs of muscle damage, but soleus muscle fibers exhibited the most damage after a combination of exercise and MDMA, with signs of muscle damage apparent 1.5 hours after exercise and still visible 24 and 48 hours later.

Both this study and the work of Gesi and colleagues, described in "Neurotoxicity", suggest that adverse events seen after ecstasy use may due at least in part to aspects of the setting where ecstasy is most frequently used. It also remains true that humans, unlike the mice in these studies, voluntarily exercise after taking MDMA, so it is possible these findings might be due at least in part to stress, and not just to the combination of exercise and MDMA. Participants in clinical trials of MDMA will not be engaged in strenuous exercise, and so risk of muscle damage should be extremely minimal.

In a study investigating the effects of MDMA on the liver, rats received a single intra-gastric dose of 5 to 40 mg/kg MDMA, or 14 daily i.g. doses of 5, 10 or 20 mg/kg MDMA (Ninkovic et al. 2004, obtained abstract only). The researchers found increased superoxide dismutase, decreased glutathione, and increased lipid peroxidation after a single dose of MDMA, and dose-dependent increases in lipid peroxidation. Chronic MDMA also dose-dependently increased lipid peroxidation and reduced glutathione, but only the highest dose regimen (20 mg/kg) increased superoxide dismutase. Study findings indicate that oxidative stress can occur in the liver after MDMA. The lower rate of lipid peroxidation and attenuated decrease in glutathione after repeated dosing may indicate tolerance to MDMA after repeated doses, or these effects may be a sign of inhibited response to oxidative stress after repeated dosing. Previous investigations have found associations between usually high doses of MDMA and signs of hepatotoxicity, with body temperature playing a role in degree of toxicity (Beitia et al. 2000; Carvalho et al. 2002; Carvalho et al. 2003; see also Baggott et al. 2001; Jerome 2004). It is unclear whether the findings from this study are relevant to hepatotoxic potential in humans. They do not increase or decrease estimated risk of liver toxicity for study participants in clinical trials of MDMA.

Connor and associates sought to uncover the cause or causes for the decrease in the immune system stimulating and proinflammatory cytokine tumor necrosis factor alpha (TNFAlpha) and increase in the immunosuppressive and anti-inflammatory cytokine interleukin-10 (IL10) in rats given MDMA (Connor et al. 2004). The authors first confirmed that MDMA did produce these effects and demonstrated that an increase in IL-10 was not causing the reduction in TNFAlpha. The researchers then manipulated levels of stress hormones, sympathetic system activity, and activity at adrenergic (norepinephrine-related) receptors. They concluded that reduction in TNFAlpha might arise as a result of stimulating the sympathetic nervous system or from norepinephrine release, and that MDMA increased IL-10 levels through stimulating beta adrenergic receptors. The immunological effects of MDMA are already known in humans (Pacifici et al. 2000; Pacifici et al. 2002; Pacifici et al. 2004) and rats (Connor et al. 2000). Surprisingly, Connor and colleagues did not examine the effects of serotonin release on TNFAlpha or IL-10 levels, even though a study in humans suggests that preventing serotonin release through paroxetine nearly eliminated IL-10 increase (Pacifici et al. 2004). Since this report studied mechanisms for the immunological effects of MDMA without reporting any new effects, these findings do not alter estimated risk for humans taking part in clinical trials of MDMA.

In addition to these studies, a team of Dutch researchers published a report on a possible mutation of the CYP2D6 enzyme, known to play a role in the metabolism of MDMA (Keizers et al. 2004). The researchers created a mutant version of the enzyme and genetically modified bacteria (e. coli) to express the new enzyme. Keizers and colleagues compared the "wild-type" and mutant enzyme on known CYP2D6 substrates (compounds the enzyme helps metabolize), including MDMA. The mutant version of CYP2D6 produced the familiar MDMA metabolites DHMA (HHMA) and MDA, but it also hydroxylated MDMA, producing what the authors referred to as *N*-OH-MDMA. These findings are of uncertain relevance, as there is no record of this mutation ever appearing in humans, and to date, the unusual metabolites produced by the potential mutant form of CYP2D6 have only been detected in horse urine (Damasia 2003, cited in Keizers et al. 2004). An examination of research into well-known variants of CYP2D6 found in humans suggests that these variants are not associated with adverse events (Gilhooly and Daly 2002), and that higher doses of MDMA may impede MDMA metabolism in most people (de la Torre and Farre 2004).

Concluding Remarks

Research reports published between March, 2004 and January 2005 examined the pharmacology and toxicity of MDMA in non-human animals and in vitro, with the bulk of the research testing or elaborating on models of MDMA neurotoxicity. Recent studies also investigated long-term behavioral effects of MDMA, potential developmental toxicity, effects on thermoregulation, and toxicity in other organs and systems, such as the liver and the immune system. If replicated, some findings that call into question indicators of MDMA neurotoxicity in other rodent studies may lead to a re-examination of previous research. However, at present, these findings remain preliminary. Other research studies have found changes in anxiety, learning and memory. It is notable that not all changes were associated with lower brain serotonin or other signs of MDMA neurotoxicity. Studies continue to support the possibility of developmental toxicity of MDMA in rats. Finally, one study examined oxidative stress in the livers of rats given MDMA, and another explored the cause or causes of immunological changes associated with MDMA. None of the studies call for an increase or decrease in estimated risk for people taking part in clinical trials of MDMA.

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