MAPS' MDMA Investigator's Brochure
Update #1
A Review of Research in Humans and Non-Human Animals

January 20, 2003

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Introduction and Executive Summaries

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Approximately 200 papers referring to MDMA or Ecstasy (material represented as MDMA) have appeared in the English-language literature from Summer 2001, when MAPS' MDMA Investigator's Brochure (Baggott et al. 2001) was completed and subsequently submitted to the FDA, to December 2002, when the writing of this Update #1 was begun. These reports address pharmacology, acute effects in human and non-human animals, demographics of Ecstasy use, toxicity, and long-term effects. This summary will only examine original research in humans and in non-human animals directly related to potential risks, with a particular focus on studies in humans. Reviews and studies of pharmacology and behavioral pharmacology will not be addressed in this report.

With a few exceptions, there have not been any new advances in knowledge or avenues of investigation appearing since the completion of the Investigator's Brochure. The research conducted in the past year and a half lends additional support to our conclusion that there is a minimal risk of functionally significant adverse effects presented by administering two doses of 125 mg MDMA to humans within the context of the proposed clinical study of the use of MDMA-assisted psychotherapy in treatment-resistant PTSD patients.

This review will begin by examining studies in humans. Clinical trials of MDMA in humans will be examined first, followed by an examination of studies of regular Ecstasy users, with Ecstasy referring here to material represented as MDMA. Research in non-human animals will be examined and related to research in humans whenever findings are directly relevant. We will refer to Phase I clinical and preclinical research as providing information about the effects of MDMA, while we will refer to research in Ecstasy users as shedding light on the effects of illicit consumption of Ecstasy, which often contains compounds in addition to or completely other than MDMA.

Texts of the accompanying papers can be accessed on a compact disc (CD) submitted along with this Update #1, and can also be located through the use of MAPS' on-line MDMA bibliography (http://www.maps.org/wwwpb/). In several cases, we present new unpublished data supplied to us by researchers for submission only to the IRC and the FDA. Texts referred to in this review and contained on the CD represent approximately 80% of all papers published from Autumn 2001 to December 2002. Most of the missing texts are reviews or pharmacology and behavioral pharmacology articles, and none of them appear to contain new information critical to assessing risk in humans.
The review of clinical trials in humans will first describe shared features of clinical trials, including methods and samples used in these studies. After presenting an overview of

clinical trials, we will discuss study findings and relate them to the potential risks and benefits faced by research participants. The order of presentation was intended to follow the format used in the Investigator's Brochure. Physiological effects will be discussed first, followed by subjective effects, neuroendocrine effects, immunological effects, electroencephalographic measures and pharmacokinetics. Most findings relevant to risk assessment will be found in the first four sections. Clinical Phase I research conducted subsequent to the completion of the Investigator's Brochure extends and reinforces findings already described in previous research. Preliminary results from a prospective study of the effects of MDMA in drug-naïve volunteers have failed to find changes in the amount of serotonin transporter in brain or in cognitive function. Studies continue to find that up to two doses of MDMA is well-tolerated in human volunteers, often in doses exceeding 125 mg, without causing cognitive impairment or persisting psychological or physiological problems of any kind. No acute adverse events are reported in any of the recently published studies.

Preliminary data from a MAPS-sponsored study in Spain into the effects of MDMA-assisted psychotherapy in PTSD patients will also be presented and discussed in relation to the proposed study. [Data not presented in publicly available review.] Unfortunately, the data set is very small (N=6), formal analyses cannot be conducted, and involves the single administration of only 50 mg, 75 mg or placebo. Nevertheless, this data does offer some information on the possible range of effects that might be produced by MDMA-assisted psychotherapy in a sample of people with treatment-resistant PTSD.

Studies in Ecstasy users will be reviewed next, with attention to findings directly affecting risk assessment. The general characteristics of these studies will be briefly discussed. Since both the Investigator's Brochure and previous reviews and critiques have addressed methodological issues relating to retrospective studies of Ecstasy users, readers are referred to this literature for a more thorough discussion. Findings relating to possible long-term effects of Ecstasy use on neurocognitive function are first described and reviewed, including examinations of verbal memory, visual memory and executive function. Investigations of the effects of Ecstasy use on neuropsychological function are next addressed, including findings on affect (general mood) and diagnosable psychiatric disorders, self-reported psychological problems, aggression and impulsivity. The body of recently published studies seeking to detect changes in serotonin function are described and reviewed, followed by studies using other methods of comparison, including electroencephalography and neuroendocrine challenge studies. Lastly, a preliminary report on long-term effects of repeated Ecstasy use on immune function is discussed.

Studies of Ecstasy users published after the completion of the Investigator's Brochure extend and support conclusions made after examining previous findings, but in a few cases, findings from recently reported studies either contradict earlier findings or offer new information concerning the potential risks of MDMA. Most studies comparing Ecstasy users to controls continue to find that Ecstasy users have selective impairment on measures of verbal recall and executive function. However, other studies have failed to find impaired verbal memory in Ecstasy users and/or have found that differences disappear once verbal intelligence and concurrent marijuana use has been taken into

account. Comparing findings across studies in Ecstasy users also suggests that changes in cognitive function may not necessarily reflect changes in serotonin function, calling into question the hypothesis that changes into cognitive function are related to Ecstasy's effect on serotonin, or to Ecstasy itself. Despite the appearance of some studies that have failed to find the expected effects of Ecstasy use on memory, and more information about possible confounding factors, risk of long-term effects on cognitive function remains a concern.

Findings reported in a growing number of recently published papers, including a large prospective epidemiological study, suggest that the association between Ecstasy use and psychiatric disorders, when apparent, is more likely due to pre-existing psychiatric disorders prior to first use of MDMA and is more strongly associated with other variables, such as cannabis use or age of onset of drug use. A majority of recently published reports strongly suggest the relationship between regular Ecstasy use and increased psychiatric complaints is complex and less certain than associations between regular Ecstasy use and neurocognitive deficits.   While an association between regular Ecstasy use and increased anxiety or depression cannot be dismissed altogether, recent research has not lent any additional support to this claim. Generally speaking, recent research would suggest that the risk of developing psychological problems or mental disorders after Ecstasy use is less likely than it first appeared.

Newly reported findings suggest that continued use of Ecstasy impaired specific immune functions over time. These findings are not yet reported in detail, and it is possible that they are the result of other factors associated with Ecstasy use, such as use of other drugs. Because the effects describe result from continued use, and not acute effects, the findings do not increase risk assessment already associated with acute immunological effects of MDMA.

Studies of Ecstasy users at best constitute the upper limits of risks faced by subjects participating in clinical research. Overall, findings from recently published reports in Ecstasy users do not substantially affect our assessment of minimal risk to participants in the proposed study receiving two doses of 125 mg of pure MDMA in a clinical setting.

After reviewing findings in humans, a section will briefly address relevant findings from studies conducted in vitro or in non-human animals, chiefly rodents and primates. This section is restricted to addressing studies that might have a direct impact on risk assessment rather than addressing the entire field of research. Studies of MDMA neurotoxicity in rodents are addressed first, with a focus on research into behavioral markers of MDMA neurotoxicity. Most studies of behavioral markers of MDMA neurotoxicity investigated the effects of MDMA on anxiety and social interaction, or examined learning and memory. Of particular interest is a primate study that demonstrated that even massive reductions in serotonin in the neocortex were not associated with cognitive dysfunction, calling into question the role of MDMA in neuropsychological findings in human Ecstasy users.

Studies of neuroendocrine effects of a neurotoxic dose regimen and imaging studies are also discussed. Developmental effects are considered separately from effects on adult animals. Effects on other physiological systems, including studies of toxicity to organs, are considered next, and the review ends with a consideration of recently conducted investigations of MDMA self-administration in rats and monkeys.

Nearly all animal studies of neurotoxicity conducted after the completion of the Investigator's Brochure merely extend or elaborate on previous findings, without either increasing or decreasing the degree of risk extrapolated to humans. The one exception is a primate study showing damage to dopamine neurons. The relevance of this study to humans is subject to considerable dispute, since research in Ecstasy users has so far failed to find any indication of decreased dopamine function except when people have also used amphetamines. Though findings of developmental effects remain inconclusive, in part due to variance in the species examined and research method employed, findings in rodents lend more support to potential developmental risks than did previous findings. These results suggest that women who are pregnant or not using effective means of birth control should still be excluded from study participation. Examinations of other physiological systems may offer some insight into cardiovascular effects seen in humans, but do not increase assessed risk. Though there are some new findings concerning effects on cardiac tissue, findings of cardiotoxicity appear only after high doses and under specific conditions. In all other cases, research into toxicity in other organs and systems replicate and extend findings already described and considered in the Investigator's Brochure

In summary, the research conducted in the past year and a half lends additional support to our conclusion that there is a minimal risk of functionally significant adverse effects presented by the administration of two doses of 125 mg MDMA in humans within the clinical context of the proposed study of the use of MDMA-assisted psychotherapy in treatment-resistant PTSD patients.

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Human Studies: Clinical Trials

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Human trials with MDMA that were ongoing or had been completed from the latter half of 2001 to December 2002 were conducted by six different research teams, including two in the United States and four in Europe. Research has been conducted in the US (Harris et al. 2002; Tancer and Johanson 2001), Spain (Hernandez-Lopez et al. 2002; Navarro et al. 2001; Pacifici et al. 2001; Pichini et al. 2002; Pizarro et al. 2002; Segura et al. 2001), Switzerland (Frei et al. 2001; Vollenweider et al. 2000), the UK (Forsling et al. 2001) and the Netherlands (Samyn et al. 2002). Researchers in Spain were in the process of conducting a MAPS-sponsored investigation into the safety and efficacy of MDMA-assisted psychotherapy in women with PTSD arising from sexual assault (before that study was halted in the Summer of 2002 as a result of pressure on the hospital from the Madrid Anti-Drug Agency, despite ongoing permission from the Ministry of Health (Bouso, personal communication). Research from nearly every team was represented in the Investigator's Brochure (e.g. Henry et al. 1999; Lester et al. 2000; Mas et al. 1999; Vollenweider et al. 1998), or unpublished data was presented within it (e.g. that of Tancer). The only teams without any publications appearing prior to the completion of the Investigator's Brochure were those of Samyn and colleagues and Bouso and colleagues. In some cases, recent publications describe findings in samples that have already been examined in previous reports (Frei et al. 2001; Harris et al. 2002).

Nearly all studies utilized a placebo-controlled, single-blind or double-blind study design, though Forsling and colleagues used a non-blinded design without placebo controls. Studies were either randomized (e.g. Frei et al. 2001) or pseudo-randomized in studies employing more than one dose condition (Harris et al. 2002; Tancer and Johanson 2001), with participants receiving the lower dose prior to the higher dose, though in each case participants remained unaware of the study design. Nearly all studies used a within-subjects design, with participants enrolled in every condition, but the study conducted by Tancer and Johanson used a between-subjects design, and the study conducted by Bouso and colleagues employed a between-subjects design.

Eleven published reports of clinical trials of MDMA in humans have appeared from Autumn of 2001 to December 2002. Unpublished data from Swiss and US studies under review for publication and preliminary data from a clinical trial in women with PTSD will also be described. [This data is not presented in the publicly available document.] Three reports describe the acute psychological and physiological effects of MDMA (Harris et al. 2002; Hernandez-Lopez et al. 2002; Tancer and Johanson 2001), and two reports address neuroendocrine effects (Forsling et al. 2001; Harris et al. 2002). One report describes EEG recorded after MDMA (Frei et al. 2001) and another reported

immunological effects (Pacifici et al. 2001). Five of eleven published studies are chiefly concerned with pharmacokinetics and drug detection in various body fluids (Navarro et al. 2001; Pichini et al. 2002; Pizarro et al. 2002; Samyn et al. 2002; Segura et al. 2001).

Research participants taking part in these studies are similar to participants who took part in the clinical trials reviewed in the Investigator's Brochure. They include male and female participants with ages ranging from 18 to 40. However, in some cases participation was restricted to males (Forsling et al. 2001; Hernandez-Lopes et al. 2002; Navarro et al. 2001; Pacifici et al. 2001; Segura et al. 2001), and all participants in the study of MDMA-assisted therapy in Spain were women (Bouso 2003, personal communication). Participation was restricted to those who were healthy, often as established through medical history, psychiatric interview and medical examination, though the six participants in the study in Spain were diagnosed with posttraumatic stress disorder. Most research teams selected participants who reported having used "Ecstasy" in the past, but participants studied by two research teams were mostly or wholly drug-naïve (Forsling et al. 2001; Frei et al. 2001; Vollenweider et al. 2000; 2003).

Doses of MDMA ranged from approximately 35 mg (0.5 mg/kg, reported in Harris et al. 2002) to between 100 and 145 mg (Bouso, personal communication; Harris et al. 2002; Hernandez-Lopez et al. 2002; Navarro et al. 2001; Pacifici et al. 2001; Pichini et al. 2002; Pizarro et al. 2002; Segura et al. 2002; Tancer and Johanson 2001), with most doses ranging from 100 to 140 mg. In their study of arginine vasopressin release, Forsling and colleagues administered 40 mg (47.6 of hydrochloride salt), and Samyn and colleagues administered 75 mg. All doses were well tolerated by study participants, and no adverse events occurred in any of the published reports.

MDMA was found to act as a sympathomimetic, stimulated the release of several hormones, and produced stimulant-like and hallucinogen-like effects in humans. There is no support for long-term effects on serotonin transporter site density or cognitive function after one or two doses of MDMA. Findings in some studies investigated newly considered neuroendocrine effects (Harris et al. 2002), detected additional MDMA metabolites (Segura et al. 2001; Pizarro et al. 2002), and sought to formally measure entactogenic effects (Harris et al. 2002).

The acute risks posed by the administration of MDMA are in most cases similar to those of other psychostimulants, such as amphetamine or methamphetamine, two compounds that have been utilized in human research with drug-naïve (e.g. Gouzoulis-Mayfrank et al. 1999a; 1999b; Justice and DeWitt 1999) or drug-experienced participants (e.g. Mas et al. 1999). Some potential long-term effects of MDMA may also be shared by psychostimulants such as methamphetamine (Davidson et al. 2001; Ernst et al. 2000), a fact already noted and discussed in the Investigator's Brochure. None of the recently conducted studies have uncovered any new or formerly unknown risks of MDMA administration in humans. There are a number of attendant risks to consuming Ecstasy in uncontrolled settings that have never been reported during laboratory studies (Henry and Rella 2001). Nonetheless, any clinical trials with MDMA must still prepare for handling the unlikely occurrence of these events.

Physiological Effects

Physiological effects of MDMA were reported in four studies. Participants included eight men (Forsling et al. 2001), eight men and women (Harris et al. 2002), nine men (Hernandez-Lopez et al. 2002) and 15 men and women (Tancer and Johanson 2001). Participants in all of the above studies were at least moderately experienced Ecstasy users. Findings from recent publications are comparable to those discussed in the Investigator's Brochure. At doses above 40 mg, MDMA elevates both systolic and diastolic blood pressure and increases heart rate (Harris et al. 2002; Tancer and Johanson 2001). Maximum increase in systolic blood pressure when compared to placebo was 20 to 38 mm Hg, and 15 to 19 mm Hg for diastolic blood pressure (Tancer and Johanson 2001) in doses ranging from approximately 75 mg to 145 mg MDMA. Heart rate was also significantly increased after approximately 75 and 105 mg MDMA, and a trend for elevated heart rate occurred with approximately 145 mg MDMA, lending support to a previously described non-linear effect on heart rate. Doses below 75 mg did not significantly elevate blood pressure or heart rate (Forsling et al. 2001; Harris et al. 2002). These findings compare well with previously reported examinations of cardiovascular effects (Grob et al. 1996, Unpublished; Lester et al. 2000; Mas et al. 1999; Vollenweider et al. 1998).

An assessment of body and skin temperature conducted in one study found a non-significant decrease in skin temperature of 5 ± 4.1 C after approximately 105 mg MDMA (Harris et al. 2002). Previously reported data from the same research team found an increase in body temperature of 0.1 ± 0.4 C after approximately 35 mg and an increase of 0.3 ± 0.4 C after approximately 105 mg MDMA (Lester et al. 2000).   As has been previously reported, researchers have failed to find significant changes in body temperature in most cases (de la Torre et al. 2000; Liechti et al. 2000).

Other physiological effects reported, such as increase in pupil size (Harris et al. 2002) were also previously reported (Cami et al. 2000). Likewise, changes in extra-ocular muscle tension reported previously (Cami et al. 2000) are replicated in a subsequent study conducted the same team (Hernandez-Lopez et al. 2002). None of the reports describe previously unreported physiological changes.

None of the findings reported are in conflict with previous research, and all reports found that MDMA could be safely administered to human volunteers. There were no reports of hypertension occurring in participants taking part in any of the studies, and there were no occurrences of adverse events during any of the clinical trials reported.

Subjective Effects and Side Effects

The acute subjective effects of MDMA were assessed in four studies (Frei et al. 2001; Harris et al. 2002; Hernandez-Lopez et al. 2002; Tancer and Johanson 2001). Participants included 16 men and women (Frei et al. 2001), eight men and women (Harris et al. 2002), nine men (Hernandez-Lopez et al. 2002), and 15 men and women (Tancer

and Johanson 2001). Participants in these studies, except that conducted by Frei and colleagues, were experienced Ecstasy users; most participants in Frei et al. (2001) had never taken Ecstasy. The findings reported by Frei and colleagues are also presented in a previously published report (Gamma et al. 2000) and will not be discussed in this review. All studies reviewed employed self-report measures administered during the experience of drug effects or immediately after they had subsided, and one study also employed observer-scored measures of psychotic symptoms. Two studies employed the Addiction Research Center Inventory (ARCI) (Hernandez-Lopez et al. 2002; Tancer and Johanson 2001), though a Spanish-language translation was used in one study (Hernandez-Lopez et al. 2002). Also used were the Hallucinogen Rating Scale, or HRS (Tancer and Johanson 2001), the Profile of Mood States, or POMS (Tancer and Johanson 2001), the Subjective Drug Effects Questionnaire, or SDEQ (Harris et al. 2002), and author-constructed visual analog scales (Harris et al. 2002; Hernandez et al. 2002; Tancer and Johanson 2001). The Positive and Negative Syndrome scale was used as an observer-scored measure in one study (Harris et al. 2002). Previous studies have already used the ARCI, POMS and HRS (Cami et al. 2000; Grob et al. 1996). The SDEQ has not been employed in research conducted prior to the completion of the Investigator's Brochure, but scores appear to be in concordance with those reported in other measures of mood and alteration in consciousness.

As previously reported (e.g. Cami et al. 2000; Downing 1986; Grob et al. 1996; In preparation; Liechti et al. 2000), MDMA induced both stimulant and hallucinogen-like effects in human volunteers. Stimulant-like effects included increases in energy, euphoria, and cognitive improvement (Hernandez-Lopez et al. 2002; Harris et al. 2002; Tancer and Johanson 2001), and hallucinogen-like effects include changes in perception, some dysphoria, changes in thought process and reduction in concentration (Harris et al. 2002; Hernandez-Lopez et al. 2002; Tancer and Johanson 2001).

Most subjective effects were only reported at doses of approximately 75 mg or higher (Harris et al. 2002; Hernandez-Lopez et al. 2002; Tancer and Johanson 2001). However, participants receiving approximately 35 mg MDMA did report increases in tension, relaxation and elation, indicating that even this low dose is capable of producing subtle but noticeable effects. Informal analysis (visual inspection) of data from 3 ascending doses of MDMA suggest that effects were not dose-dependent (Tancer and Johanson 2001). An earlier report from the Spanish team found that 125 mg MDMA, as opposed to 75 mg MDMA, produced some sedating effects along with energizing effects in eight male Ecstasy users (Cami et al. 2000), indicating that higher doses of MDMA may in some cases produce qualitatively different effects from lower doses.

Participants experienced dysphoria and anxiety along with positive mood (Harris et al. 2002; Hernandez-Lopez et al. 2002; Tancer and Johanson 2001). However, no participant reported extreme anxiety or required clinical intervention. Furthermore, research employing an observer-scored measure of psychotic symptoms failed to report an increase in psychotic symptoms after approximately 35 or 105 mg MDMA (Harris et al. 2002). Some subjective effects of MDMA are unpleasant, but none were greatly distressing.

None of the studies addressed in the Investigator's Brochure attempted to formally measure the entactogenic effects of MDMA, though informal reports were described (e.g. Greer and Tolbert 1986; Vollenweider et al. 1998). An attempt to assess these effects after approximately 35 and 105 mg MDMA was made by examining specific items in the visual analog scales (Harris et al. 2002). The researchers failed to find any significant increases in feelings of closeness to others. Participants in another study reported increased feelings of friendliness after approximately 75, 110 and 145 mg MDMA (Tancer and Johanson 2001).

Self-reported side effects collected in recent reports do not differ from side effects listed in earlier studies (Harris et al. 2002; Hernandez-Lopez et al. 2002), and included dry mouth or throat, difficulty concentrating, unusual somatic sensations, dizziness and loss of appetite after 100 and approximately 105 mg MDMA. A dose of approximately 35 mg MDMA produced a few side effects in approximately half of the sample, including hot or cold sensations, dizziness and odd somatic sensations (Harris et al. 2002). No newly reported side effects appear in these recently published studies.

Only one report examined subacute side effects appearing 24 hours after MDMA administration (Harris et al. 2002). The side effects reported after receiving approximately 105 mg MDMA did not differ significantly from sub-acute side effects assessed in previous studies (Vollenweider et al. 1998; Liechti et al. 2000; Liechti and Vollenweider 2000). These included lack of appetite, dry mouth and hot or cold sensations. Far fewer sub-acute effects were reported after approximately 35 mg MDMA; these included difficulty concentrating in one participant and feeling as if the heart was beating faster in another subject. This study extends the investigation of sub-acute side effects to lower doses of MDMA. However, no new sub-acute effects are reported that have not been previously described.

With the exception of data on entactogenic effects, all findings concerning subjective effects and side effects reported acutely after MDMA are similar to previous findings and do not contradict them. As already indicated, MDMA was found to alter mood and perception and produces somatic side effects.

Neuroendocrine Effects

Researchers assessed release of arginine vasopressin (AVP) (Forsling et al. 2001), cortisol (Forsling et al. 2001; Harris et al. 2002; Pacifici et al. 2001), dehydroxyandrosterone (DHEA) (Harris et al. 2002), follicle stimulating hormone (FSH) (Harris et al. 2002), progesterone (Harris et al. 2002) and prolactin (Harris et al. 2002). Studies involved eight men (Forsling et al. 2001), eight men and women (Harris et al. 2002) and 17 men (Pacifici et al. 2001). Research findings previously exist for all hormones except for DHEA, FSH and progesterone. Findings reported in research published after the completion of the Investigator's Brochure are in agreement with previously reported findings. MDMA is associated with the release of AVP, cortisol,

DHEA and prolactin, with higher plasma values measured after MDMA than at baseline or after placebo.

Research findings by Forsling and colleagues were already presented in an initial report in 1999 (Henry et al. 1999). Plasma AVP was assessed at baseline and again after MDMA in a sample of eight drug-naïve men. Significant increases from baseline levels of AVP were reported, and these were not related to cortisol release. Additionally, this study reported a negative association between plasma MDMA and AVP levels, and negative associations between plasma R-MDMA and AVP release. Thus it would appear that a metabolite of MDMA may be involved in stimulating AVP release, a finding later supported in studies conducted on isolated rat tissues (Fallon et al. 2002), wherein it was found that the MDMA metabolite 4-hydroxy-3-methoxy-methamphetamine (HMMA) was more effective than MDMA at stimulating AVP release from rat hypothalamus.

Cortisol was released by 35 mg to 105 mg MDMA (Forsling et al. 2001; Harris et al. 2002; Pacifici et al. 2001), with higher doses associated with greater plasma cortisol. These findings are in line with previous reports of increased cortisol after MDMA (Grob et al. 1996, In preparation; Mas et al. 1999; Vollenweider 1998). Plasma cortisol could be increased again when a second dose of 100 mg MDMA was administered four hours after an initial dose of 100 mg (Pacifici et al. 2001), with elevation appearing just as cortisol values had begun to decline. Elevated plasma cortisol was associated with drug liking in one study (Harris et al. 2002), whereas elevated salivary cortisol was not associated with any measure of subjective effects.
Prolactin was found to be released after approximately 105 mg MDMA, but not 35 mg MDMA. It would appear that both prolactin and cortisol release are affected by MDMA dose. Prolactin release was also associated with increased heart rate, but was inversely related to self-reported giddy excitement (SDEQ "LSD").

There was a trend for release of the hormone DHEA after approximately 35 mg MDMA, with significant elevation appearing after approximately 105 mg MDMA (Harris et al. 2002). This finding was not previously reported, and its significance is currently unclear. Increase in DHEA was not associated with any change in physiological effects, but it was associated with elevated reports of euphoric mood and overall euphoria. Neither 35 nor 105 mg MDMA increased levels of FSH and progesterone (measured in women only).

Most neuroendocrine effects reported here are extensions or replications of earlier findings. There are a few new findings concerning effects of MDMA on the release of DHEA, FSH and progesterone, and with the relationship between hormone release and specific subjective effects. Elevated AVP release might play a role in causing hyponatremia reported in Ecstasy users, but is not liable to cause harm in controlled settings, where participants can be provided with electrolyte-containing liquids like Gatorade and liquid consumption can be monitored.

Immunological Effects

The Spanish team continued to investigate the effects of MDMA on immunological function in two separate studies, one in eight male Ecstasy users and one in nine male Ecstasy users (Pacifici et al. 2000; 2001a). Previous studies had indicated that like ethanol, MDMA reduced CD4 cells (Pacifici et al. 2001). Both 75 and 100 mg MDMA affected immunological function. An extension of these findings examined the effects of two doses of MDMA administered within 4 and 24 hours of each other (Pacifici et al. 2001b). Pacifici and colleagues found that 100 mg MDMA reduced CD4 cell number and CD4/CD8 ratio, reduced lymphocyte stimulation and increased NK cell number, providing a generally immunosuppressive profile that peaked at 1.5 hours after drug administration. Immunological effects of one 100 mg dose had largely subsided 24 hours later. A second dose of 100 mg MDMA administered four hours after the first dose produced the same immunological changes and enhanced the effects produced by the previous dose. Duration of immunological effects was also lengthened when a second dose of MDMA was given four hours after the first, with immunosuppressive effects still seen 24 hours after initial drug administration. This is the only study in which a second, or "booster," dose was administered after an initial dose.

Administering a second dose of 100 MDMA 24 hours after an initial dose of 100 mg also produced similar immunological changes (Pacifici et al. 2001). However, the second dose produced a greater reduction in CD4 cells and a greater increase in NK cells than the first dose, and CD4 numbers were still reduced when assessed 48 hours after initial drug administration. Plasma MDMA was also higher after the second dose than after the first, possibly as a result of inhibited metabolism of MDMA produced by the first dose. Hence two repeated doses of MDMA may have a greater impact on immunological function than a single dose.

A report of immunological function after two repeated doses of MDMA support and extend findings previously described by the same team of researchers, and suggest caution in using MDMA in people with immunocompromising conditions. Repeated doses of MDMA appear to exacerbate this effect, suggesting that increased risk of suppressed or disrupted immune function after repeated doses of MDMA. Immunological studies continue to use small samples, with no more than nine participants taking part in any given study, and all investigations so far have only examined male participants. Hence it is possible that effects seen in these studies may not accurately predict expected effects in women.

Electroencephalography

The first investigation of the encephalographic effects of MDMA was reported in 2001 (Frei et al. 2001). Quantitative EEG using low-resolution brain electromagnetic tomography (LORETA) was performed on the same sample of 16 mostly drug-naïve men and women studied in a positron emission tomography (PET) imaging study of the acute effects of MDMA on cerebral blood flow (Gamma et al. 2000). Comparisons were made between eyes-open and eyes closed conditions measured during placebo and after approximately 119 mg MDMA (1.7 mg/kg MDMA). Frei and colleagues reported finding that MDMA produced an increase in all beta activity and a concomitant decrease

in alpha activity. Activity in the alpha and delta bands was decreased in the frontal area, and beta activity increased in the frontotemporal area, findings interpreted as possible indications of disinhibition of frontal circuits and increased excitation. Theta activity was globally decreased around the cingulate, a pattern also seen after the administration of the stimulant d-amphetamine and the noradrenergic agonist tandamine. Reduced Alpha activity seen in posterior parietal, cingulate, occipital and temporal cortex with eyes open and globally in frontal and cingulate regions with eyes closed may be an indicator both of increased alertness and increased anxiety. However, the authors note that the function of alpha activity is not fully understood. Surprisingly, the authors report that other studies measuring EEG after acute administration of selective serotonin uptake inhibitors (SSRIs) like fluvoxamine produce nearly opposite effects. As already noted from the previous report, MDMA was well-tolerated in this sample, and the researchers were able to perform PET imaging and electroencephalography during peak drug effects.

So far, this is the only investigation relying on functional EEG as a means to assess the effects of MDMA on brain activity. However, the authors state that results are largely similar to what might be expected from functional EEG studies using other serotonergic and noradrenergic drugs. While these findings might be of interest to the neuroscientist, their relevance to risk assessment is unclear. It does appear that at least healthy volunteers experiencing the subjective effects of MDMA are able to tolerate undergoing imaging techniques such as PET and LORETA.

Potential Long-term effects

Currently unpublished data presented in the Investigator's Brochure from Dr. Charles Grob's Phase I study already indicate that performance on tests of cognitive function administered to 14 Ecstasy users after two separate doses of MDMA was not significantly different from performance before MDMA administration in this sample. Participants in this study were drawn from a larger sample of 18, and doses used in this larger sample ranged from 16 mg to 204.8 mg, with eight individuals receiving at least one dose equal to or higher than 125 mg in the course of the study.   More recently, preliminary data from Dr. Vollenweider and a report prepared for publication have failed to find any changes in cognitive function or in apparent amount of serotonin receptors as assessed through positron emission tomography (PET) scans in previously drug-naïve participants who received up to approximately 119 mg MDMA. This data has been collected in a sample of at least eight drug-naïve men. These recent reports are extensions of data first presented in 2000 and described in the Investigator's Brochure.

Because studies of Ecstasy users tend to share the same methodological flaws, such as retrospective study design, difficulties in obtaining genuinely matched controls differing only in the use of MDMA, non-random selection of participants and reliance on self-reported drug use histories, findings from prospective studies would seem to offer some advantages over retrospective studies. However, at least one investigator has studied the effects of MDMA by performing assessments at baseline and after one or two doses of MDMA. As noted in the Investigator's Brochure, findings reported by Vollenweider are derived from a comparison of measures taken after MDMA administration with measures

taken at baseline (Vollenweider et al. 2001; 2000). Doses employed ranged from approximately 120 to 140 mg (1.5 to 1.7 mg/kg). Assessments included PET with the radioactive drug, trans- 1,2,3,5,6,10-beta-hexahydro-6-[4-(methylthio)phenyl[pyrrolo-[2,1- a]isoquinoline, or McN5652 in eight participants and assessment of neurocognitive function in at least six participants. McN5652 has been used to assess amount of serotonin transporter sites in the brain in Ecstasy users and non-user controls (McCann et all 1998), with findings of lower McN5652 binding in Ecstasy users. As noted in the study protocol, these investigations have so far failed to find any effects of up to two doses of MDMA on McN5652 binding or on measures of memory and cognitive function. Findings from this ongoing investigation suggest that the long-term effects of a few doses of MDMA administered in controlled settings are minimal.

Aside from an investigation in a very small sample of male Ecstasy users (Obergriesser et al. 2001), the imaging study is one of the first studies that has failed to find any changes in serotonin function after MDMA administration. A failure to find changes in cognitive function after MDMA administration in a drug-naïve sample is in agreement with findings reported by Grob and colleagues and presented in the Investigator's Brochure. Ongoing research continues to support minimal to low risk of long term effects after the administration of one or two doses of MDMA at doses close to those proposed for use in this study.

Pharmacological Effects

Five investigations of the metabolism and pharmacology of MDMA were conducted after the completion of the Investigator's Brochure. Four of five investigations were conducted on small samples of men by a team of researchers in Barcelona (Navarro et al. 2001; Pichini et al. 2002; Pizarro et al. 2002; Segura et al. 2001), and one investigation was undertaken by a team of researchers in the Netherlands (Samyn et al. 2002). The Dutch team conducted their research on a sample of 12 men and women reporting at least some prior experience with Ecstasy. Most of the findings reported in these studies extend previously reported findings, though one study found potentially interesting differences in metabolism of MDMA versus the metabolite 4-hydroxy-3-methoxy-methamphetamine (HMMA) (Pizarro et al. 2002), and another describes the first reported detection of the MDMA metabolite). 3,4-dihydroxymethamphetamine (HHMA or DHMA) (Segura et al. 2001). Other studies indicated that MDMA could be reliably detected in saliva and sweat as well as in plasma and urine after the administration of 75 or 100 mg MDMA. No adverse events were reported during the course of any of the studies (Navarro et al. 2001; Pichini et al. 2002; Samyn et al. 2002; Segura et al. 2001).

100 mg MDMA was found to produce peak plasma MDMA in eight male volunteers at one to two hours (Navarro et al. 2001), a finding similar to others reported previously by this team (Mas et al. 1999). Peak plasma MDMA after 75 mg assessed in four male volunteers appears slightly later, at two to four hours after drug administration (Samyn et al. 2002). Half-life calculated after 100 mg MDMA was administered to eight volunteers (gender unspecified) was 8.49 ± 0.54 hours (Pizarro et al. 2002), similar to previously reported values (Mas et al. 1999). HMMA was the most prevalent metabolite detected in

plasma and urine (Navarro et al. 2001; Pizarro et al. 2002), followed by 4-hydroxy-3-methoxyamphetamine (HMA) and 3,4-methylenedioxyamphetamine (MDA). The same team of researchers described the successful detection of the reactive metabolite DHMA, called HHMA by the authors (Segura et al. 2001). The metabolite was detectable in plasma and urine and peaked in plasma at 1.2 ± 0.3 hours after administration of 100 mg MDMA. DHMA was detected in urine as well and made up 17.7% of recovered drug.

Examination of the two enantiomers (chiral forms) of MDMA and its metabolite HMMA offer evidence for the involvement of other non-enantioselective enzymes, such as CYP 1A2, CYP2B6 and CYP3A4, in the metabolism of 100 mg MDMA in six male volunteers (Pizarro et al. 2002). While the ratio of the more rapidly metabolized S-(+)-MDMA and the less rapidly metabolized R-(-)-MDMA changed over time, the researchers found little indication of a similar change in the ratio of R-(-)-HMMA to S-(+)-HMMA over time, with only a very slight shift toward greater R-(-)-HMMA. These findings suggest that one of the enzymes involved in the metabolism of MDMA is not the enantioselective CYP 2D6. A recent review of previous pharmacological studies of MDMA in humans arrived at the same conclusion (Kraemer and Maurer 2002). It is possible that MDMA itself saturates CYP 2D6, a finding suggested in the Investigator's Brochure and supported to some degree by assessments of plasma MDMA made after a second dose is administered (Pacifici et al. 2001).

Three studies found that MDMA was detectable in saliva (Navarro et al. 2001; Samyn et al. 2002; Segura et al. 2001). One study reported great inter-individual variability in salivary MDMA after 75 mg (Samyn et al. 2002). Research also established that MDMA was detectable in sweat after the administration of 75 mg (Samyn et al. 2002; Study 1), as well as after uncontrolled self-administration of Ecstasy (Samyn et al. 2002; Study 2). The MDMA metabolite MDA and HMMA were also detectable in saliva after 100 mg MDMA, with a salivary peak for MDA of 1.5 to 4 hours after drug administration, compared to a peak of 4 to 6 hours in plasma (Navarro et al. 2001).   Only trace amounts of the metabolite HMMA were found in saliva.

None of the research findings contradict previously reported findings. However, a few modest but significant advances in knowledge were made concerning the metabolism of MDMA and its detection in body fluids.

MDMA in Women Diagnosed with PTSD – Preliminary Data

To date, there are no published reports of the effects of MDMA in people diagnosed with serious mental illnesses. Research participants in the clinical trials described above were for the most part free from mental illness, with the exception in some cases of substance abuse with regard to Ecstasy. However, a MAPS-sponsored team of researchers in Spain has been investigating MDMA-assisted psychotherapy in women diagnosed with PTSD resulting from sexual assault. Though the study has been temporarily halted due to political pressure from the Madrid Antidrug Agency, some data has already been gathered from this study.

Conclusion

All recent studies indicate that MDMA can be safely administered to human volunteers. . No long-term deleterious physiological, psychological or neurocognitive effects were reported in any research subject and no acute adverse events occurred during any of the new studies reported after the completion of the Investigator's Brochure. MDMA is a sympathomimetic drug producing acute subjective effects somewhat similar to psychostimulants and psychedelics. MDMA administered in doses ranging from 35 to 171.8 mg were well tolerated in samples of healthy volunteers. Preliminary results from a prospective study of the effects of MDMA in drug-naïve volunteers failed to find changes in the amount of serotonin transporter in brain or in cognitive function. Recent findings generally support and extend initial findings in the Investigator's Brochure of minimal risk to prescreened research subjects receiving pure MDMA in a controlled setting.

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MAPS' MDMA Investigator's Brochure
Update #1
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Human Studies: Effects of Regular Ecstasy Use

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Since the completion of MAPS' initial MDMA Investigator's Brochure in July 2001, researchers have continued to examine neuropsychological and neurocognitive function in regular Ecstasy users, with Ecstasy referring to material represented as MDMA and sold as such on the black market. The following review focuses on this body of research into the neurocognitive and psychological effects of regular Ecstasy use, and on attempts to measure serotonin function in human Ecstasy users.

Executive Summary

Most studies of neurocognitive function continue to find differences on some measures of memory and executive function between Ecstasy users and controls, with these differences generally minor.   In contrast, two studies failed to find any differences between Ecstasy users and controls. In one study, differences in performance on a test of memory were more closely related to verbal intelligence and concurrent cannabis use than they were to Ecstasy use. As stated in the Investigator's Brochure, we continue to be concerned with the potential effects of regular Ecstasy use on verbal and visual memory and executive function, but results from more well-controlled studies will be required to separate out the effects of Ecstasy from possible confounds

Findings reported in a number of recently published papers suggest that the association between Ecstasy use and psychiatric disorders, when apparent, is most likely due to pre-existing psychiatric disorders prior to first use of MDMA and is more strongly associated with other variables, such as cannabis use or age of onset of drug use. A majority of recently published reports strongly suggest the relationship between regular Ecstasy use and increased psychiatric complaints is complex and less certain than associations between regular Ecstasy use and neurocognitive deficits. While an association between regular Ecstasy use and increased anxiety or depression cannot be dismissed altogether, recent research has not lent any additional support to this claim. Generally speaking, recent research would suggest that the risk of developing psychological problems or mental disorders after Ecstasy use is less likely than it first appeared.

Overall, imaging and neuroendocrine studies have found differences between Ecstasy users and people reporting no use of Ecstasy. While most imaging and neuroendocrine studies of Ecstasy users largely replicate previously reported findings, a few studies offer potentially new or contradictory findings. Dissociations between apparent measures of serotonin function and measures of neurocognitive function raise questions about these measures as direct indices of Ecstasy neurotoxicity. Recent publications have presented

critiques of imaging studies and of using number of serotonin transporter sites as a measure of serotonin function (Kish 2002)

All research addressed in this report was conducted in people self-reporting use of Ecstasy and in one or more control groups, including abstinent Ecstasy users, cannabis (marijuana) users, polydrug users with no self-reported use of Ecstasy, and people with no reported history of illicit drug use. A majority of the studies employed selection criteria that required Ecstasy users to report a lifetime consumption of at least 20 or more tablets. All studies except one relied on non-representative samples and recruited participants through "snowball" sampling or through advertisements placed in magazines or venues (such as clubs or at a university).

Nearly all studies were retrospective in design, meaning that samples were selected on the basis of previous drug use, and sample sizes tend to be small. Most studies examined both men and women, but a few studies examined men only. Unlike some previously conducted studies, current studies have generally used more appropriate control groups. Nevertheless, most of the methodological limitations noted in previous studies and described in the Investigator's Brochure are found again in the research published after its completion. These limitations include retrospective research design, use of non-random and non-representative sampling, small samples, reliance on self-reported drug use information, and use of the same sample or samples including the same people in subsequent publications. These methodological flaws render it difficult to draw definite conclusions about the cause of differences seen between Ecstasy users and controls, as pointed out in a recent critique of studies of long-term effects of Ecstasy (Cole et al. 2002).

Neurocognitive Function

Studies comparing neurocognitive function in regular Ecstasy users and non-user controls continue to find impairments in verbal recall, (Bhattachary and Powell 2001; Fox et al. 2001a; Morgan et al. 2002; Reneman et al. 2001a; 2001b), visual recall (Fox et al. 2002; 2001a) and executive function (Bhattachary and Powell 2001; Heffernan et al. 2001; Morgan et al. 2002; Zakzanis and Young 2001). One research team failed to find any long-term effects of Ecstasy use on attentional processes, finding lower performance on one task only (Zakzanis et al. 2002). Some authors found a relationship between lifetime exposure to Ecstasy (number of tablets taken over a lifetime) and performance on tests of recall or executive function (Bhattachary and Powell 2001; Fox et al. 2001; Reneman et al. 2001a). However, one study found that cannabis use and verbal intelligence were stronger predictors of verbal recall than Ecstasy use (Simon et al. 2002). This study failed to find any significant differences in memory assessed in Ecstasy users and cannabis user controls. Most studies noted an association between regular Ecstasy use and reduced verbal recall (Bhattachary and Powell 2001; Fox et al. 2001b; Morgan et al. 2002; Reneman et al. 2001a; b), a finding that is in agreement with previous reports (e.g. Gouzoulis-Mayfrank et al. 2000; Morgan 1999). One recently published study also found that regular Ecstasy users showed deficits in visual working memory (Fox et al. 2002). While reports continue to find a relationship between regular Ecstasy use and deficits in

executive function (Bhattachary and Powell 2001; Heffernan et al. 2001; Morgan et al. 2002; Zakzanis and Young 2001), recent reports do not always support this relationship (Fox et al. 2001a; Morgan et al. 2002). Study limitations include very small samples (Reneman et al. 2001b), reporting data from identical or related samples in separate reports (Fox et al. 2001a; 2001b; Reneman et al. 2001a; 2001b), and differences between Ecstasy users and controls in use of other drugs (Bhattachary and Powell 2001). Nevertheless, research conducted subsequent to our initial review continues to lend support to findings of a hypothesized link between regular Ecstasy use and minor impairments in a few specific domains of cognitive function.

Verbal Memory

Seven studies published within the last year and a half assessed verbal memory in Ecstasy users (Bhattachary and Powell 2001; Fox et al. 2001a; 2001b; Morgan et al. 2002; Reneman et al. 2001a; 2001b; Simon et al. 2002), and one study examined visual memory (Fox et al. 2002), with verbal recall assessed through word list or story recall tasks. Not included are studies solely relying on self-reported memory function (Rodgers et al. 2001). A majority of these studies found that Ecstasy users performed less well in immediate and delayed recall of word list pairs, as assessed via the RAVLT or similar list-learning tasks (Fox et al. 2001b; Reneman et al. 2001a; b). In one study, immediate, but not delayed, recall was associated with total lifetime consumption of Ecstasy in both 22 current and 16 abstinent users reporting an average lifetime consumption of 485 tablets (Reneman et al. 2001a). Another study found that 14 subjects who reported using Ecstasy for fewer than five years (with an average lifetime consumption of 224 tablets) did better than 14 subjects who had used Ecstasy for at least five years (with an average lifetime consumption of 811 tablets) (Fox et al. 2001b). Both groups of Ecstasy users had lower scores on measures of verbal recall than did 14 subjects who reported using other drugs, but not Ecstasy. A study in a very small sample of 8 male Ecstasy users with a lifetime consumption of at least 50 tablets and 7 gender-matched controls reported lower scores in Ecstasy users on the delayed recall section of the RAVLT (Reneman et al. 2001b). This sample possibly may consist of a sub-sample of Ecstasy users sampled in other reports.

Studies that assessed memory through prose or story recall also had mixed results (Bhattachary and Powell 2001; Fox et al. 2001a; Morgan et al. 2002). One study found that 18 current Ecstasy users with a total lifetime consumption of 303 tablets and 15 former Ecstasy users with a total lifetime consumption of 512 tablets had lower scores on this task than did 15 non-drug user controls (Morgan et al. 2002). Former Ecstasy users performed less well than 16 polydrug controls on this task. An investigation employing 18 novice users (lifetime consumption 1-5 times), 20 regular users and 16 abstinent users (lifetime consumption approximately 30-51 times) and non-drug using controls found that non users performed better on immediate and delayed story recall (Bhattachary and Powell 2001). This study also found an approximate relationship between lifetime Ecstasy consumption and verbal recall. However, another research team failed to find differences in performance between a sample of Ecstasy users and polydrug user controls on prose recall, both when Ecstasy users were divided on the basis of experiencing self-

reported problems and when divided on the basis of lifetime Ecstasy dose ("low," "medium" or "high") (Fox et al. 2001a). It is notable that the same team of researchers did find regular Ecstasy use affected verbal recall when measured via recall of word lists. Perhaps these conflicting findings are best explained if reduced prose recall is due in part to use of other drugs, since controls in the second study performed by Fox and colleague were permitted to use substances other than Ecstasy, whereas Bhattachary and Powell selected controls with no history of illicit drug use.

One recently published study in Ecstasy users and polydrug user controls suggests that the effects of regular Ecstasy use on recall are not restricted to verbal memory. Fox and her colleagues found that 20 regular Ecstasy users reporting an average lifetime consumption of 172 tablets did not perform as well as 20 polydrug user controls on a computerized assessment of visual memory (Fox et al. 2002). Most of the differences were only seen in the most difficult tasks, indicating that visual memory deficits in Ecstasy users were subtle and selective. A majority of investigations of memory function in Ecstasy users have found verbal memory effected, while only a few have found effects on visual memory (see Investigator's Brochure), making this recent finding somewhat unusual. It is possible that regular Ecstasy use affects a process involved in verbal recall and visual working memory, but that the process is more important in verbal than in visual tasks.

Not all researchers found differences in verbal memory. Fox and colleagues found that when lists of word pairs were organized into categorical groups prior to presentation, 40 Ecstasy users with a total lifetime consumption of 365 tablets performed no differently than 20 polydrug-user controls (Fox et al. 2001a). Simon and colleagues found only a trend for 40 Ecstasy users with an average lifetime consumption of 258 tablets to do less well on sub-tests of immediate and delayed auditory memory on the Wechsler Memory Scale (WMS) than 37 cannabis users. Subsequent to this finding, a regression analysis indicated that this trend was more likely due to differences in verbal intelligence and cannabis use. Current Ecstasy users did less well than former Ecstasy users in one study evaluating verbal recall (Reneman et al. 2001b), suggesting that recent use of Ecstasy or a factor associated with recent Ecstasy use affects verbal memory. Hence it remains possible that findings of neurocognitive deficits in regular Ecstasy users are at least in part due to use of other drugs, such as cannabis, and that neurocognitive function might be affected independently by residual effects of recent Ecstasy use and cumulative effects of regular Ecstasy use. Findings reported by Morgan and colleagues (2002) and by Reneman and colleagues (2001a) indicate that there may be at least some recovery of function on measures of verbal recall, as impaired verbal recall was either less pronounced or not detected in former users when compared with current users.

Several studies relied on self-reported memory difficulties or cognitive failures in Ecstasy users and controls (Heffernan et al. 2001; Rodgers et al. 2001). One research team gathered data from an on-line survey (Rodgers et al. 2001), and the other gathered data from study participants completing questionnaires (Heffernan et al. 2001). 155 Ecstasy users completing an on-line survey indicated greater difficulties in remembering to perform tasks or behaviors they regularly performed, whereas 192 cannabis (marijuana)

users were more likely to report difficulties remembering things in the past (Rodgers et al. 2001). Lifetime Ecstasy consumption is not reported in this study but approximately two thirds reported it to be between 1 and 99 tablets, and a third reported it to be 100 or more tablets. Two other studies conducted by the same research team sought to validate and apply an instrument measuring self-reported memory for performing planned or routine tasks, or prospective memory (Heffernan et al. 2001). Forty-one Ecstasy users in one study reported greater memory difficulties on all scales of this measure than 41 drug-user controls without indicating any greater likelihood of providing socially desirable answers. However, 15 Ecstasy users participating in a second study described in the same report failed to report a greater number of cognitive failures than did 15 drug-user controls. These last findings suggest that the authors' measure of prospective memory may be more sensitive to Ecstasy-related changes in memory, or that Ecstasy users are more liable to feel comfortable admitting to difficulties presented in the prospective memory measure than reporting general cognitive failures.

In contrast, findings from two studies indicate that self-reported difficulties attributed to Ecstasy use are a poor predictor of objectively assessed cognitive function (Fox et al. 2001a; Heffernan et al. 2001, Study 2). Fox and colleagues found that lifetime exposure to Ecstasy was a stronger predictor of performance on tests of spatial memory and executive function than number of self-reported problems. Lower scores on assessments of memory and executive function were associated with lifetime Ecstasy consumption, with lower scores on these tasks found in "medium" and "high" lifetime consumers, and not in those reporting "low" lifetime consumption. "Low" dose users (14) reported an average lifetime consumption of 38 tablets, average lifetime consumption in "medium" dose users (14) was 236 tablets and in "high" dose users was 918 tablets (14). The research team of Heffernan also failed to find a relationship between task performance on assessments of memory and executive function and self-reported cognitive problems in a sample of 30 Ecstasy users (lifetime consumption not reported) and 37 drug-user controls (Heffernan et al. 2001). These findings call into question the relevance of greater self-reported memory problems in Ecstasy users reported by Rodgers (Rodgers et al. 2001) and Heffernan (Heffernan et al. 2001).

Executive Function

Executive function in most studies was measured either via word fluency tasks or Tower of London tasks. In the first task, participants are required to list as many words as they can think of within a minute's time that satisfy a specific requirement, such as beginning with a specific consonant. Tower of London tasks require people to move a series of actual or virtual 'balls" from one peg to another, and planning is often measured as well as time to correct solution. Two of four studies found regular Ecstasy users did less well on word fluency tasks (Bhattachary and Powell 2001; Heffernan et al. 2001). Bhattachary and Powell (2001) found that all three of their Ecstasy user groups fared less well than did non-drug user controls on the word fluency task. In another study, Heffernan and colleagues found that 30 Ecstasy users (lifetime consumption not reported; frequency of use reported as 5.6 tablets per month) did not perform as well as drug-user controls on all three word fluency tasks (letter, category and combined) (Heffernan et al.

2001). However, two of four reports failed to find significant differences in executive function (Fox et al. 2002; Morgan et al. 2002). Another study found 20 Ecstasy users reporting an overall lifetime consumption of 172 tablets only had lower scores on one of three measures of word fluency when compared with 20 drug-user controls (Fox et al. 2002). Morgan and colleagues used the same sample of current and former Ecstasy users, polydrug users and non-drug user controls described previously, and found no differences in verbal fluency except in one area; current Ecstasy users did less well at generating category words (Morgan et al. 2002).

Several studies found regular Ecstasy use was associated with increases in planning time or lower scores on Tower of London tasks (Fox et al. 2002; 2001a; Morgan et al. 2002). Neither study employing the Tower of London found that regular Ecstasy use affected all aspects of the Tower of London task (Fox et al. 2001a; Fox et al. 2002), though Ecstasy users reporting lifetime consumption of 918 tablets and those with self-reported problems did take longer to plan their moves on this task. In their earlier study, Fox and associates failed to find any differences between 40 Ecstasy users and drug-user controls on Wisconsin Card Sort (WCST) performance (Fox et al. 2001a), regardless of whether Ecstasy users were categorized by lifetime consumption or by presence versus absence of self-reported problems. However, a later study found regular Ecstasy use was associated with poorer performance on the most difficult trials of a WCST-like task (Fox et al. 2002).

A comparison of 24 regular Ecstasy users reporting a lifetime consumption of approximately 39 tablets and 24 drug user controls found that ecstasy users had lower global (profile) scores on a comprehensive measure of executive function (Zakzanis and Young 2001). Effects of regular Ecstasy use were particularly apparent in tests of planning and organization, and several parameters of Ecstasy use, including duration of use and lifetime consumption, were related to performance on executive function tasks. Because of their potential involvement in working memory and executive function, one study sought to assess attentional processes in Ecstasy users. These researchers assessed attentional processes in 24 regular users reporting a lifetime consumption of approximately 28 tablets and 30 drug user controls, finding differences in performance on only one of eight sub-tests contained in the Tests of Everyday Attention (TEA) (Zakzanis et al. 2002).

Ecstasy Use, Anxiety, Depression and Psychological Problems

The association between regular Ecstasy use and mood or psychological health has been examined in comparisons of Ecstasy users and non-users. A number of studies continue to find that regular Ecstasy use is associated with increased anxiety, depression, or other psychological problems or diagnoses (Dughiero et al. 2001; Gerra et al 2002; Lieb et al. 2002; MacInnes et al. 2001; Morgan et al. 2002; Parrott et al. 2002; 2001). However, several studies also found that use of other drugs, age of onset of drug use or pre-existing psychiatric disorders rather than Ecstasy use (Daumann et al. 2001; Lieb et al. 2002; Morgan et al. 2002; Parrott et al. 2001) may better explain this association. A majority of recently published reports strongly suggest the relationship between regular Ecstasy use

and increased psychiatric complaints is complex and less certain than associations between regular Ecstasy use and neurocognitive deficits, calling into question conclusions made about this relationship on the basis of previous research. One study failed to find any differences between SCL-90-R scores of Ecstasy users and drug-user controls (Simon et al. 2002), despite finding differences in self-reported physical health assessed through a different instrument.

A study using on-line surveys found that number of self-reported complaints attributed to Ecstasy increased with lifetime consumption of Ecstasy use (Parrott et al. 2002b). This study compared 109 "novice" users (lifetime consumption 1-9 times), 136 "moderate" users (10-99 times) and 37 "heavy" users (100 or more times) on number of self-reported health complaints. Higher lifetime consumption was associated with greater likelihood of reporting anxiety, depression, poor concentration, memory problems and other health complaints. However, respondents were not objectively assessed for the presence of these health complaints, and the retrospective study design prevented researchers from establishing the sequence of occurrences in this sample. Participants are also requested to report only those symptoms they attribute to Ecstasy use without specifying how the respondents were meant to draw this conclusion. Without clear directions, it is possible that Ecstasy users may have relied on their beliefs about the effects of Ecstasy to determine association with Ecstasy use rather than relying on symptom appearance after Ecstasy use. In addition, other recently conducted studies suggest that self-report measures of problems are unreliable (Fox et al. 2001a; Heffernan et al. 2001).

Regular Ecstasy use was often associated with a greater number of complaints on the SCL-90, a self-report measure of mental health complaints (Daumann et al. 2001; Dughiero et al. 2001; Parrott et al. 2001; Morgan et al. 2002). 43 regular Ecstasy users with average lifetime consumption of 233 tablets were more likely to report anxiety, sleep disturbances and obsession-compulsion like problems than 77 non-users (Dughiero et al. 2001). Both "light" and "heavy" Ecstasy users in Italy and England were more likely to report suffering from anxiety, anger and obsessive-compulsive problems and to experience more bodily complaints (Parrott et al. 2001). This study compared 150 non-drug users, 185 alcohol-only users, 97 alcohol and cannabis users, 102 polydrug users, 115 "light" Ecstasy users (lifetime exposure of 1-20 doses) and 119 "heavy" users (lifetime use higher than 20 doses). A study using a revised version of the SCL-90 in 18 regular Ecstasy users, 15 former Ecstasy users, 16 polydrug users and 15 non-drug users reported that current Ecstasy users were more likely to indicate experiencing anxiety, depression, feeling paranoid, appetite problems and bodily complaints, and that former users still reported being anxious, bodily complaints, and altered appetite and sleep (Morgan et al. 2002).

However, the association between regular Ecstasy use and self-reported psychological problems is qualified in each of the three studies. Self-reported health complaints are associated with use of illicit drugs, not use of Ecstasy alone, since most statistically significant differences reported in this study were between all polydrug user groups (polydrug-no Ecstasy, light and heavy Ecstasy users) and groups reporting little or no substance use (no substances, tobacco and alcohol only, cannabis). Current or previous

Ecstasy use did not affect overall severity scores on the SCL-90-R (Morgan et al. 2002), and a stepwise regression performed on this data found that use of amphetamines, cannabis and psilocybin explained as much or more variance in self-reported health complaints as did regular Ecstasy use. In addition, gender differences may have complicated responses to the SCL-90 in the study performed by Dughiero and colleagues (2001).

One investigation using a revised version of this measure, the SCL-90-R, found no statistically significant differences between 28 Ecstasy users (with an average lifetime consumption of 93 tablets), 28 cannabis users, and 28 non-drug user controls after controlling for cannabis use and age of onset of drug use (Daumann et al. 2001). While Ecstasy users did list a greater number of health complaints and reported a greater degree of certain types of anxiety (phobic anxiety, paranoid ideation, obsessive or compulsive behavior) than non-drug using controls, their scores were not significantly different from those of cannabis users. When a regression analysis was performed on this sample with age of onset of drug use and cannabis user as factors, Ecstasy users no longer differed from non-drug using controls. The research described above and findings from other recently published studies indicate that regular substance use in general, rather than Ecstasy use in specific, is associated with a greater number of self-reported psychological problems.

The same research team that reported a failure to find any effects of regular Ecstasy use on memory also failed to find any differences in SCL-90-R scores between Ecstasy users and drug-user controls (Simon et al. 2002). 40 Ecstasy users with a lifetime consumption of 258 tablets scored similarly to drug-user controls on the SCL-90-R. However, the Ecstasy users also reported more problems with general health, pain, physical health and physical role on the SF-36 measure than controls.

Assessing depression through the Beck Depression Inventory, a self-report measure of depression-related symptoms, MacInnes and colleagues found higher BDI scores in 29 regular Ecstasy users with a lifetime consumption of 527 tablets than in 29 non-drug user controls (MacInnes et al. 2001). In contrast, Daumann and colleagues found no statistically significant differences in depression scores in 28 ecstasy users reporting a lifetime use of 93 tablets, 28 cannabis users and 28 non-drug user controls, with depression assessed through another self-report measure (German-language Depression Scale). 12 male Ecstasy users with a lifetime consumption of 52.3 tablets had higher depression scores on the Hamilton Depression Rating Scale than did 12 gender-matched controls (Gerra et al. 2002), but all Ecstasy users were selected from amongst people seeking information on or treatment for drug abuse, and they may not be representative of the population on general.

As well as measuring self-reported symptoms and depression, Daumann and colleagues also compared responses on specific measures of anxiety and general health complaints in regular Ecstasy users, cannabis users and non-drug user controls. Both regular Ecstasy users and cannabis users had more general health complaints than non-drug user controls, but there were no significant differences between the number of health complaints listed

by Ecstasy users and cannabis users. Ecstasy users, cannabis users and non-drug users scored similarly on a measure of anxiety (the STAI).

A longitudinal study of approximately 2400 young people living in or around Munich, Germany sought to determine whether Ecstasy use precedes or follows diagnosis with a psychiatric disorder (Lieb et al. 2002). Unlike the studies described above, this study established diagnosis through a clinical interview. Comparing responses on the second or third interview with that of the initial interview, the researchers found that it was more likely for people previously diagnosed with a mental disorder to commence using ecstasy than it was for people who had begun using Ecstasy to develop a mental disorder subsequent to Ecstasy use. While Ecstasy users were found to have greater rates of depression and anxiety disorders, the causal relationship between Ecstasy use and mood disorders is unclear. Findings from this prospective analysis suggest that any relationship between Ecstasy use and psychiatric diagnosis may arise because people with pre-existing disorders are more prone to using Ecstasy than people without pre-existing diagnoses.

Aggression

Three studies assessed degree of aggression reported or displayed by regular Ecstasy users (Daumann et al 2001; Gerra et al 2001; Gerra et al. 2002). Daumann and colleagues assessed trait aggressiveness by administering a German-language version of the Buss-Durkee Hostility Inventory to the sample of Ecstasy users, cannabis users and non-drug using controls described earlier. Cannabis users were found to have higher aggression scores than either Ecstasy users or non-user controls, though total lifetime consumption of Ecstasy, duration and frequency of users were also related to aggression scores. Behavioral aggression in Ecstasy users was investigated by comparing male Ecstasy users and gender-matched non-drug using controls on their response to a fictitious opponent who subtracted points from participants in a computerized competition referred to as the Point Subtraction Aggression Paradigm, or PSAP (Gerra et al. 2001). Trait aggression was also measured by an Italian-language version of the Buss-Durkee Hostility Inventory. 12 Ecstasy users seeking information or treatment at a substance abuse treatment facility and reporting a lifetime consumption of 77.9 tablets were more likely to subtract points from their opponent than were 20 gender matched controls. They were also more likely to protect their points. Ecstasy users also scored higher on direct aggression and irritability scales of the Italian-language version of the BDHI. Measures of blood pressure and heart rate found that elevations in systolic blood pressure and heart rate appearing during the competition remained elevated longer after the session was complete in Ecstasy users. Differences in plasma cortisol, ACTH, norepinephrine and epinephrine associated with each session are possibly indicative of greater experienced stress or sensitivity to the procedure. Trait aggression was also measured in 12 Ecstasy users and 12 non-drug user controls in a sample described in more detail below. In this second study, repeated Ecstasy use was associated with increases in different sub-scales of the psychometric measure than were noted in the earlier study. Unlike participants in other studies, those taking part in the studies conducted by Gerra's research team are closely monitored for drug use via two to three

weekly urinary screens. However, it should also be noted that the Ecstasy users and matched controls are not selected from the same population and may not share the same circumstances or personality characteristics. The two studies offer somewhat contradictory reports of the association between Ecstasy use and aggression. In previous studies, a decline in trait aggression is reported in Ecstasy users after 12 months of abstention from use (Gerra et al. 2000); participants in this study had only been abstinent for three weeks.

Impulsivity

Impulsivity in Ecstasy users and controls was assessed in four studies (Daumann et al. 2001; Fox et al. 2002; Morgan et al. 2002; Rodgers et al. 2001). Researchers employed psychometric and behavioral measures of impulsivity, with behavioral measures usually assessing rapidity of response and degree of inhibition for an inappropriate response. There was only a trend for current and former Ecstasy users to have higher impulsiveness scores than polydrug user or non-drug user controls on the Impulsiveness, Venturesomeness and Empathy Scale (Morgan et al. 2002). However, in contrast, 28 Ecstasy users (lifetime consumption of 93 tablets) scored higher than 28 cannabis users or 28 non-drug user controls on the Barratt Impulsiveness Scale (Daumann et al. 2001). Mixed results also appear with behavioral measures. Researchers using a Go/No Go task, wherein participants must inhibit responding to non-target items, found no differences in performance for Ecstasy users with 20 lifetime consumption of 172 tablets and 20 drug-user controls (Fox et al. 2002). However when Morgan and colleagues employed a measure relying on rapidity of response and error rate (the Matching Familiar Figures, or MFF, task) found that 18 current and 15 former Ecstasy users (lifetime consumption of 303 and 512 tablets respectively, as described earlier) responded more rapidly and made more errors than either polydrug user or non-drug user controls (Morgan et al. 2002). Likewise, Ecstasy-using respondents to an on-line survey were more likely to send in incomplete surveys or make errors in response (Rodgers et al. 2001). It is unclear why some measures detect differences in impulsiveness while others fail to detect any differences, but it seems very likely differences in samples and in variance in aspects of impulsiveness measured may be some of the reasons for the lack of consistent findings across studies. The relationship between Ecstasy use and impulsivity and its relevance as an indicator of changes in the serotonin system is also uncertain, and this problem is discussed in the Investigator's Brochure.

Imaging Studies of Serotonin Function

Eight published reports used imaging techniques to compare the brains of Ecstasy users with those of controls, often with the goal of finding indicators of MDMA neurotoxicity or reduced serotonin function. There were three SPECT imaging studies using the radioligand [123I]-2-beta-carbomethoxy-3-beta-(4-iodophenyl) tropane (Beta-CIT) (Reneman et al. 2001a; Reneman et al. 2001b; Reneman et al. 2002), one SPECT study using the serotonin 5HT2A agonist R91150 (Reneman et al. 2002), two proton magnetic resonance spectroscopy (MRS) studies (Obergriesser et al. 2001; Reneman et al. 2001b) and two PET studies of brain glucose metabolism with fluoro-deoxy-glucose (FDG)

(Buchert et al. 2001; Obrocki et al. 2002). Findings from the studies using Beta-CIT are complex, but indicate that current heavy Ecstasy users may have fewer serotonin transporter sites than abstinent or moderate Ecstasy users. Findings reported in the study using R91150 found lower binding in current users and higher binding in former users. The two MRS studies produces conflicting results, and both relied on very small samples. A few group differences may have been found in the FDG studies, but both studies may be utilizing data from nearly identical samples. Overall, imaging studies have found differences between Ecstasy users and people reporting no use of Ecstasy. However, recent publications have presented critiques of imaging studies and of using number of serotonin transporter sites as a measure of serotonin function (Kish 2002).

Beta-CIT is a radioactive drug that binds to the serotonin and dopamine transporter; studies use time after injection and selected brain area as means to study serotonin and dopamine binding separately. All studies using this compound were performed by a team in the Netherlands. The first SPECT study found lower Beta-CIT binding in 22 current Ecstasy users with a lifetime consumption of 485 tablets than in drug user controls (Reneman et al. 2001a). No significant differences were apparently found between either of these groups and 16 former users with a lifetime consumption of 268 tablets. It should be noted that this study failed to find any region-specific differences in Beta-CIT binding. This study is also discussed earlier in the section on cognitive function, as the authors measured memory with the RAVLT.

In a second study relying on some of the same samples, the authors found women classified as current "heavy" ecstasy users (lifetime consumption of at least 50 tablets) had lower Beta-CIT binding in frontal, temporal-parietal and occipital areas than women reporting moderate use (lifetime use of 1-50 tablets) or no use for at least one year prior to study (Reneman et al. 2001b). Men in all groups were found not to differ on Beta-CIT binding, regardless of lifetime consumption of Ecstasy or time elapsed since last use. These findings were reported in a study of 15 moderate users (6 women) with lifetime consumption of 28.6 tablets and abstinent for approximately 15 weeks (108 days), 23 heavy users (11 women) with lifetime consumption of 530 tablets who were abstinent for approximately 10 weeks (69 days), 16 former Ecstasy users (8 women) with lifetime consumption of 268 tablets and abstinent for approximately 2.4 years (870 days), and 15 drug using controls (8 women). Fewer areas of lower Beta-CIT binding were found between female former users and non-user controls, a finding suggestive of recovery after a period of abstinence. It appears that the same group of former users appear in both studies, and that substantially similar samples of current heavy ecstasy users are used in the first and the second studies.

The third study used Beta-CIT to assess striatal dopamine transporter binding in people reporting Ecstasy use alone and people reporting intentional use of amphetamines (Reneman et al. 2002). This study reported that 29 ecstasy users reporting a lifetime consumption of 324 tablets and nine ecstasy and amphetamine users with a lifetime consumption of 358 tablets had higher striatal Beta-CIT binding than did 15 controls who reported the use of other drugs, but not Ecstasy or amphetamines. However, those reporting use of Ecstasy and amphetamines had lower striatal Beta-CIT binding than did

the Ecstasy users reporting no intentional use of amphetamines. Regression analyses found that neither past Ecstasy use nor past cannabis use affected striatal Beta-CIT binding. These findings are comparable to those reported in a previous study of Ecstasy users and drug-user controls that also found no differences in striatal Beta-CIT binding (Semple et al. 1999). The findings reported by Reneman and colleagues stand in contrast to recently reported findings of dopamine neurotoxicity in monkeys and baboons after a repeated dose regimen of MDMA. Instead, along with previously reported findings, they suggest that Ecstasy use has minimal no significant effects on the dopamine system.

SPECT scans using R91150, a radioactive drug used to bind to serotonin 5HT2A receptors, were conducted in 17 current ecstasy users reporting a lifetime consumption of 224 tablets, 7 former users with lifetime consumption of 274 tablets and drug user controls (Reneman et al. 2002b). Current ecstasy users had last used the drug 23.1 days, and time elapsed since last use of Ecstasy was reportedly 137.2 days. Lower R91150 binding was found in current users when compared with controls, and higher ligand binding was seen in the former users. Changes in number of 5HT2A receptors over time may reflect compensatory changes in the serotonin system in response to damage to serotonin neurons, or they might reflect recovery of serotonin function. The authors also reported a correlation between time elapsed since last use and degree of R91150 binding, with amount of binding increasing with longer time since last use. The authors reported similar findings in rats, though it appears that R91150 binding is more rapidly increased in rats. Findings reported here are in agreement with earlier findings reported by the same research team. However, there are some indications that the researchers have published reports based on data from samples containing at least some and possibly most of the same samples (e.g. Reneman et al. 2000; Reneman et al. 2001).

All three studies of Beta-CIT binding suggest that the relationship between repeated use of Ecstasy and possible changes in serotonin transporter binding are complex, with effects dependent on lifetime consumption and gender. It should be noted that earlier studies of Ecstasy users that relied on the same radioligand found lower Beta-CIT binding in men (Semple et al. 1999). A critique of imaging studies discusses the issue of relying on serotonin transporter binding as a measure of serotonin function (Kish 2002) . Changes in neuroendocrine function, in mood and gender-related differences are all considered potential modulators of brain serotonin transporter. Comparative studies of 5HT2A receptors in rats were made by the same research team (Reneman et al. 2002b) and are discussed in the section on in vitro and non-human animal studies. While these findings suggest that changes in 5HT2A receptor density may be indicative of changes in serotonin function produced by MDMA, interspecies differences in time needed before this change appears are also apparent. Some recently reported discoveries had not been described before, as was the case for dose and gender effects in Beta-CIT binding in Ecstasy users (Reneman et al. 2001c). No changes in Beta-CIT binding were seen in men or women who took less than 20 ecstasy tablets,

Three more studies using MRS have been published since the appearance of the research performed by Chang and colleagues (Chang et al. 1999). Two of these studies employed very small samples, with less than ten individuals per condition. Researchers used a

larger sample in the third study, but all participants in this study were male. Each study reported different results. One comparison of five male Ecstasy users with a lifetime consumption of between 120-350 tablets) and five non-drug user controls did not find any measurable differences in presence of N-acetylaspartate (NA), creatine (Cr) or choline (Cho) in hippocampus (Obergriesser et al. 2001). This study also reported no differences in performance on assessments of memory or executive function, but fail to report scores or statistical tests used to analyze scores, and participants may have been selected on the basis of test performance. Another study reported by Reneman and colleagues (Reneman et al. 2001b) did find an association between delayed recall scores and Na/Cr ratios in pre-frontal cortex of 8 male Ecstasy users reporting a lifetime consumption of 901 tablets. However, while memory in ecstasy users was compared with memory in 7 gender-matched drug using controls, no MRS was performed on controls. Hence it is unclear as to whether N-acetylaspartate, a measure of neuron damage, is in fact increased in this sample of very heavy Ecstasy users and controls. Findings concerning verbal recall are also discussed in the section on cognitive function. Subsequent to this publication, the same research team reported lower NA/Cr and NA/Cho ratios in mid-frontal gray matter in 15 male ecstasy users with a lifetime use of 723 tablets, when compared with gender-matched controls (Reneman et al. 2002b). In contrast to initial findings, these findings establish a difference in NA levels in ecstasy users. However, the researchers failed to assess neurocognitive performance, and so it is unclear what association, if any, these findings have with impaired memory in ecstasy users.

Since each research team employing MRS used a slightly different study design and two of three studies assessed brain areas also considered in previous research, it is very hard to draw conclusions from comparisons of all studies. As well, findings reported by Reneman and colleagues in 2002 (Reneman et al. 2002b) may include some of the same participants examined in an earlier study by the same team (Reneman et al. 2001b). The earlier study of Chang employed larger samples, reported lifetime Ecstasy consumption, and employed controls (Chang et al. 1999), making it a stronger effort than either of the more recently conducted studies. Findings in one study are somewhat in agreement with those of Chang and colleagues, whereas findings in the other reports are somewhat in conflict. Stronger conclusions may await fuller reports by both Obergriesser and Reneman.

Two studies of PET scans using radiolabeled fluorodeoxyglucose (FDG) as a measure of brain glucose metabolism (Buchert et al. 2001; Obrocki et al. 2002), with both studies performed on 93 regular Ecstasy users reporting a lifetime use of 483 tablets, and an age-matched sample of 27 cancer patients with no history of illicit drug use. Both reports, which appear to be relying on data from identical samples, report finding lower FDG values in putamen and caudate, and in the left, but not right, amygdala. However, there was no single region that reliably distinguished between Ecstasy users and cancer patient controls. Amount of FDG taken up into right amygdala was related to time period since last use of Ecstasy, and reduced global FDG uptake was associated with being younger than 18 years on first use of Ecstasy. In contrast to studies attempting to image serotonin transporter sites, this study failed to find any associations between lifetime Ecstasy

consumption and FDG uptake globally or in any given region. The relevance of glucose metabolism to long-term effects of MDMA remains unclear, and the only earlier reports examining cerebral glucose metabolism were also conducted by the same research team (e.g. Obrocki et al. 1999). It is also surprising that two studies reporting nearly identical findings in identical samples were published in two different reports.

Other Putative Measures of Serotonin and Dopamine Function

Two research teams investigated the use of electroencephalography or electro-oculography to detect differences in Ecstasy users and controls (Croft et al. 2001; Gijsman et al. 2002), one team examined plasma monoamine concentrations in Ecstasy users and controls (Stuerenburg et al. 2002), and two teams examined measured neuroendocrine response after pharmacological challenge with drugs affecting the serotonin (Gouzoulis-Mayfrank et al. 2002) or dopamine (Gerra et al. 2002) systems. These studies represent various attempts to establish other means to measure effects of Ecstasy on serotonin function or investigate potential long-term effects through measuring neurotransmitter content in blood or neuroendocrine response to a dopaminergic drug. Methods are not comparable across studies, and results are not always readily interpretable in terms of supporting or refuting long-term effects of Ecstasy use.

Differences in auditory evoked potential (AEP) in response to variation in stimuli volume were used as an indirect measure of serotonin function in a study of 22 Ecstasy users, with a lifetime consumption of 225.9 tablets, 19 cannabis (marijuana) users and 20 non-drug using controls (Croft et al. 2001). Croft and colleagues found that degree to which AEPs varied in response to stimuli volume (intensity dependence) was greater in Ecstasy users than in cannabis users or non-drug user controls, a finding the authors considered evidence that Ecstasy use had affected the serotonin system. A positive association was also found to exist between lifetime Ecstasy consumption and amount of intensity dependence in AEP. One previous study had also found similar, but not identical results (Tuchtenhagen at al. 2000). In contrast, a d-fenfluramine challenge in male Ecstasy users and controls failed to produce an expected attenuation of d-fenfluramine initiated changes in the electro-ocular measure of peak saccadic velocity (Gijsman et al. 2002). Instead, dexfenfluramine increased peak saccadic activity in 21 heavy Ecstasy users (lifetime consumption of 741 tablets taken on 230 occasions), 21 moderate users (lifetime consumption of 169 tablets on 73 occasions) and 20 drug-user controls, and the expected decreases in posterior/occipital delta and theta activity appeared in all three groups of men. Furthermore, rather than being less affected by d-fenfluramine challenge, reduction in frontal/central alpha activity after d-fenfluramine challenge was even greater in heavy Ecstasy users. This report describes some novel findings, but their relevance to assessing risk of long-term effects remains unclear. As described below, failure to find changes in peak saccadic velocity after d-fenfluramine challenge and findings of reduced cognitive function have been previously reported in the same sample (Verkes et al. 2000).

Plasma monoamines (serotonin, dopamine, norepinephrine and dopamine) were assessed in a sample of 107 Ecstasy users, 11 abstinent users and 41 drug-user controls

(Stuerenburg et al. 2002). Ecstasy user participants were divided into groups of "low" users (lifetime exposure of 100 or fewer tablets), "medium" users (lifetime consumption of 100-499 tablets) and "high" users (lifetime consumption of 500-2500 tablets), and no information is provided on period of abstinence required for inclusion as an abstinent user. Light Ecstasy users had higher amounts of plasma epinephrine than abstinent users and controls, and plasma norepinephrine was higher in both "light" and "medium" Ecstasy users when compared to abstinent users and controls. Plasma norepinephrine values were related to lifetime Ecstasy consumption, Ecstasy consumption in the past month and the past year prior to study. Plasma dopamine levels were higher in "medium" users when compared with drug user controls, and there were no differences between groups in plasma serotonin levels. Since plasma monoamine values may or may not be related to brain monoamine values and since substantial information concerning drug use and demographic make up of samples is not reported in this paper, further interpretation of study findings remains unclear.

The research team led by Gouzoulis-Mayfrank examined levels of the hormone prolactin after fenfluramine in 24 Ecstasy users with a lifetime consumption of 79.6 tablets, 7 male cannabis users and 11 female non-drug user controls. Due to small sample sizes, data from both female cannabis users and the three male non-drug users was not considered in this study.) The increase in prolactin release after fenfluramine was blunted in cannabis users when compared to Ecstasy users or non-drug user controls. However, degree of prolactin release after fenfluramine was related to some parameters of Ecstasy use, such as duration of use and average dose per use. Stronger relationships still existed between parameters of cannabis use and prolactin release after fenfluramine. This would suggest that changes in neuroendocrine response cannot be read as direct indicators of serotonin neurotoxicity arising from repeated use of Ecstasy. However, it should be noted that drug use variables were strongly confounded with gender in this study, so that participant gender may have played a role in producing the observed pattern of differences.

Prolactin and growth hormone concentrations were measured after challenge with the dopamine D2 receptor agonist bromocryptine in 12 male Ecstasy users and 12 non-drug user controls reporting an average lifetime consumption of 52.3 tablets and verifiably abstinent for an average of 27 days prior to study (Gerra et al. 2002). Both groups showed prolactin release after bromocryptine, but growth hormone release after bromocryptine was lower in Ecstasy users than it was in controls. Lifetime Ecstasy consumption was inversely associated with growth hormone release, with higher lifetime consumption associated with less growth hormone release after bromocryptine. The authors interpret findings as indicative of direct effects of Ecstasy use on the dopamine system, but acknowledge that effects may also be caused by changes in 5HT2A receptors (Reneman et al. 2001c) or to pre-existing differences in the dopamine system. It is the only report offering any conceivable support to claims made by some that repeated Ecstasy use harms dopamine neurons, but study results are amenable to alternate explanations.

Serotonin function and Neurocognitive Performance

While recent findings continue to support a relationship between regular Ecstasy use and reduced performance on tests of verbal memory, visual memory and executive function, these findings do not always match findings of differences on proposed indices of serotonin function. For instance, reduction in serotonin transporter sites as measured by the radioactive marker Beta-CIT has been found in women only, and not men (Reneman et al. 2001c). Yet gender differences in the impact of Ecstasy use on cognitive function have only been found in one study, and in this case, women seemed to be less affected (Bolla et al. 1998). In a study that may even have examined a subset of the same individuals assessed in recent publications, Reneman and colleagues failed to find any gender differences in the effects of regular Ecstasy use on memory performance (Reneman et al. 2001a). Though Gijsman et al (2002) found no differences in dexfenfluramine-induced changes in saccadic peak in moderate or heavy Ecstasy users and controls, a previous report examining data drawn from the same sample of men found that both moderate and heavy users had lower scores on measures of verbal and visual memory (Verkes et al. 2000). It is possible that peak saccadic velocity after dexfenfluramine and Beta-CIT binding is not an accurate gauge of serotonin function. As noted earlier, some researchers have critiqued the use of imaging studies as evaluations of MDMA effects on serotonin function (Kish 2002). It may also be that lower task performance in regular Ecstasy users is unrelated to changes in serotonin function but still somehow related to ecstasy use. While these dissociations have no direct impact on conclusions drawn about study findings, they suggest that caution should be used in relying on changes in one domain (such as serotonin transporter density) as indicators of changes in another domain (such as that of cognitive function).

Immune Function in Ecstasy Users

In addition to conducting studies on the acute and sub-acute effects of one or more doses of MDMA on the immune system, discussed earlier in this review, a team of researchers in Spain have also studied the effects of repeated Ecstasy use on immune function (Pacifici et al. 2002). While these findings have so far only appeared in a report covering all of their research without presenting any one study in great detail, their work has found that continued Ecstasy use may affect immune function, though the reasons for this are not altogether clear. In the first of two studies, complete immune cell profiles were conducted in 30 male Ecstasy users and 24 gender-matched non-drug user controls, with Ecstasy users probably all participants in their MDMA trials. In contrast to changes seen immediately after MDMA (Pacifici et al. 2001), NK cells measured at baseline were lower than in controls. There was a trend for levels of lymphocytes, T cells and CD4 cells to be lower in Ecstasy users than in non-drug user controls. The second of two studies examined complete blood profiles of six male Ecstasy users and eight non-drug using blood donors over a two-year period, with profiles taken at baseline, one year later and two years later. This study found that numbers of several immune cells declined over time, with number of lymphocytes and CD4 cells at lower levels one and two years after baseline in Ecstasy users, and with CD 19 B cells lower than controls two years after baseline. The authors do not have an explanation for their findings, but note that T cell production is partially mediated by serotonin. However, they do not rule out the impact of other acute effects of repeated Ecstasy use. It should be noted that no information is

provided concerning average lifetime Ecstasy consumption in either sample, although the authors said that participants in the second study reported having used between five and 50 tablets in a lifetime at baseline. No information is also provided on number of Ecstasy tablets consumed in the intervening time between each blood profile. Furthermore, use of other drugs by Ecstasy users might have played a role in these generally immunosuppressive effects, since the comparison group reported no use of any illicit drug, whereas Ecstasy users reported use of cannabis and other drugs. Further research and reports that present results in more detail are needed before these effects can be better understood. Nevertheless, it would appear that continued regular use of Ecstasy and other drugs over an extended period of time might impair immune function.

Conclusions

All studies located during this time period describe findings in regular Ecstasy users, with participants in most studies reporting an average lifetime consumption of at least 20 tablets. Statistically significant, but subtle and selective differences in some cognitive domains between Ecstasy users and non-user controls are often noted in samples of Ecstasy users with a total lifetime consumption of 20 or more tablets, though this is not always the case. As stated in the Investigator's Brochure, we continue to be concerned with the potential effects of regular Ecstasy use on verbal and visual memory and executive function, but results from more well-controlled studies will be required to separate out the effects of Ecstasy from possible confounds.

An increasing number of studies suggest that concern over long-term effects of regular Ecstasy use on psychiatric health is unwarranted, as several recently published studies failed to find a strong association between regular Ecstasy use and self-reported complaints or clinically diagnosed mental disorders. While imaging studies continue to find differences in possible measures of serotonin function, such as Beta-CIT binding, findings in other studies are at times contradictory (as in research using MRS) or insufficient (as in research measuring glucose metabolism with FDG). At least one imaging study also suggests that men and women who have taken 20 or fewer Ecstasy tablets do not show significantly lower Beta-CIT binding (Reneman et al. 2001c). Complex and somewhat mixed results open to at least one alternate interpretation are also found in neuroendocrine challenge studies. Recent reports of reduced numbers of immune cells in people using Ecstasy repeatedly over a period of two years also suggests that regular use of Ecstasy might increase susceptibility to infection, though findings are preliminary at present.

After considering all published studies in Ecstasy users published in the last 15 months, our conclusion remains unchanged that there is minimal risk of producing adverse long-term effects in subjects administered two doses of 125 mg MDMA.

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MAPS' MDMA Investigator's Brochure
Update #1
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Investigations of MDMA Toxicity in Non-Human Animals

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Extensive studies in rats and monkeys have found that high or repeated doses of MDMA can oxidatively damage serotonergic axons originating in the dorsal raphe nucleus of the brainstem, whereas high or repeated doses of MDMA in mice harm dopamine neurons. This research has been thoroughly reviewed in the Investigator's Brochure (e.g. Baggott et al. 2001) and elsewhere (Baggott and Mendelson 2001). Most of the research performed from late 2001 to the present consists of studies intended to support a specific model of MDMA neurotoxicity or to refute a competing model. No new major advances in knowledge have been made in adult animals, though studies of developmental effects present new evidence both for and against the presence of such effects. All findings continue to support the exclusion of pregnant women and women not using an effective means of birth control in human trials with MDMA. Researchers continue to find that high or repeated doses of MDMA damage serotonin neurons while still failing to find significant differences in cognitive assessments of MDMA-treated animals and controls. Starting in late 2001 and continuing on through 2002, a number of studies producing contradictory findings concerning the long-term effects of MDMA exposure on anxiety in rodents were published. Heart, liver and kidney toxicity have also been examined in rodents, with research mostly extending previous findings. A study reporting damage to dopamine axons in non-human primates after a repeated-dose regimen of MDMA appears to contradict previous findings in non-human primates (Ricaurte et al. 2002). However, speculations made concerning the application of these findings to human Ecstasy users are not supported by other studies in humans.

Neurotoxicity

Most recently published studies addressing the issue of MDMA neurotoxicity in non-human animals seek to support a specific model of MDMA neurotoxicity or to refute a competing model. These studies employ pharmacological and non-pharmacological treatments either intended to protect or exacerbate MDMA-induced neurotoxicity, and usually evaluate brain serotonin, serotonin transporter or another marker of serotonin function. However, one study sought to demonstrate that MDMA-induced changes in serotonin axons result from disrupted function and not down-regulation. Research concerned with discovering behavioral changes produced after MDMA administration, including those markers specifically associated with neurotoxicity, also continue in rodents and monkeys. A few researchers have also employed neuroendocrine challenge studies or sought to validate imaging methods used in human studies. Recent investigations in non-human animals have not raised any new concerns relating to clinical

trials with MDMA, though some study findings may end up revising ideas about the long-term effects of high or repeated doses of MDMA.

Ten studies have been conducted in rats and five in mice since the completion of the Investigator's Brochure. A neurotoxic dose regimen of MDMA produces effects in serotonin in rats and dopamine in mice. Since effects in rats are closer to those in primates and perhaps humans, studies in rats may be somewhat more informative about potential neurotoxicity in humans. A study using a number of pharmacological interventions offers at least one case for differences in mice and rats (Colado et al. 2001), suggesting that mouse neurotoxicity may arise from oxidative stress produced by a specific metabolite not involved in rat MDMA neurotoxicity. Some support for the importance of dopamine release in MDMA neurotoxicity in mice was reported by Camerero and colleagues (Camerero et al. 2002), though one dopamine uptake inhibitor used in this study also had antioxidant properties.

Arguments over the significance of changes in serotonin axons after MDMA were addressed in an in vitro study of rostral raphe neurons (serotonin neurons) taken from rats given twice-daily doses of 20 mg/kg MDMA for four consecutive days (Callahan et al. 2001). Effects were compared with the known serotonin neurotoxin 5,7-DHT and fenfluramine. Anterograde (forward) transport in neurons was similar after all three compounds. This is the first study to date that employed this technique to measure function in serotonin axons after MDMA. It implies that axonal swelling and other changes in serotonin neurons seen after high or repeated doses of MDMA in neurons are not due to downregulation but to disrupted axonal function.

Research conducted since the completion of the Investigator's Brochure has sought evidence in support of at least four overlapping hypotheses concerning the causes of MDMA neurotoxicity in rodents. Most explanations of MDMA neurotoxicity rest on oxidative stress, wherein metabolism of MDMA or released neurotransmitters produces reactive products that damage serotonin axons. Some explanations posit that specific metabolites, such as glutathione conjugates or products of dopamine breakdown, are involved. The other major set of hypotheses relates to energy exhaustion or bioenergetic stress. The effects of high ambient temperature and hyperthermia are generally taken into account, and most models looking at neuroprotection also assess body temperature.

Various candidates for producing oxidative stress have been investigated in the past year and a half, including monoamines and conjugated metabolites. While MDMA metabolites remain candidates, no studies specifically addressing them were published in this time period. Several studies manipulating monoamine metabolism, as through the use of reserpine (Yuan et al. 2002a), MAO-B antisense (Falk et al. 2002) or MAO knockout mice (Fornai et al. 2001), were performed, with most finding at least some attenuation of MDMA neurotoxicity when metabolism of monoamines was inhibited. Glutathione depletion in rats proved to be neuroprotective in one case and did not

exacerbate MDMA neurotoxicity (O'Shea et al. 2001), and when the norepinephrine system was damaged in mice, it exacerbated dopaminergic toxicity after MDMA in mice (Fornai et al. 2002). Support for a role of dopamine metabolites in MDMA neurotoxicity in rats was reported in a study using dopamine transporter antisense (Kanthasamy et al. 2002), though no attempt was made to measure extent of oxidative stress seen after antisense administration. Dopamine transporter antisense was neuroprotective without reducing MDMA-induced hyperthermia. A study in rats offered some support for effects related to bioenergetic stress (Darvesh et al. 2002), though alternative hypotheses are possible. However, another study intended to find support for this model (Yuan et al. 2002b) found that glucose depletion did not exacerbate neurotoxicity.

Adrenalectomy reduced MDMA neurotoxicity in rats but also induced hypothermia, whereas a peripheral nicotinic antagonist (chlorisondamine) reduced MDMA neurotoxicity without significantly altering body temperature (Fernandez et al. 2002). Similarly, research in female mice found that corticosterone exacerbates MDMA neurotoxicity (Johnson et al. 2002a). Fluoxetine was found to be a more potent neuroprotective agent than fluvoxamine in a study with Dark Agouti rats, probably by directly acting on the transporter (Sanchez et al. 2001). In vivo microdialysis and studies in synaptosomes comparing 4-methylthioamphetamine (MTA), m-chlorophenylpiperazine (mCPP), p-chloroamphetamine (pCA) and MDMA found that synaptosomal serotonin release might be a marker of neurotoxicity, though they did not measure effects of these compounds on brain serotonin (Gobbi et al. 2002).

Comparisons across species demonstrate discrepancies in several areas. For instance, MDMA produces little or no effect on learning or working memory in rodents and monkeys, yet studies in humans find that repeated use of Ecstasy is associated with memory and executive function in humans. On the other hand, both neurotoxic and non-neurotoxic MDMA dose regimens usually seem to increase anxiety in rats, whereas an association between Ecstasy use and self-reported anxiety and other psychological problems appears to be less strong and more complex. It is possible that some of the differences between findings reported in humans and non-human animals may derive from species-specific effects of MDMA on neuronal function, and other differences may result from study design and methods. For instance, Ecstasy users have chosen to self-administer the drug and may have done so as a result of pre-existing traits or conditions not found in non-users, whereas laboratory animals given MDMA in the course of research have not chosen to take the drug. Furthermore, the dose regimens used in many studies in non-human animals do not resemble patterns of exposure seen in human Ecstasy users.

Learning and cognitive function

While a few researchers have found that non-human animals given MDMA show reduced performance on tasks requiring memory (e.g. Marston et al. 1999), most researchers have

failed to find any functional consequences of serotonergic neurotoxicity in rodents or non-human primates (Baggott and Mendelson 2001, also see Investigator's Brochure). Recently, studies were performed in squirrel monkeys, rhesus monkeys and several strains of rat. Neurotoxic doses of MDMA had little or no effect on learning and memory in monkeys, but the effects of MDMA in rats are less clear. Most of this research is consistently with previous attempts to demonstrate reduced learning or memory after exposure to MDMA, though there are now a greater number of studies describing effects.

Researchers sought to use drug challenge conditions to unmask previously hidden deficits or markers in monkeys (Taffe et al. 2002; Winsaur et al. 2002). Monkeys in this study received 2.5 mg/kg MDMA twice daily for four days, and a subsequent dose regimen following the same pattern, but with 5 mg/kg. MDMA-treated squirrel monkeys did not perform differently from saline-treated monkeys on a sequential learning task at baseline when tested 4 to 13 days after MDMA dose regimen. This task requires each monkey to learn a sequence of key presses associated with a sequence of colored key lights through repeated presentation. This study also failed to find differences between the two groups after they were challenged with the benzodiazepine triazolam, the serotonin releaser fenfluramine and the 5HT2C agonist, serotonin releaser and 5HT1A agonist mCPP (Winsaur et al. 2002). Performance on a number of tasks was assessed in rhesus monkeys given 10 mg/kg MDMA daily for four days, with assessments taking place 13 months after drug administration (Taffe et al. 2002). Tasks included a measure of working memory (self-ordered spatial search), sustained attention in goal-directed behavior (progressive ratio reinforcement), reaction time (RT) and bimanual dexterity (removing raisins from a transparent plastic board with holes). At baseline, task performance by MDMA-treated monkeys did not differ from controls, and the two groups performed similarly after challenge with the 5HT2A antagonist ketanserin and the 5HT1A agonist 8-hydroxy-aminotetralin (8-OHDPAT). Only challenge with the serotonin releaser and 5HT2C agonist mCPP produced group differences, with MDMA-treated animals showing greater sensitivity to the RT-slowing and progressive ratio reducing actions of mCPP. Yet despite minimal effects on test performance, rhesus monkeys given MDMA had significantly reduced levels of serotonin in all areas of cortex. Both studies suggest that a dose regimen of MDMA capable of producing neurotoxicity did not affect working memory or dexterity, and only affected attention under mCPP challenge.

Inconclusive findings of MDMA-induced impairment of learning or memory also appear in studies of rodents. While there was an association between exposure to MDMA and reduced time spent exploring a novel object and more time investigating a novel rat on second presentation (Morley et al. 2001; Pompei et al. 2002), both measures can be interpreted as measures of anxiety or of sociality. Rats given 5 mg/kg MDMA three times a day for two days spent less time exploring a novel object placed near a previously presented object than did controls or rats given 1 mg/kg MDMA four times a day for two days (Morley et al. 2001), with testing performed 12 weeks after drug administration. A

lower dose of MDMA (5 mg/kg once a day for two days) produced no significant differences in object exploration when compared to all other groups, indicating perhaps a slight decrease in time spent investigating the novel object. These findings are interpreted as indicators of reduced memory, with a high dose of MDMA decreasing memory. However, it is possible that exploratory behavior is also partially controlled by levels of anxiety, and rats in this study also displayed greater anxiety than controls (see below for discussion). It should also be noted that brain serotonin levels or serotonin system function were not assessed in this study. Recall for social information was assessed through comparing time spent interacting with a rat on second presentation on the eighth and final day of drug administration, with greater interaction considered a measure of less recall (Pompei et al. 2002). Rats given 1 and 5 mg/kg for eight days spent more time interacting with the rat on second presentation than controls or rats given 10 mg/kg. However, all rats tested, including controls, spent a greater amount of time with the novel rat on second presentation, suggesting that social interaction might increase with familiarity. If this is the case, an alternate interpretation of the findings reported above is that lower doses of MDMA either did not change or reduced social anxiety, whereas higher doses might have increased social anxiety.

MDMA-induced reductions in learning, memory or cognition in non-human animals are elusive. Studies in monkeys have found that MDMA dose regimens that reduce brain serotonin have little or no effect on measures of learning or memory, a pattern consistent with earlier findings (e.g. Frederick et al. 1999). While a few previously conducted and recently conducted studies in rats have found that MDMA may affect memory-related behaviors, their relation to reduction in serotonin function is not entirely clear, and the measures used are open to alternative interpretations. In contrast, impaired performance on measures of verbal recall and executive function in regular Ecstasy users are reported in many studies (see Investigator's Brochure and related discussion in this report). This discrepancy remains to be explained, but may arise for several reasons, including species-specific differences in MDMA effects, pre-existing conditions influencing drug-use behavior and task performance in regular Ecstasy users, or effects produced by use of other drugs. The last hypothesis has been addressed in the Investigator's Brochure, where support was found in some studies (Croft et al. 2001) but not in others (Gouzoulis-Mayfrank et al. 2000). Recently published studies are similar to prior publications in their general failure to find MDMA-induced impairments in learning and memory in non-human animals. However, there are now a few more studies finding at least potentially relevant behavioral differences (Morley et al. 2001; Pompei et al. 2002), though the significance of these findings is not completely certain.

<u>Anxiety and social interaction</u>

Several research teams examined the association between exposure to MDMA and changes in anxiety, producing sometimes contradictory study findings (Fone et al. 2002; Gurtman et al. 2002; Mechan et al. 2002; Morley et al. 2001). There are three reports of

increased anxiety (Fone et al. 2002; Gurtman et al. 2002; Morley et al. 2001) and one report of reduced anxiety (Mechan et al. 2002). Additionally, one study intended to measure social memory may also have found increased anxiety at a high dose level and decreased anxiety at lower doses (Pompei et al. 2002). Anxiety was assessed with the elevated plus maze (Gurtman et al. 2002; Mechan et al. 2002; Morley et al. 2001), emergence from an enclosed "hide box" (Gurtman et al. 2002; Morley et al. 2001), the open field test (Mechan et al. 2002) and social interaction tests (Fone et al. 2002; Gurtman et al. 2002; Morley et al. 2001; Pompei et al. 2002). Greater anxiety on the plus maze was seen in Wistar rats given 5 mg/kg MDMA three times a day for two days (Morley et al. 2001) or four times on an hourly basis for two days (Gurtman et al. 2002). Morley and associates assessed anxiety 12 weeks after MDMA administration, and Gurtman and associates assessed anxiety over a period of four to nine weeks post-MDMA. Dark Agouti rats given a single dose of 12.5 mg/kg MDMA displayed less anxiety (Mechan et al. 2002) when tested 80 days after drug administration. However, assessment of brain serotonin and the serotonin system was only reported by Gurtman and colleagues, who found evidence for lower levels of serotonin. Likewise, the Wistar rats were less likely to emerge from an enclosed box, whereas the Dark Agouti rats were more likely to explore an open field (Mechan et al. 2002; Morley et al. 2001). Both groups of Wistar rats described by Gurtman and Morley, and Lister Hooded rats given 7.5 mg/kg MDMA twice daily for three days showed reduced social interaction with a strange rat, with no signs of reduced serotonin observed in rats killed 24 days after MDMA administration (Fone et al. 2002).

In addition to studying rats receiving the repeated dose regimen of 5 mg/kg three times a day, the study conducted by Morley and colleagues also gave rats a single dose of 5 mg/kg MDMA once daily for two days or four doses of 1 mg/kg amphetamine for two days (Morley et al. 2001). Lower doses of MDMA still produced greater anxiety in the plus maze than controls, and slightly more anxiety than controls on the hide box emergence task. However, rats in all three drug administration conditions interacted less with a strange rat than controls. These findings suggest that the effects of MDMA on anxiety may increase with dose in at least some rat strains, and that amphetamine may produce some similar effects.

Considering all research findings, it appears that repeated doses of MDMA can either increase or decrease anxiety in rodents, and that a demonstrably non-neurotoxic dose of MDMA may also alter level of anxiety (Fone et al. 2002). One attempt at reconciling these findings proposes that neurotoxic doses of MDMA increase anxiety in less anxious rat strains and decrease anxiety in more anxious strains, and that moderate doses of MDMA might alter anxiety more than higher doses (Green and MacGregor 2002). Increased anxiety was noted in Wistar, and Lister Hooded rats (Fone et al. 2002; Gurtman et al. 2002; Morley et al. 2001), whereas decreased anxiety was noted in Dark Agouti rats (Mechan et al. 2002). However, this analysis cannot account for findings of greater anxiety after a non-neurotoxic dose of MDMA (Fone et al. 2002), a finding that suggests

that changes in neural function can occur in MDMA-treated animals without being related to neurotoxicity.

Neuroendocrine challenges and imaging studies

Several studies have found altered neuroendocrine response to serotonergic drugs in regular Ecstasy users (Gerra et al. 2000; McCann et al. 1999; Verkes et al. 2000). Researchers have conducted similar studies in non-human animals, using the same or similar pharmacological interventions. Neuroendocrine response to challenge with mCPP was examined in squirrel monkeys given 5 mg/kg MDMA twice daily for four consecutive days (Hatzidimitrou et al. 2002). Challenge was performed under anesthesia either 2 weeks or 3.5 years after MDMA administration, and challenge was also performed on monkeys given an identical dose regimen of fenfluramine. Prolactin response to mCPP was blunted two weeks after receiving MDMA, but 3.5 years after MDMA treatment, prolactin release after mCPP was at even greater levels than in controls. Study findings suggest that it takes longer for human neuroendocrine responses to return to normal values, or that MDMA-induced changes in response to mCPP are not comparable across species. However, because baseline prolactin responses are not measured, group differences may arise from inter-individual variability in neuroendocrine response.

In line with discoveries made in human trials (Forsling et al. 2001), a study in cultured rat hypophyseal cells found that MDMA and several of its metabolites cause the release of vasopressin and oxytocin (Forsling et al. 2002). It would seem that MDMA can lead to release of both hormones in rodents as well as humans, though there are as yet no published reports of in vivo studies of vasopressin or oxytocin release in non-human animals given MDMA.

Four studies in non-human animals relied on radioactive compounds, or radioligands, that bind with the serotonin 5HT2A receptor or with the serotonin transporter. One investigation compares imaging of 5HT2A receptors done in rats and humans (Reneman et al. 2002) and the other studies sought to validate the use of radioligands for measuring the serotonin transporter (Boot et al. 2002; Reneman et al. 2002c; Szabo et al. 2002). The investigation of 5HT2A transporter density employed the radioligand R91150 in rats and humans, and the two serotonin transporter drugs were compared in baboons in one study and in rats and rhesus monkeys in the other.

Rats in the study of 5HT2A receptors were given 10 mg/kg twice daily for four days, and were then tested 6 hours, 3 days or 30 days later, with imaging compared to measures of serotonin and its metabolite 5HIAA (Reneman et al. 2002). The same radioligand was used in SPECT imaging of 17 current and seven former Ecstasy users, as well as drug-using controls. (See section on humans for greater discussion). Rats given MDMA 6 hours prior to 5HT2A receptor measurement showed reduced binding for R91150, whereas amount of receptor binding was no longer different from controls 30 days after MDMA administration. However, all rats showed a decline in serotonin and 5HIAA

content after MDMA administration, including rats given MDMA 30 days prior to measurement. While study findings are somewhat similar to those seen in humans, extrapolating from findings in rats to humans would imply that humans had lost about 80% of brain serotonin, a finding that seems incompatible with findings of only subtle differences in brain function and behavior seen in regular Ecstasy users.

The study in baboons (Szabo et al. 2002) employed a very small sample size, and only one of five animals received MDMA, two regimens of four daily doses of 5 mg/kg MDMA. Paroxetine pre-treatment was used for imaging controls, and only saline-treated animals were used as controls for measuring brain serotonin content. The research did find that both trans- 1,2,3,5,6,10-beta-hexahydro-6-[4-(methylthio)phenyl[pyrrolo-[2,1-a]isoquinoline (McN5652) and 3-(11)C-amino-4-(2-dimethylaminomethylphenylsulfanyl)benzonitrile (DASB) bound in areas highest in serotonin, and that the two compounds produced similar patterns. The authors did not did not use a comparison of different (enantiomeric) forms of McN5652 to assess non-specific (not related to serotonin) binding in this study, though they had used it in prior research in humans (McCann et al. 1998). Furthermore, McN5652 binding in some brain areas was lower than after paroxetine pre-treatment, a finding that suggests a potential interaction between paroxetine pretreatment and imaging with McN5652. While the study was intended to demonstrate the validity of this compound in humans, the study seems to be poorly designed and uses a very small sample size.

Three studies, two of them conducted in rats and one in a very small sample of rhesus monkeys, examined the validity of assessing serotonin transporter site density with the radioligand [123I]-2beta-carbomethoxy-3beta-(4-iodophenyl) tropane (Beta-CIT), a compound also used to measure serotonin function in Ecstasy users (see discussion in "Studies in Ecstasy Users"). Comparisons were made of rats given saline or 20 mg/kg MDMA twice daily for four days and between a rhesus monkey that received twice-daily doses of 5 mg/kg MDMA for four days (Reneman et al. 2002). Comparisons of radioligand binding were made at four, ten and 31 days after MDMA administration in monkeys, and after four and seven days after MDMA treatment in rats. The study found that MDMA-treated animals had lower Beta-CIT binding in sites assumed to have serotonin neurons when compared with control binding and from autoradiography with radioactively labeled citalopram. It appears that there may be a return to greater Beta-CIT binding in monkeys 31 days after MDMA administration, suggesting either that the process is reversible or that it is not only related to serotonin axon loss. The relationship between reduced serotonin transporters, as measured via Beta-CIT autoradiography and via assays with radioactively labeled paroxetine, was examined by comparing brains of rats given two 20 mg/kg doses of MDMA for four days or 300 mg/kg of the tryptophan hydroxylase inhibitor p-chlorophenylalanine followed by 100 mg/kg every other day for eight days (Boot et al 2002). In rats killed 7 and 14 days after treatment, only those given MDMA beforehand had reduced serotonin transporter sites, though rats in both conditions had reduced levels of serotonin and its metabolite 5-HIAA. However, earlier studies using P-chlorophenylalanine (Linnet et al. 1995) have reported reduced serotonin transporter sites after treatment. All three of these studies sought to validate the use of

radioactive compounds as a means to measure serotonin function. However, methods used in some studies may call at least some of the findings into question.

Possible Dopamine Toxicity in Non-Human Primates

A study in non-human primates sparked concern that repeated doses of MDMA might harm dopamine neurons (Ricaurte et al. 2002). Very small samples of squirrel monkeys and baboons received 3 doses of 2 mg/kg MDMA every three hours. Harm to dopamine neurons was established through several means of assessment, including measuring dopamine transporter density and silver staining. Drawing on study results, the authors hypothesized that humans using repeated doses of MDMA would also damage their dopamine neurons. However, a case report and other research conducted prior to 2001 (Kish et al. 2000; Semple et al. 1999) and one conducted subsequently (Reneman et al. 2002) do not lend any support to these concerns. Reneman and colleagues found that reduced dopamine transporter binding was seen only in regular Ecstasy users who reported amphetamine use, and not in those who reported Ecstasy use only (Reneman et al. 2002). It also seems highly likely that the dose regimen used in monkeys and baboons does not represent a commonly used regimen in humans, since the authors reported a 20% to 40% mortality rate in their sample, which is much higher than the estimated mortality rate in human users (well below 1%; see the Investigators' Brochure).

Developmental Neurotoxicity

Investigations into the effects of MDMA exposure in rodents have been somewhat inconclusive but suggest that exposure during early periods of an animal's life may affect brain development and behavior (Meyer et al. 2002; Morley-Fletcher et al. 2002; Whitworth et al. 2002; Won et al. 2002). Only a few studies have examined developmental effects in humans (McElhatton et al 1999), and findings of developmental effects are present but also confounded in the study. All findings continue to support the exclusion of pregnant women and women not using an effective means of birth control in human trials with MDMA.

10 mg/kg given once over three days in mice 32 days after birth, but not 28 or 52 days, resulted in reduced social interactions when tested at 80 days after birth, indicating either that this represents a period of sensitivity in mice or that the interval between MDMA regimen and testing was most sensitive to the effects of MDMA (Morley-Fletcher et al. 2002). In vitro studies of embryonic mouse and rat cells found that maternal or direct exposure to MDMA affected cell development. In one study, MDMA, cocaine and fluoxetine all altered development of rat embryonic glutamate cells, perhaps by altering levels of serotonin or norepinephrine (Whitworth et al. 2002). Maternal exposure to a regimen of very high doses of MDMA during gestational days 6 through 13 in mice (40 mg/kg twice daily for seven days) altered the neurochemistry of fetal cells extracted from the exposed dams (Won et al. 2002). Concentrations of serotonin and dopamine were elevated on cells maintained to days 22 and 36. Finally, rat pups exposed to 10 mg/kg

MDMA given twice daily for four consecutive days from post-natal days one through four had lower concentrations of hippocampal serotonin (Meyer et al. 2002).

It should be noted that somewhat contradictory findings of reduced versus increased serotonin and dopamine may relate to interspecies differences between mice and rats and differences in developmental period. Previous investigations suggested that MDMA might have developmental effects, but that the existence or nature of these effects was not clear. Current research lends support to the possibility of developmental effects of MDMA, though it should be kept in mind that the dose regimens used in these studies are high and repeated when compared to the doses employed in human clinical trials. It is unclear whether lower and less frequent doses would have the same effects. Nevertheless, it seems reasonable to continue the current measures intended to eliminate possible fetal exposure to MDMA.

Thermoregulation

While core temperature increased in rabbits given 3 or 6 mg/kg MDMA (Pedersen and Blessing 2001), cutaneous temperature measured in the rabbit's ear, or pinna, declined and showed signs of vasoconstriction. When a procedure was performed that produced adrenalectomy on one side of the animals, reduced blood flow was only seen in the pinna on the non-adrenalectomized side, suggesting a central mechanism regulating cutaneous blood flow. It is unclear whether these results are also applicable in humans, but there has been some indication (see Harris et al. 2002; Tancer et al. 2001) that humans are also affected by reduced blood flow to the skin. To date, there have been no reports of hyperthermia arising during a clinical trial of MDMA in humans.

Cardiovascular and Cardiotoxicity

The effects of MDMA on heart tissue were investigated in mice and in cultured rat and human cells. Two studies conducted by Gesi and colleagues (Gesi et al. 2002a; Gesi et al. 2002b) reported that repeated doses of 20 mg/kg (Gesi et al. 2002b) to 30 mg/kg (Gesi et al. 2002a) MDMA produced only slight and nonsignificant changes at the mitochondrial level. Significant structural alterations were produced only when MDMA was administered in combination with loud white noise in an attempt to simulate human noise exposure at nightclubs and dance events. However, humans attending dance events find the music they hear pleasurable or are at the least familiar with hearing loud noise, while the mice in this study probably experienced stress from exposure to loud noise.

1 to 100 mcM MDMA applied directly to cultured rat aortic tissue failed to inhibit contractions in the presence of serotonin (Murphy et al. 2002), a finding that may relate to its actions as a 5HT2A receptor agonist. Direct application of 10 mcM MDMA to isolated rat right ventricle potentiated norepinephrine-evoked contractions, perhaps through inhibition of norepinephrine uptake (Cleary et al. 2002), a finding the authors

suggest might be related to cardiac problems acutely after MDMA in Ecstasy users. Contraction of vascular tissue taken from rats given 20 mg/kg MDMA twice daily for four consecutive days showed changes in response to serotonin (Cannon et al. 2001). Maximum contraction of aortic rings was reduced after MDMA on 1 and 7 days post-drug, but not 14 or 21 days after MDMA. The authors interpret their results as indicative of potential cardiovascular difficulties in regular Ecstasy users.

Researchers attempting to model binge use in rats administered twice-daily doses of 3 mg/kg or 9 mg/kg MDMA for four consecutive days in three separate dose regimens conducted ten days apart (Badon et al. 2002). While largely devoted to investigating the acute effects of MDMA on the sympathetic system, examinations were made of cardiac tissue taken from rats killed after the first, second or third MDMA dose regimen. Pathologists blind to condition did not see any heart lesions after the first 9 mg/kg regimen; Tissue damage was observed after each successive dose regimen, and heart inflammation was seen in rats killed after receiving all three repeated dose regimens. It is unclear whether these findings have any relevance for Ecstasy users. However, alterations in sympathetic versus parasympathetic regulation has been reported in a small sample of Ecstasy users (Brody et al. 1998), as previously described in the literature review.

Most of the effects described in these studies involve the repeated administration of high doses of MDMA, and the dose regimens used in these studies generally do not represent common use patterns in Ecstasy users. Furthermore, effects on heart tissue are not found consistently, with one study finding effects only in combination with loud noise whereas another found effects after successive repeated-dose regimens. It is unclear what import, if any, these findings have for the administration of two doses of 125 mg MDMA separated by 3-4 weeks. Findings of cardiac and cardiovascular effects are to be expected given the sympathomimetic actions of MDMA and do not describe heretofore unknown risks to human study participants. Aside from hypertensive episodes in less than 5% of participants in previous laboratory studies described in the study protocol, no additional cardiovascular or cardiac problems have been reported, and none of the hypertensive episodes required medical intervention. Further study may indicate whether the effects described in rodent and in vitro studies described here are relevant to regular Ecstasy users.

Immunological Effects

Extending a continued investigation of the immunological effects of MDMA in non-human animals begun in mice (Connor et al. 2000a; 2000b), studies in rats found similar effects (Connor et al. 2001a; 2001b). One study found MDMA acutely reduced interleukin- (IL-) and tumor necrosis factor (TNF), and that this was not blocked by the serotonin uptake inhibitor paroxetine (Connor et al. 2001a). The other investigation in rats found that MDMA acutely reduced interferon-Gamma, but not interleukin-6 when rats were challenged with MDMA and an antigen stimulating factor (Connor et al. 2001b). When considered along with human studies (Pacifici et al. 2001; 2000) and

earlier studies in mice (Connor et al. 2000a; 200b), it appears that MDMA similarly affects the immune systems in three different species. The reported observations are in agreement with previous and current studies in humans and do not raise any new concerns about immunological effects.

Liver and Kidney Toxicity

Three studies have explored the effects of MDMA on hepatic tissue and have investigated conditions that may exacerbate these effects, such as vitamin E deficiency, in hepatotoxicity associated with MDMA, and one study examined effects of MDMA and metabolites on kidney cells. Two of the three studies are conducted by the same team that published earlier reports on the effects of MDMA on liver tissue (Carvalho et al. 2002; Monteil-Duarte et al. 2002). Injections of 10 or 20 mg/kg MDMA, but not 5 mg/kg, produced some evidence of liver damage in mice, with effects accentuated when mice were kept in a warm environment (30 versus 20 deg. C) (Carvalho et al. 2002). It is notable that the dose of 20 or 30 mg/kg given in a warmer environment increased mortality from 10% to 40%, a sign that the doses used may not reflect those employed either by illicit Ecstasy users or in human studies of MDMA. Mice given four repeated doses of either 5 or 10 mg/kg S-(+)-MDMA did not produce significant changes in liver cells (Johnson et al. 2002). Only animals maintained on a diet deficient in vitamin E demonstrated significant harm to liver cells. Lastly, cultured rat hepatocytes exposed to MDMA at 1, 3 and 5 mM induced apoptosis, with effects being dose dependent (Montiel-Duarte et al 2002). However, the level of exposure used in this study is eleven times greater than levels of MDMA expected occur in humans (Carvalho et al. 1999).

Considered together, these investigations may help generate hypotheses concerning liver disease seen in regular Ecstasy users. For instance, it would appear that both high ambient temperature and vitamin E deficiency might increase risk of liver disease after regular Ecstasy use. However, these findings do not increase the assessment of minimal risk of liver damage for clinical research participants.

Cultured rat and human renal (kidney) cells were unaffected after incubation with MDMA or MDA, at concentrations ranging from 100 to 800 mcM (Carvalho et al. 2002b). Incubation with the highest concentration of the MDMA metabolite alpha-methyl-dopamine produced significant decreases in rat and human cell viability. Glutathione conjugates of alpha-methyldopamine were also found to affect kidney cell viability. It thus appears that MDMA metabolites might affect renal cells more than MDMA itself. As was true of the in vitro studies in liver cells described above, the level of exposure far exceeds expected levels in Ecstasy users. No cases of renal problems have arisen after MDMA administration in controlled settings.

Self-administration Studies

Rhesus monkeys trained to self-administer cocaine will self-administer racemic MDMA and its two enantiomers, though methamphetamine maintained higher rates of self-administration than MDMA (Fantegrossi et al. 2002). After initially failing to find any preference for a place associated with MDMA injection in group-housed rats, Meyer and others (2002) found that socially isolated rats did prefer the drug-associated location (conditioned place preference). These findings confirm previous findings that MDMA possesses reinforcing effects and may have some degree of abuse potential. Previous studies have already demonstrated that animals trained to administer other drugs will self-administer MDMA (e.g. Lamb and Griffith 1987).

Conclusion

Researchers continue to study the effects of MDMA in non-human animals and in vitro, with most research published after the completion of the Investigator's Brochure addressing models of MDMA neurotoxicity. It still remains difficult to establish functional consequences of MDMA neurotoxicity. Changes in anxiety seem to accompany neurotoxic dose regimens, but it has also been found that a non-neurotoxic dose will also alter anxiety in rats (Fone et al. 2002). Study findings reported in recent publications suggest that exposure to MDMA during a specific critical period in development may be harmful, though findings are inconsistent across species. All findings continue to support the exclusion of pregnant women and women not using an effective means of birth control in human trials with MDMA. When given in high or repeated doses, or under certain conditions, MDMA can produce toxicity in cardiac, liver and kidney tissue, and acute immunological effects in rodents are similar to those reported in humans (see discussion in section on clinical trials.) Most study findings extend or qualify findings from previous studies. However, a study reporting damage to dopamine axons in non-human primates (Ricaurte et al. 2002) after a repeated-dose regimen of MDMA appears to contradict previous findings humans (Reneman et al. 2002; Semple et al. 1999). In contrast, speculations made concerning the application of these findings to human Ecstasy users are not supported by other studies in humans.

Some researchers have used evidence of neurotoxicity found in non-human animal studies as evidence of risk of neurotoxicity in human Ecstasy users, even after the administration of MDMA in research (Gijsman et al. 1999; McCann et al. 2001). The case for extrapolating from studies in rodents and monkeys is largely based on the assumptions of interspecies scaling, a method used for estimating the pharmacological effects of drugs across different species. However, calculating a neurotoxic dose of MDMA using interspecies scaling may be inappropriate. Interspecies scaling models may not be suited for estimating effects of extensively metabolized drugs, and there is some evidence that calculations using less than three different species are not as accurate as those using three or more species (Mahmood and Balian 1996). Like reports published before them, recently published studies continue to use doses of MDMA higher than those used by illicit Ecstasy users or in clinical trials in humans.

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