

DEFINING NEUROTOXICITY: LESSONS
FROM MDMA AND OTHER AMPHETAMINES

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“This is your brain (an egg); this is your brain on drugs (egg in frying pan).” This “fried egg/fried brain” analogy has long been used to depict the adverse consequences of drug use. But just what are the neurotoxic effects of drugs used in a recreational or a therapeutic context? Clearly, in the field of MDMA research, the term “neurotoxicity” has been very broadly applied to describe the effects of the drug in both experimental animals and man. Unfortunately, there has been very little effort to define what is meant by “MDMA neurotoxicity” much less to distinguish MDMA’s “neurotoxic” actions from its potential to cause neuropathological effects, i.e. effects associated with degenerative disorders of the nervous system. In short, everybody talks about drug-induced neurotoxicity but little attempt is made to define it in terms meaningful to the human condition. In this chapter I will briefly address specific aspects of the MDMA neurotoxicity issue by looking at definitions of this term, as well as some different types of effects of MDMA in animals (and humans), and I will talk about functional outcomes. I won’t address dosage regimen, *per se*, but I will touch on some cross-species extrapolation issues.

Brain damage vs. neurotoxicity:

So just what is neurotoxicity in the context of the effects of MDMA use? For example, can this drug damage the brain? For that matter, just what is brain damage and can chemicals/drugs damage the brain? These all are relevant issues with respect to

understanding potential risks associated with the use of MDMA or any other agent that affects the nervous system.

When one speaks of brain damage this is usually equated with neuropathology. Thus, traumatic injury or neurological diseases such as Alzheimer's, Parkinson's, Huntington's or multiple sclerosis all have distinct neuropathological underpinnings defined by changes in brain cells viewed under a microscope (Adams and Duchen, 1992; Olanow and Lieberman, 1992). These neuroanatomical abnormalities serve as the structural (brain cell) basis for the functional deficits associated with a given condition. No one will argue that the neuropathological effects underlying the devastating symptoms associated with diseases or trauma of the nervous system do NOT constitute brain damage. Likewise, on the surface, it would seem safe to assume that acute or long-term administration of MDMA would not be linked to underlying neuropathology (brain damage) in the absence of some neurological symptoms. Unfortunately, this assumption would be erroneous on at least two counts. First, owing to the functional reserve of the nervous system, damage or even near complete destruction of a given brain area is not necessarily associated with loss of brain function. For example, in victims of Parkinson's disease, loss of upwards of 70% of the target neurons is required before characteristic symptoms emerge (Jellinger, 1987). A second consideration is the fact that damage to the putative target of MDMA, the serotonin-containing neurons, may not be obvious due to our lack of understanding as to the function of this component of the nervous system. Thus, it is possible that repeated administration of MDMA over long periods of time may result in subtle brain pathology (damage) and such damage eventually may eventually be

manifested by subtle changes in mood , appetite and sexual behavior, to name a few effects associated with the serotonergic nervous system (Meltzer, 1990; Curzon, 1990; Gorzalka et al., 1990).

When one encounters the term neurotoxicity, this usually is in reference to the effects of chemical exposures in the environment (Luthman et al., 1995) or workplace (Costa and Manzo, 1998), or with self-administration of drugs of abuse (Erinoff, 1993). The specter of chemical-induced carcinogenesis and birth defects has long dominated public perception of the hazards of exposure to specific chemicals and drugs (the “lumps and stumps” mentality). There is, however, more than ample evidence in the literature for the propensity of all classes of chemicals to damage the developing (O’Callaghan and Miller, 1989) and adult nervous system (O’Callaghan, 1993; O’Callaghan et al., 1995), as well as to cause cancer and birth defects. Viewed in these terms, neurotoxicity is synonymous with chemical-induced brain damage. This does not discount the fact that chemicals and drugs have actions on the nervous system that change its biochemistry, indeed, by design that is how drugs achieve their therapeutic actions. Yes, unwanted short to long-term changes in brain chemistry can be viewed as “neurotoxic” because these changes may represent undesired effects of the compound (ethanol would certainly fit into this definition) (O’Callaghan, et al., 1995). Viewed in these latter terms, however, neurotoxicity cannot be equated with brain damage in the absence of evidence for neuropathology. This is the crux of the arguments surrounding the effects of MDMA (see below). For the purposes of the arguments put forth here, a chemical will be considered to be neurotoxic if it causes brain damage.

Neurotoxic episodes in man have been well documented following the ingestion of tainted food (e.g. domoic acid)(Perl et al., 1990), the application of tainted acne medication (triethyltin)(Kimbrough, 1976) or the exposure to industrial solvents and metals to name a few (e.g. mercury and carbon disulfide)(Costa and Manzo, 1998). All of these incidents were associated with deaths and were linked to the neuropathological effects of the offending agent. It is not a question as to whether chemicals are neurotoxic but rather which chemicals preferentially attack the nervous system and at what exposure levels. Moreover, owing to the extreme cellular complexity of the nervous system, one cannot predict which brain area or cell type will be vulnerable to a given neurotoxic chemical or whether symptoms of exposure will be overt or hidden (see O'Callaghan, et al., 1995). Nor can one assume that the neurotoxic effects of a drug are just dose-related extensions of its pharmacology. For example, therapeutic dosages of a drug known as MK-801, an anti-seizure medication, antagonize the toxic actions of excessive levels of the neurotransmitter glutamate by blocking its receptors throughout the brain (Monaghan et al., 1989). At high dosages, MK-801 has been shown to destroy neurons in a small area of cerebral cortex, a brain region unrelated to the sites of its therapeutic actions (Fix et al., 1994; 1995). By analogy, even though the psychostimulant actions of MDMA may be mediated through the serotonergic nervous system, there is no reason, a priori, to assume that serotonergic neurons would be affected at doses that might be toxic to other brain systems (or other organs). All of this may seem confusing but the facts of the matter are dictated by our neurobiological make-up which, in turn, predicts the following:

- 1) chemicals/drugs can damage the brain; 2) areas of the brain that mediate the desired

pharmacological effects may not be the areas vulnerable to toxicity and 3) subtle brain damage can occur in experimental animals and in man in the absence of overt symptoms.

How do you detect subtle damage of the brain?

If one accepts the notion that chemicals can damage the brain and that damage constitutes neurotoxicity, then all that is needed to assess neurotoxicity are the appropriate techniques. As with the evaluation of traumatized or diseased brains, neuroanatomical methods have remained the dominant means for detection and characterization of neurotoxicity (see O'Callaghan et al., 1995). Where cells are killed outright by the offending agent, it is possible to visualize the damaged areas with tissue stains that have been in common usage for more than a century. Of course, under these circumstances, the functional deficits associated with the loss of cells already may have provided the clues to point to a neurotoxic exposure. This situation is unlikely to describe the real-world situation. Here, the greatest concern is directed toward detecting and preventing the cumulative damage that occurs following protracted exposures to chemicals or drugs whose damage is not detected using traditional neuroanatomical stains. Examples would be drugs or chemicals that kill only a few cells, where the surviving cells would be in far greater numbers than those that were destroyed (i.e. like looking for the "needle in the haystack"). Perhaps an even more likely situation would be chemical destruction of parts of neurons with sparing of the nerve cell itself. This is the case put forth for MDMA neurotoxicity (see below). Under both of these scenarios, the selective and discrete nature of neurotoxic effects dictates the need for special

techniques/indicators to identify cells damaged (but not necessarily killed) by a given neurotoxic agent (O'Callaghan and Jensen, 1992). This is not an easy task because, as mentioned above, while the targets of neurotoxic insults may be limited to a very small area of the nervous system, any area of the nervous system may be affected. For the small drop of damaged brain to be detected within the sea of unaffected tissue, requires an indicator of neurotoxicity that possess several features, as follows: 1) it must reveal diverse types of injuries to any area of the nervous system, 2) it must be sensitive to low levels of damage, 3) it must be specific to the damage (neurotoxic) condition so that therapeutic effects of drugs are not scored positive. Succinctly stated, the ideal neurotoxicity endpoint would be an indicator of damage at any level anywhere in the nervous system that would not pick up therapeutic actions of drugs (O'Callaghan et al., 1995).

The propensity of the damaged brain to cause enlargement of a specific cell type known as the astrocyte and for damaged neurons to become impregnated with silver (argyrophilia) are two of only a handful of generic indicators of brain damage, regardless of the causative agent (O'Callaghan, 1993; O'Callaghan and Jensen, 1992). Astrocyte enlargement, known as astrogliosis, refers to the reaction of this brain cell type to all types of brain injury (O'Callaghan, 1993). The hallmark of this response is the accumulation of a protein within astrocytes known as glial fibrillary acidic protein (GFAP). Increases in GFAP, therefore, serve as an indicator of astrogliosis and, by extension, of neurotoxicity. Increased GFAP expression can be examined in slides of brain tissue with antibodies that recognize this protein. Alternatively GFAP levels in

samples of brain tissue can be measured by sensitive immunoassays. Elevations in GFAP are already widely accepted as indicators of brain damage associated with neurological diseases such as Alzheimer's and multiple sclerosis. More recently, enhanced expression of GFAP has been validated as an indicator of neurotoxicity by using a wide variety of prototype chemical neurotoxicants (O'Callaghan, 1993; O'Callaghan, et al., 1995). These include agents that damage many regions of the brain and many different cell types within a brain region, as would be expected to occur under "real-world" conditions. Moreover, increases in GFAP reveal subtle damage to neurons, such as loss of nerve endings, under conditions where traditional neuropathological stains fail to reveal the damage. Importantly, GFAP levels do not change with pharmacological agents administered at therapeutic dosages. Thus, GFAP assessments fulfill the desired requirements for an indicator of neurotoxicity.

As with the increases in GFAP associated with chemical-induced neurotoxicity, staining of brain cells with special silver degeneration stains can be used to show regions and cell types damaged by neurotoxic exposures (O'Callaghan and Jensen, 1992). Silver stains are not as extensively validated as GFAP as indicators of neurotoxicity. Where silver stains have been used, however, they show at least equal sensitivity to GFAP, they reveal sites of damage in the absence of overt cytopathology as assessed by traditional neuroanatomical methods and drug effects do not screen positive. When analysis of GFAP is coupled with mapping of argyrophila using silver stains, there is a remarkable correspondence between the regional and cellular patterns of neurotoxicity revealed by the two techniques. Thus, it is likely that the two approaches to neurotoxicity assessment

represent specific and sensitive methods for the assessment of all types of neurotoxic exposures.

What is serotonin neurotoxicity?

In light of the points made above, it would seem sensible to apply GFAP assays and silver degeneration stains to determine whether MDMA is neurotoxic. This has been done in a number of laboratories and I will elaborate on some of the findings below. First, however, it is useful to review the context within which MDMA is considered to be neurotoxic. In almost all studies using experimental animals and humans, MDMA is described as a serotonin neurotoxin (see McKenna and Peroutka, 1990; Steele et al., 1994; Green et al., 1995). What does this mean? Serotonin neurotoxicity implies that MDMA damages the serotonergic nervous system. Because MDMA was known to release serotonin from the nerve endings of serotonin containing neurons in experimental animals, these serotonergic neurons were viewed as the presumed targets of any neurotoxic effects of the drug. Indeed, subsequent measurements of serotonin levels after administration of high dosages of MDMA to rats showed weeks-long decreases in this neurotransmitter. Further, measurements of the enzyme that catalyzes the synthesis of serotonin (tryptophan hydroxylase) and of the protein that transports the released serotonin back into the nerve endings (serotonin transporter) also showed reductions as a result of high doses of MDMA. Because these three constituents of serotonin nerve endings all were reduced for long periods of time (weeks to even months) as a result of

large doses of MDMA, these changes were viewed as evidence of serotonin neurotoxicity, i.e. MDMA-induced brain damage (see reviews by, McKenna and Peroutka, 1990; Steele et al., 1994; Green et al., 1995; Sprague, et al., 1998). There is little argument that the protracted decreases in the constituents of serotonergic neurons resulting from the acute or chronic administration of MDMA are not drug-like (subjective) actions of the compound that serve as the basis for its self-administration. Moreover, the persistent nature of the decreases in these serotonergic endpoints could be considered manifestations of toxicity, at least at a metabolic level within serotonergic neurons. Over the past decade, however, there has been broad recognition of the malleability of the adult nervous system. This "plasticity" certainly extends to the serotonergic nervous system and to its components affected by MDMA. For example, it is now known that treatment with antidepressants such as paroxetine or fluoxetine can decrease the serotonin transporter (Piñeyro et al., 1994) as can a condition that does not even involve exposure to a drug: a food restriction diet (Huether et al., 1997). Pharmacotherapy with antidepressants such as fluoxetine (Prozac®) or dieting is not conditions one often associates with neurotoxicity. Because MDMA and Prozac share the propensity to decrease the serotonin transporter suggests that MDMA can be viewed as much as an antidepressant agent as a "serotonin neurotoxin." Taken together, these observations indicate that changes (even long-term changes) in markers of serotonergic neurons are likely a reflection of neuronal plasticity, i.e. adaptive changes that occur in response to drug therapy in an otherwise intact neuron. Thus, alterations in parameters associated with the functioning of serotonin neurons can not be taken as evidence of neurotoxicity, in the absence of evidence for serotonin neuron pathology. Not only are such "markers"

of serotonergic neurons not useful as stand-alone measures of neurotoxicity, it also is quite likely that current pharmacotherapies may induce changes in these markers and that such changes would be the expected effects of the long-term therapeutic actions of these drugs.

Application of silver stains and GFAP analysis for the assessment of MDMA-induced neurotoxicity

As noted above, one way to resolve the controversy as to whether MDMA is neurotoxic to serotonin neurons would be to use sensitive and selective indicators of neurotoxicity such as silver stains and GFAP analysis (O'Callaghan and Jensen, 1992). When a dose of MDMA (20 mg/kg) that caused 50% decreases in brain serotonin was administered to the rat, it failed to increase GFAP or result in silver staining at any time point after dosing (O'Callaghan and Miller, 1993). Daily dosages of up to 30 mg/kg for a week also did not increase GFAP (O'Callaghan and Miller, 1993). Only when given at fairly enormous dosages to the rat (4 x 50 mg/kg over 24 hours) was evidence of damage obtained (O'Callaghan and Miller, 1993). Even under these circumstances, however, increases in GFAP and silver staining were observed in the cortex but the damaged areas were not those associated with serotonin neurons (O'Callaghan and Miller, 1993; Jensen et al., 1993). These findings indicated that only massive doses of MDMA can cause damage to the brain of the rat and that the damage that occurs is not related to the serotonin nervous system. The implication of these findings is two-fold: 1) changes in markers of serotonin neurons can occur independent of damage to these neurons and 2) large doses of MDMA are required to damage the nervous system of the rat, approximately 100 times the human dosage taken in a recreational context.

One easy explanation for the failure to see damage to serotonin neurons after MDMA, as assessed by assaying GFAP or using silver stains, was that the techniques were not sensitive enough. To address this issue, the known serotonin neurotoxin, 5,7-dihydroxytryptamine, was administered to the rat at a dosage that produced decreases of serotonin equivalent to those seen with MDMA. This resulted in large increases in GFAP (40-100%) in the areas of the brain where serotonin was decreased and these effects were accompanied by silver staining (O'Callaghan and Miller, 1993). These findings indicate the sensitivity of GFAP and silver staining as indices of chemical-induced damage to serotonin neurons. Thus, hallmarks of brain damage occur after damage to serotonin neurons but are absent following administration of very high dosages of MDMA.

Lessons from other compounds and from man.

Lessons learned using experimental animals don't always apply to man, therefore, the absence of evidence (negative data) cited above is not evidence for absence of neurotoxic effects of MDMA in man. In no small measure this often is why sub-human primates are used in an attempt to model more closely the effects presumed to occur in humans.

Unfortunately, different species of sub-human primates also provide different responses to drugs, including MDMA (compare Ricaurte et al., 1988 with Insel et al., 1989). There are, however, lessons that can be learned from human exposures to other compounds that can be applied to MDMA. These compounds are 1-methyl-4-phenyl-1,2,3,6-

tetrahydropyridine (MPTP), methamphetamine and dexfenfluramine. In the early 1980's a group of individuals self-administered what they assumed was an analogue of meperidine (a synthetic narcotic). The compound administered turned out to be MPTP, an unintended contaminant that had devastating consequences: many of the individuals exposed to MPTP developed symptoms of Parkinson's disease (see review by Sayre, 1989). Subsequent research clearly demonstrated that MPTP damaged dopamine-containing neurons, the same ones affected in Parkinson's disease. The MPTP episode raised the specter that similar damage would occur in another cohort of humans, methamphetamine users, because methamphetamine acts on the same dopamine neurons damaged by MPTP and Parkinson's disease. Given the widespread usage of methamphetamine in the 1960's and 1970's, the potential for this drug to have damaged dopamine neurons should now be manifested with age, as is the case for Parkinson's disease (Langston et al., 1992). Because the prevalence of Parkinson's disease has not increased over the intervening decades suggests that methamphetamine is unlikely to have had a neurotoxic effect on human dopamine neurons. A recent study of humans exposed to methamphetamine (Wilson et al., 1996) bears more directly on this issue and provides data more relevant to human MDMA users. This study involved post-mortem examination of brains from verified methamphetamine users. Marked decreases in markers of dopamine neurons were found in these brains including, dopamine, the enzyme that catalyzes its formation (tyrosine hydroxylase) and the dopamine transporter. If the decreases in these markers were a reflection of damage to dopamine neurons, then this would have been manifested as symptoms of Parkinson's disease prior to death. None of these individuals had such symptoms. Thus, as was the case for markers of

serotonin neurons in rats (and, recently, humans) exposed to MDMA (McCann et al., 1998), the data for dopamine markers in human methamphetamine users was indicative of an adaptive change in response to the drug rather than a neurotoxic action on the neuron. The anorectic agent, dexfenfluramine, is the final example of a human exposure relevant to MDMA. Although this compound recently was taken off the market due to reports of abnormal heart function, it was also the subject of a controversy involving neurotoxicity (e.g. see McCann, et al., 1997). This stems from the fact that in rats, MDMA and dexfenfluramine have nearly identical actions on serotonin neurons (e.g. see Zaczek et al., 1990). As a consequence, dexfenfluramine, like MDMA, became a suspect "serotonin neurotoxin (e.g. see McCann et al., 1997)." Unlike MDMA, dexfenfluramine or its racemate, fenfluramine, have been taken by millions of patients worldwide for 20 years. Extensive post-marketing patient surveillance has yet to reveal any effects of dexfenfluramine that can be linked to adverse effects on the nervous system (e.g. see Guy-Grand et al., 1989). The above data for human exposures to methamphetamine and fenfluramine are consistent with results of research on these agents using experimental animals. They indicate that these drugs have the potential to alter biochemical markers of dopamine and serotonin neurons without causing neurotoxicity. Given the similarities between the effects of these compounds and MDMA, it is likely that we can infer that MDMA shares similar actions in man.

Despite nearly two decades of research on the neurotoxic properties of substituted amphetamines, including MDMA, no definitive data have been obtained to indicate that these compounds are in fact neurotoxic to man. This interpretation of existing data does

not constitute an endorsement of the recreational or therapeutic use of these compounds. Rather, it is a call for continued research on the adaptive responses that these compounds engender in their target neurons. If we are to understand the potential for these drugs to cause neurotoxicity, then we must understand the significance of their long-term effects in relation to their potential to damage the nervous system.

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