MDMA in somewhat high doses has been shown to be a neurotoxin in experimental animals. The applicability of these results to human use, however, still remains unclear. The data from animal studies does not provide conclusive evidence either way, although it is sufficient to raise legitimate concerns. The human studies, while raising other concerns, appear to not provide much support to the notion that serotonin systems of MDMA users are not structurally intact. Of crucial consideration are the validity of some of the markers used to determine neurotoxicity, and how the results from those tests are interpreted.

neurochemical markers and **mdma** neurotoxicity

LAMONT GRANQUIST

There are several different methods of examining changes in brain state following MDMA. The most direct methods include 1) observing silver-impregnated, degenerating neurons via silver staining techniques 2) observing structural abnormalities in serotonin-immunoreactive neurons indicative of grossly deformed and degenerating neurons 3) observing elevations in GFAP (glial fibrillary acidic protein) which are presumed to occur as a reaction to neural damage. Needless to say, these methods are invasive, but they provide the best evidence.

There are several different methods of examining changes in brain state following MDMA.

Rather more indirect methods assay the levels of "neurochemical markers." These methods include assaying levels of central 5-HT (serotonin) and central 5-HIAA (5-hydroxyindoleacetic acid), and measuring [^{3}H]paroxetine binding — thought to be a marker of 5-HT nerve terminal integrity. Again, these methods are invasive. The most indirect route is via assaying changes in the level of 5-HIAA in the CSF (cerebrospinal fluid). Levels of CSF 5-HIAA have been found to correlate well to levels of central 5-HT and 5-HIAA[1], and it is on this basis that CSF 5-HIAA has been used as an indicator of 5-HT structural integrity in humans. Unlike the direct methods, it is not at all certain that these methods produce good evidence of structural injury to the 5-HT system, and it is this point which will be investigated further.

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Dosage Regimens

The route and frequency of dosage is also of crucial consideration. Many animal models use a "subchronic" regimen of MDMA, where a particular dose (usually 5 - 20 mg/kg) is given twice a day for four days (8 doses given approximately every 12 hours). MDMA has been shown to have increasing toxicity on repeated exposure, therefore these regimens probably do not entirely accurately model most human use. Humans also tend to take MDMA orally (p.o.), while animals are generally administered MDMA subcutaneously (s.c.) or intramuscularly (i.m.). It has been shown in primates that s.c. administration tends to increase toxicity 2-3 times over p.o.[2] MDMA is also administered in humans at levels of 1.7-2.7mg/kg while animal experiments generally use 5mg/kg of MDMA or higher.

Non-Human Primate Data

In general extrapolating from animal studies to humans is difficult. One study has found that 2.5mg/kg of MDMA given p.o. once every two weeks for a total of eight doses over 4 months did not produce any neurotoxic response in 5-HT and 5-HIAA assays of eight

brain regions.[3] If human and animal doses were identically equivalent this would seem to provide a "no effect" level for any possible MDMA neurotoxicity which was close to human levels. Extrapolating from animal studies directly, however, is a difficult task at best. First, it isn't known exactly how much the toxicity is increased from the usual subchronic dosing route. It has also, however, been pointed out that non-human primates are more sensitive to MDMA than non-primates[4], and therefore humans may be even more sensitive. Also, based on the increased sensitivity of humans vs. non-human primates to neurotoxins like MPTP[5,6], the "safety margin" between doses used in the laboratory animals and doses used in humans might be closed considerably. In general, the extrapolations from animals will be used in this paper to simply show that there is room for doubt. The firmer rationale for doubt will come from the human studies.

Several studies have examined p.o. administration of MDMA.[1,7,8] Only one published study so far has examined the effects of a single oral dose of MDMA of 5mg/kg in nonhuman primates (thereby roughly approximating cautious human use).[1] That study only assayed levels of central 5-HT and found that they were decreased to 83% of controls in the hypothalamus and 79% of controls in the thalamus with levels in the frontal cortex, hippocampus, putamen and caudate remaining unchanged. There is no published report of [^{3}H]paroxetine binding assays, or any other assays done using the p.o. route in nonhuman primates.

The remaining studies of MDMA in the primate have relied on subchronic i.m. or s.c. administration. Three studies are particularly notable. Two of these used subchronic 5mg/kg s.c. MDMA in non-human primates and found fairly good direct evidence of neurotoxicity using immunohistochemical techniques.[9,10] This strongly suggests that there is a level beyond which MDMA is neurotoxic. It should, however, be emphasized that these studies used the s.c. and not p.o. route, and followed a subchronic regimen. Cumulatively, the monkeys were dosed with an amount 32-72 times the human dose. Adding an arbitrary factor of 5 to account for increased sensitivity and other extrapolation difficulties brings this range down to 6-14 doses. At this level, it would be of concern perhaps in the same way that the neurotoxicity of alcohol is of concern mostly to those who use to excess. Using an arbitrary factor of 10 would reduce it to 3-7, which begins to be more of a concern for most users. The risk does appear to be contingent on how one massages the data, which leaves room for doubt (although raising valid concerns).

Another study of MDMA used subchronic 2.5mg/kg i.m. MDMA in non-human primates.[11] This study is notable since it produced decreases of approximately 50-70% of control levels of central 5-HT and 5-HIAA, while [^{3}H]paroxetine binding was not changed, and in some areas showed statistically insignificant increases. The lack of reduction in [^{3}H]paroxetine binding strongly suggests that 5-HT presynaptic terminals were intact in these animals, although 5-HT and 5-HIAA were dramatically reduced. This study would seem to indicate a lower boundary on MDMA neurotoxicity using doses which were 2-6 times as large as a single human dose, with a cumulative dose of 16-54 human doses. At a higher subchronic dose of 10mg/kg i.m., it was found that 5-HT and 5-HIAA levels did not return to control. This is not surprising, given the finding that 5 mg/kg s.c. levels almost certainly damaged neurons. Recovery of 5-HT and 5-HIAA was not investigated at the lower level of 2.5mg/kg.

Human Data

It must be concluded from this study that levels of central 5-HT and 5-HIAA (and therefore levels of 5-HIAA in CSF) are not useful markers for the structural integrity of serotonin neurons. Without [^{3}H]paroxetine binding data on the study examining single oral doses of MDMA in nonhuman primates, the mild decreases in 5-HT and 5-HIAA tend to suggest, by comparison with the above study, that those animals had structually intact serotonin neurons. Similarly, the results of the recent studies in humans showing decreases to approximately 55-80% of controls (20-45% reductions) in CSF 5-HIAA do not convey any useful information about structural integrity of the 5-HT neurons of those subjects.

Furthermore, the utility of [^{3}H]paroxetine binding as a measurement of neurotoxicity itself can be called into question. It has been observed that subchronic administration of antidepressants reduces binding to the serotonin transporter[12,13], and reduces expression of 5-HT transporter mRNA.[13,14] Based on the similarities between MDMA and SSRIs, it would be reasonable to assume that MDMA might cause the same alterations. Since

extrapolating from animal studies to humans is difficult

it is not believed that antidepressants are serotonergic neurotoxins, the decreases in [^{3}H]paroxetine binding of 80-90% of controls observed after antidepressant treatment should be considered to be indicative of neurocompensatory or neuroregulatory changes and not neurotoxicity. It would appear that reductions of greater than 80-90% of control levels of [^{3}H]paroxetine binding would be necessary to positively conclude that serotonin neurons have been damaged.

Given that the animal studies are inconclusive, the human studies of MDMA users should be considered. As previously noted, the lower CSF 5-HIAA levels do not appear to be useful proof of neurotoxicity, and the other results from the human studies should be considered. First, it should probably be noted that there are some methodological concerns in the *Neuropsychopharmacology* study of human users.[15] One of those is that it is difficult to establish that psychological and biological factors do not predispose one to using MDMA (mixing up correlation and causality). Another is that the MDMA group was substantially more experienced with non-MDMA amphetamines than controls (43% vs. 14%). It is therefore not totally clear that the controls were appropriate without more information. Given those problems, however, the results that MDMA users had decreased impulsivity and hostility, as well as increased harm avoidance and constraint, would tend to be the opposite of what would be expected from damage to the 5-HT system.

The admissible human data shows no evidence of damage in psychological and physiological tests.

Sleep Data

The other published human study examined the sleep EEGs of human users[16] because of the function of serotonin in sleep. MDMA was found to not cause gross abnormalities in the quality of sleep in human users — suggesting that the systems responsible for sleep were intact. MDMA also did not change REM (rapid eye movement) or stage 3 and 4 SWS (slow-wave sleep) periods. As mentioned in the *Sleep* article, this is not what would be expected from experience with chemical or anatomical lesioning of the serotonergic systems in animals. Also, the fact that MDMA does not reduce REM and SWS, while reducing the lighter stage 2 sleep, may indicate that MDMA users experience better quality sleep. REM and SWS are considered important states in sleep (being linked to memory and psychiatric health), while stage 1 and stage 2 sleep are not generally regarded as being important.

Therefore, it does not appear that there is evidence to support the thesis that MDMA causes gross irreversible structural damage of the 5-HT system in "ordinary" human use. The animal studies do not show convincing evidence of damage in doses which extrapolate well to human use. The admissible human data shows no evidence of damage in psychological and physiological tests. In fact, the human data are entirely consistent and support the thesis that 5-HT systems in human users are structurally intact.

Neurochemical Effects

There is, however, some cause for concern over neurochemical changes, even if there are no gross structural abnormalities produced by the normal human use of MDMA. The neurochemical changes induced by MDMA would presumably result from a decrease in TPH (tryptophan hydroxylase) activity[17] occuring in otherwise intact 5-HT neurons. Since there is evidence that 5-HIAA levels are depressed in MDMA users, this should be of concern. One possibility might be that disruptions in 5-HT synthesis might produce psychological side effects ranging from the post-MDMA "burnout," to the psychiatric effects which have been observed in some (presumably ideosyncratically sensitive) MDMA users.[18,19] It also, however, might be possible that reductions in TPH activity in structurally intact 5-HT systems could be psychologically beneficial. The degree to which this effect of MDMA is qualitatively "bad" or "good" needs to be determined. In general, the question must always be raised as to whether the changes induced in the brain by MDMA are "toxic" or "therapeutic."

One important consideration is whether the reduction in TPH activity is preventable and reversible. Permanent changes in brain function have been found at levels of MDMA known to be neurotoxic to monkeys.[20] If TPH levels were found to similarly not recover at lower doses, then an argument could be made that this should be considered "damage." Should studies show that lower levels of MDMA produce permanent decreases in 5-HT and 5-HIAA levels, it is still not entirely clear what to interpret. One possible objection is that the long-term changes might reflect an MDMA-precipitated adaptive alteration in the brains of these animals to their environment. The extent to which the changes are permanent, irregardless of external variables, would have to be examined. Similarly, the possibility that the environment affects re-innervation of destroyed

axons at neurotoxic doses should be examined.

Effects of Prozac

Fluoxetine (Prozac) and other SSRIs have been shown to prevent the reduction in TPH activity caused by MDMA.[21] Should the reductions in TPH activity caused by MDMA become of concern, there may be a role for SSRIs in preventing or reversing these effects. It has been reported that fluoxetine administered concurrently with MDMA may prevent the "burnout" in human users, without dimin-ishing the entactogenic effects of MDMA.[22] Fluoxetine may also be useful in treating those users who experience adverse psychiatric side effects — perhaps making up for an inability on the part of the user's neurochemistry to handle temporary alterations in 5-HT function. It should be carefully pointed out that prevention of MDMA "burnout" by fluoxetine and the MDMA "burnout" phenomenon itself are not good indications of structural changes in nerve cells.

Summary

In summary, evidence does not suggest that neurochemical markers are good indicators of neurotoxicity. 5-HT and 5-HIAA appear not to be useful, and [^{3}H]paroxetine binding appears problematic, as indicators of 5-HT system integrity. In light of these considerations, MDMA has only been shown to be neurotoxic at somewhat high levels in experimental animals, and the evidence in humans suggests a lack of neurotoxicity. There do appear to be at least some temporary changes in brain function caused by MDMA, but the exact nature of the changes remains to be determined, and the possible role of SSRIs in preventing or reversing the changes merits further examination. The possibility that the changes are permanent in humans needs to be addressed. To what extent the neurochemical changes induced by MDMA are responsible for therapeutic or pathological changes in psychology also needs to be determined. The frequency and severity of post-MDMA psychiatric effects (including "burnout") should also be assessed. And in light of the somewhat narrow safety margin, the effects of MDMA in higher doses needs to be investigated to the extent possible. Hopefully, the clinical studies using MDMA in human volunteers will help to answer some of these questions.•

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Hopefully, the clinical studies using MDMA in human volunteers will help to answer some of these questions.