Mapping Toxicant-Induced Nervous System Damage With a Cupric Silver Stain: A Quantitative Analysis of Neural Degeneration Induced by 3,4-Methylenedioxymethamphetamine

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INTRODUCTION

Evidence continues to accumulate implicating toxic substances in the etiology of a variety of neurological diseases. Consequently, the process of identifying, understanding, and regulating neurotoxic substances remains a pressing challenge. This challenge is complex because toxicants can injure the nervous system in a variety of ways. In addition, knowledge of the structure and function of the nervous system is not sufficient to entrust a single endpoint with predicting the neurotoxic potential of a substance. The purpose of structural assessments is to provide a convincing picture of the location and extent of damage to the nervous system. Silver stains that selectively reveal neural degeneration hold particular promise in this regard. Evidence that such histological procedures can selectively stain degenerating neurons has emerged from more than 25 years of anatomical tract tracing experimentation (Beltramino et al., this volume). In this chapter, the authors describe preliminary results using a recently developed cupric silver stain (de Olmos et al., in press) to delineate areas of the brain damaged by administration of 3,4-methylenedioxymethamphetamine (MDMA).

MDMA causes dramatic and long-lasting neurochemical alterations in the brain (Fuller 1989; Commins et al. 1987*a*; McKenna and Peroutka 1990; Schmidt 1987; Ricaurte et al. 1988; Scallet et al. 1988; Bittner et al. 1981; Schmidt and Taylor 1987; Stone et al. 1987; Davis et al. 1987; Schmidt et al. 1986). The extent to which MDMA-induced neurochemical alterations reflect neural degeneration remains controversial. To address this issue, the authors have mapped regions of the brain stained with the cupric silver method following administration of MDMA. Although a better understanding of the chemical

basis of the stain's selectivity for degenerating neurons is needed before the observed staining can be uniquely associated with particular degenerative events, the present results demonstrate that the cupric silver stain is a useful tool in determining the location and extent of structural alterations resulting from exposure to a toxicant.

ANALYSIS

MDMA (0, 25, 50, 150 mg/kg as salt) was administered to Long-Evans rats four times, with a 12-hour interdosing interval. There were four animals per dose group. The animals were housed individually in wire-bottom hanging cages (to prevent hyperthermia-induced mortality) and allowed food and water ad libitum. At various times after the last dose, animals were anesthetized with pentobarbital, perfused intracardially with saline followed by 4 percent paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4). After perfusion, the brains were removed and a 4-mm sagittal block of the cerebrum was frozen and sectioned at 40 microns. All sections were collected and allocated to one of six sets. Sets of sections were stained with either cresyl violet or the cupric silver stain. Regions of silver staining unique to the treated animals were traced from four homologous sections from a 0.72 mm sample of the sagittal block and reconstructed using a computerized serial section reconstruction system. A survival time of 48 hours was employed for the dose-response analysis. For the time-course analysis, MDMA (100 mg/kg) was administered four times, with a 12-hour interdosing interval, except for animals in the group labeled "2x," in which MDMA (100 mg/kg) was administered only twice. Four animals were sacrificed at each survival time (18 hours, 48 hours, 60 hours, 7 days, and 14 days). An additional four animals were treated with fluoxetine (5 mg/kg) or MK-801 (1 mg/kg) 30 minutes prior to each of four doses of MDMA (100 mg/kg). Control groups included either fluoxetine or MK-801 or MDMA alone. Neurochemical evaluations of animals from corresponding cohorts are reported by O'Callaghan and Miller (this volume).

RESULTS

Evidence of cupric silver staining could be observed in several regions in control animals. Consistent and intense staining was apparent in the olfactory bulb. Diffuse and very light terminal-like staining was occasionally observed in the hippocampus. Scattered stained neurons occasionally could be seen in the forebrain. With the exception of the olfactory bulb and hippocampus, staining in control animals was sufficiently minimal to allow for a systematic comparison of different brain regions following administration of MDMA.

In animals treated with MDMA and sacrificed 48 hours later, the intensity of staining in the neocortex is dose related (figures 1 and 2). The staining is most prominent in the frontoparietal region of the neocortex. The majority of stained elements within the neocortex are axons and terminals. At lower doses (4x25 to

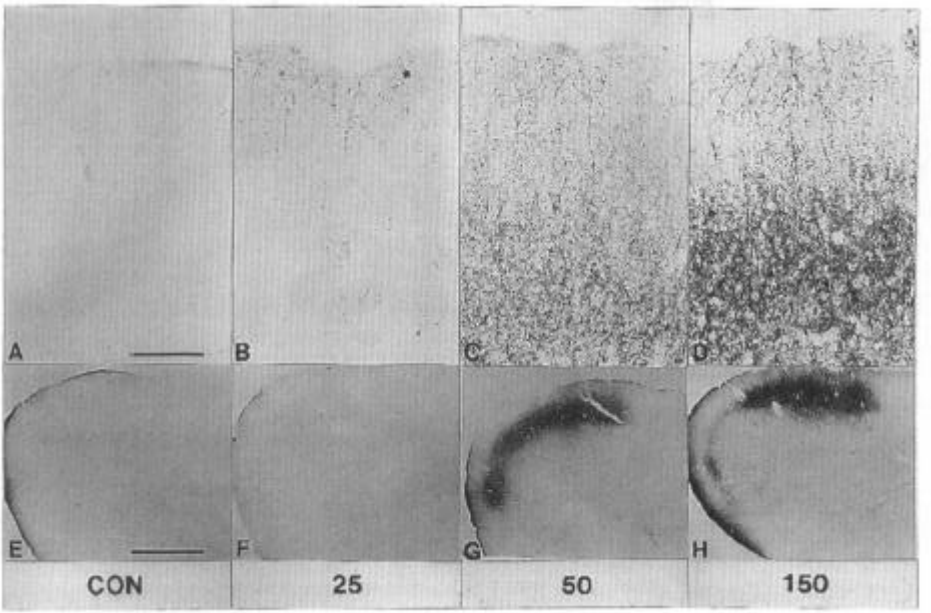

FIGURE 1. *Silver staining in sagittal sections of frontoparietal cortex in animals sacrificed 48 hours following various doses of MDMA. High magnification of upper neocorticel layers. Panels A through D: scale bar=100 mm. Lower magnification of frontoparietal cortex. Panels E through H: scale bar=1 mm. Control (panels A and E). four doses of 25 mg/kg given 12 hours apart over 2 days (panels B and F).*

4x50 mg/kg), staining is primarily in the upper layers, whereas at higher doses (4x75 to 4x150 mg/kg), it reaches deeper layers. Perikaryal staining is more pronounced at the higher doses.

Silver staining is not confined to the frontoparietal neocortex. In some cases, pronounced staining occurs in other brain regions, including the striatum and thalamus (figures 3 and 4). Such staining does not occur in all animals in any one dose group. For example, one animal receiving 4x75 mg/kg MDMA exhibited extensive staining within the striatum and thalamus (figure 3, panels A and D, and figure 4), whereas another animal receiving the same dose and exhibiting the same degree of damage within the neocortex did not exhibit staining in the striatum (figure 3, panels C and D). The predominant type of staining differed in the various brain regions. Although terminal, axonal, and perikaryal staining all were present in the neocortex, striatal staining appeared to be almost exclusively of terminals, and the thalamus exhibited staining of both axons and terminals.

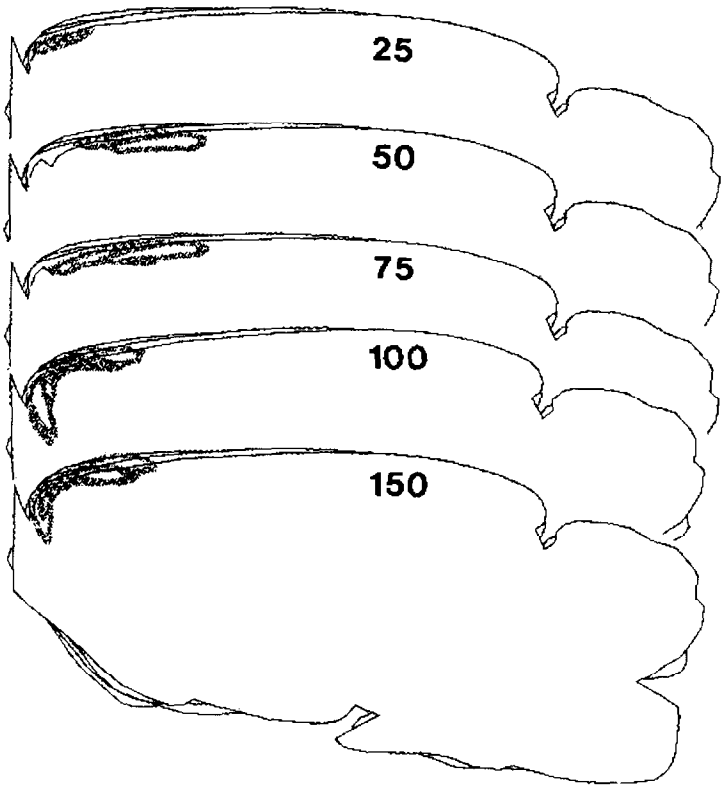

FIGURE 2. *Volumetric reconstructions of regions of intense silver staining (shaded areas) in the frontoparietal neocortex from four Sagittal sections after various doses in mg/kg of* MDMA.

Silver staining was apparent after the lowest dose (4x25 mg/kg), and the volume of staining in the neocortex and striatum was dose related (figure 5). The volume of degeneration was evaluated at various times after repeated doses of 100 mg/kg (figure 6). Substantial staining was evident as early as 18 hours after only two doses of 100 mg/kg. The extent of staining was only slightly smaller than that observed 16 hours after four doses of 100 mg/kg. Staining was maximal at 18 hours and declined thereafter but was still detectable at 14 days.

The effects of fluoxetine (5 mg/kg) and MK-801 (1 mg/kg) on MDMA-induced (4x100 mg/kg) silver staining in the frontoparietal cortex also were examined (figure 7). Neither fluoxetine nor MK-801 induced intense silver staining when administered alone Fluoxetine reduced by approximately half the volume of tissue stained and dramatically reduced the intensity of staining throughout the

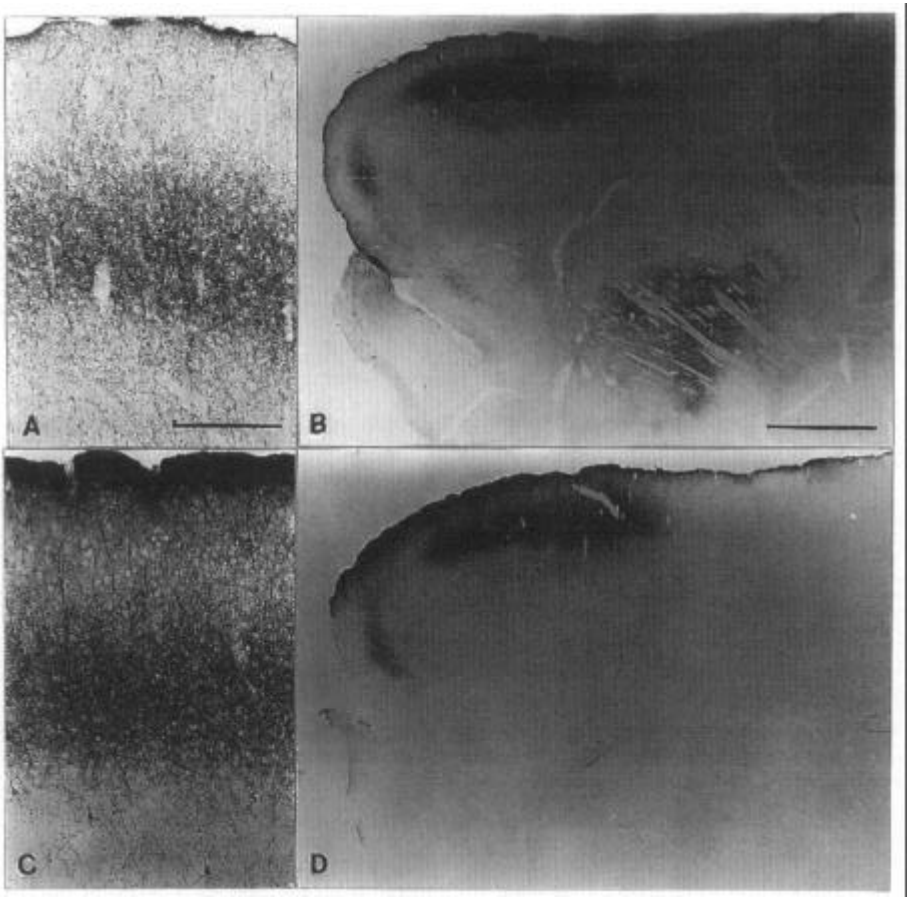

FIGURE 3. *Silver staining in the frontoparietal cortex and striatum 48 hours following the last dose of MDMA (4x75 mg/kg.) Panels A and B: sagittal section from an animal exhibiting damage to striatum. Panels C and D: sagittal section from an animal not exhibiting any silver staining in striatum. Panels A and C: high power of neocortex from sections shown in panels B and D. Note that the extent of silver staining in the neocortex is about the Same in both animals. Thus, it is unlikely that either variation in dosing or technical considerations pertaining to the histological method account for the differences. Scale bar=0.2 mm for panels A and C. Scale bar=1 mm for panels B and D.*

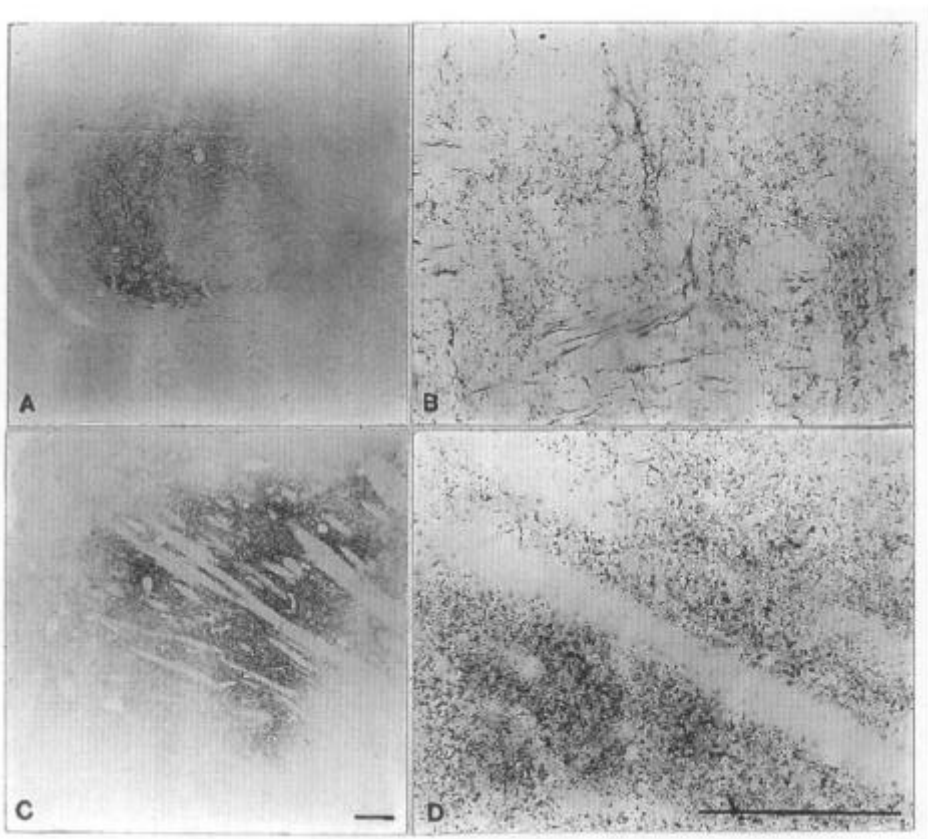

FIGURE 4. *Thalamic and striatal silver staining 48 hours after the fourth dose of MDMA (75 mg/kg). Panels A and B: section through thalamus showing both axonal and terminal degeneration. Panels C and D: section through striatum. From the same animal as in figure 3, panels A and B. Scale bar=0.2 mm for panels A and C. Scale bar=0.1 mm for panels B and D.*

affected regions. The reduction in intensity of staining was not associated with a preferential reduction in the staining of terminals, axons, and perikarya. In contrast to fluoxetine, MK-801 virtually eliminated evidence of MDMA-induced silver staining.

CONSIDERATIONS FOR THE INTERPRETATION OF SILVER STAINING

The authors' observations confirm a previous report (Commins et al. 1987*a*) that MDMA induces terminal, axonal, and perikaryal staining in the frontoparietal neocortex. We further assessed the volume of this staining within the neocortex

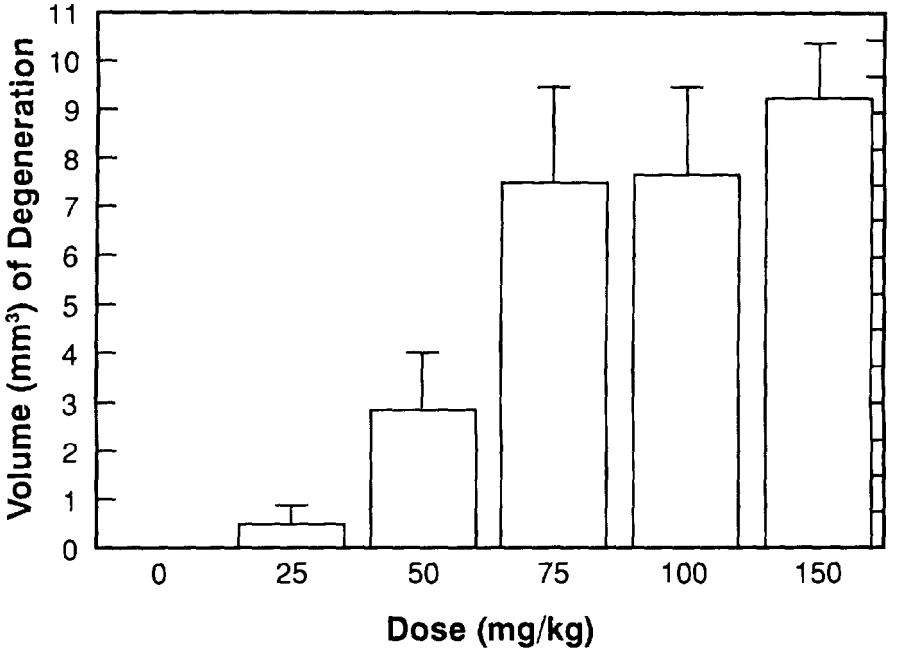

FIGURE 5. *Dose dependence of the volume of MDMA-induced neocortical and striatal silver staining. Each group included four animals. All animals received each dose of MDMA twice a day for 2 days (a total of four times). All animals were sacrificed 48 hours after the last dose. Standard errors indicated by vertical bars.*

and demonstrated its dose dependence. Despite the dramatic nature of this MDMA-induced silver staining, there are several considerations that need to be borne in mind when interpreting the present findings.

First, all doses of MDMA used in the present study produced intense silver staining. We therefore do not know a dose level at which MDMA produces no evidence of silver staining. The time-course data indicate that optimal staining occurs less than 48 hours following MDMA administration, and significant staining could be induced by only two doses of 100 mg/kg MDMA. Thus, when attempting to determine the highest dose of MDMA that does not induce silver staining, a survival time shorter than 48 hours may be more appropriate. In addition, a single rather than a multiple, dose also may provide a better estimate of the highest dose that does not induce silver staining.

Second, the chemical basis for the stain is not currently known. Consequently, silver staining cannot be specifically associated with particular degenerative

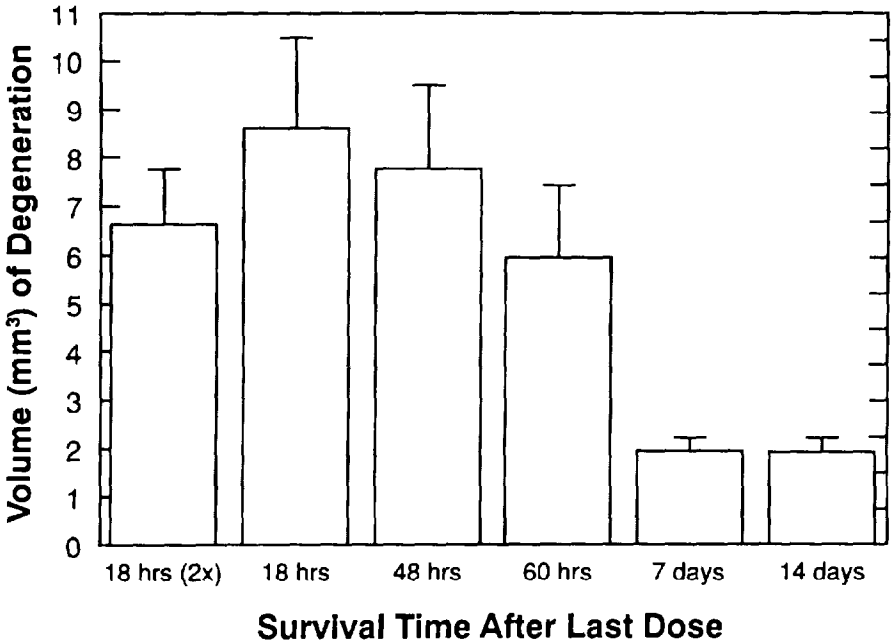

FIGURE 6. *Time course of the changes in the volume of MDMA-induced neocortical and striatal silver staining. All animals recieved 4x 100 mg/kg MDMA and were sacrificed at various times after the last dose except those in the group marked "2x," which received only 2x100 mg/kg MDMA and were sacrificed 18 hours after the second dose. Each group included four animals. Standard errors indicated by vertical bars.*

events. The extent to which the stain may recognize reversible as well as irreversible alterations also is not known. Longitudinal experiments that include estimates of the total number of neurons in specific brain regions will be important in addressing such questions.

Third, we have not measured the intensity of staining; rather, we have relied on estimates of the volume of intense staining. Consequently, there remains a subjective component to the volume determination. However, the authors' impression is that intensity and volume are highly correlated and the subjective component has only a minor, if any, influence on estimates of the volume of degeneration. We are currently developing an automated process for objectively determining the intensity and volume of staining.

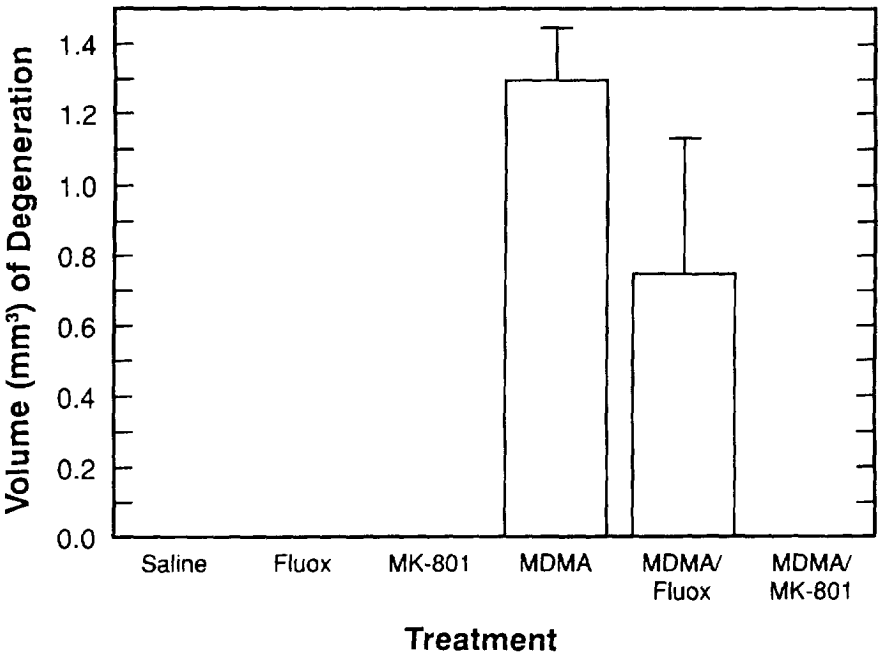

FIGURE 7. *Effect of fluoxetine (fluox) (5 mg/kg) and MK-801 (1 mg/kg) given 30 minutes prior to each of four doses of MDMA (100 mg/kg) on MDMA-induced silver staining. All groups included four animals. Animals received either saline or MDMA and were pretreated with either MK-801 or fluoxetine 30 minutes prior to MDMA. All animals were sacrificed 48 hours after the last dose. Note that fluoxetine pretreatment partially blocked MDMA-induced silver staining; MK-801 eliminated MDMA-induced silver staining. Standard errors indicated by vertical bars.*

Finally, this analysis was not exhaustive because the survey was restricted to selected sections through a segment of the cerebrum in which unambiguous staining was present. More extensive studies addressing the extent and nature of staining in a more precise manner are needed to fully characterize MDMA-induced silver staining.

CONCLUSIONS

Nonetheless, our current results show that maps of silver staining can demonstrate dose-response relationships. These maps of silver staining render a profile of MDMA-induced damage that differs from that engendered by evidence from neurochemical and immunohistochemical studies with respect

to two important features. First, MDMA-induced injury as revealed by silver staining is not restricted to serotonergic neurons. Second, the spatial pattern of injury as revealed by silver staining does not correspond to the spatial pattern of MDMA-induced alteration in serotonergic innervation. These two features of MDMA-induced injury highlight the potential involvement of nonaminergic mechanisms in MDMA neurotoxicity.

MDMA-Induced injury as Revealed by Silver Staining Is Not Restricted to Serotonerglc Neurons

Consistent with a previous report (Commins et al. 1987*a*), we have observed that MDMA induces silver staining of cell bodies, axons, and terminals in the neocortex. Because immunohistochemical studies have not revealed serotonergic perikarya within the neocortex (Lidov et al. 1980; Steinbush 1981) the staining of perikarya constitutes evidence of MDMA-induced injury to nonserotonergic neurons.

Additional evidence that nonserotonergic processes are also injured by MDMA comes from the observation that fluoxetine blocked part, but not all, of the MDMA-induced staining. Fluoxetine pretreatment reduces the volume of intensely stained tissue by approximately half and also reduces the intensity of staining throughout the stained region. Staining of terminals, axons, and cell bodies was still observed after fluoxetine pretreatment. Although we have not established a dose-response relationship for fluoxetine blockage of MDMA-induced silver staining, the dose of fluoxetine used, 5 mg/kg, was sufficient to prevent the serotonin depletion produced by the dose, 100 mg/kg of MDMA (J.P. O'Callaghan, D.B. Miller, and K.F. Jensen, unpublished observations). These observations support the conclusion that a substantial proportion of the degenerating terminals, axons, and cell bodies are nonserotonergic.

The Spatial Pattern of MDMA-Induced Injury as Revealed by Silver Staining Does Not Correspond to the Spatlal Pattern of Alterations In Serotonin Innervation

The regional pattern of reduction in serotonin is consistent with the idea that MDMA's effect on serotonergic neurons is selective for their axons and terminals (Wilson et al. 1989; Mamounas and Molliver 1988; O'Hearn et al. 1988; Ricaurte et al. 1988; Molliver et al. 1990). The MDMA-induced loss of uptake binding sites is largely in agreement with this idea (De Souza et al. 1990; Battaglia et al. 1987, 1991; Battaglia and De Souza 1989; De Souza and Battaglia 1989; lnsel et al. 1989). Molliver and coworkers (Wilson et al. 1989; O'Hearn and Molliver 1984; Mamounas et al. 1991) have proposed that MDMA is even more selective, damaging primarily a subpopulation of serotonergic axons. They report that fine serotonergic axons originating in the dorsal raphe are preferentially vulnerable to MDMA, whereas coarse serotonergic axons from median raphe are resistant to

MDMA. Differences in the pattern of innervation between the fine and coarse fibers are most striking in the olfactory bulb, hippocampus, and entorhinal cortex where immunohistochemical studies have demonstrated a preferential loss of staining of these fine fibers following para-chloroamphetamine (PCA) or methylenedioxyamphetamine (MDA) (Mamounas et al. 1991).

The regional distribution of MDMA-induced silver staining does not correspond to the regional distribution of the vulnerable fine fibers. MDMA-induced silver staining is limited primarily to the frontoparietal cortex. It sometimes involves regions of posterior neocortex and striatum and is rarely observed in thalamus. Because terminal-like staining occurs frequently in the olfactory bulb and sometimes in the hippocampus of control animals, the possibility of MDMA-induced injury in these regions cannot be excluded. However, the alterations in serotonergic innervation revealed by neurochemical and immunohistochemical studies are far more widespread than the restricted damage to the frontoparietal cortex revealed by silver staining.

Within the neocortex, the vulnerable fine fibers can be seen throughout the cortical lamina but are particularly dense in layer IV (Blue et al. 1988; Kosofsky and Molliver 1987). MDMA, MDA, and PCA appear to selectively eliminate the staining of these vulnerable fine fibers (Steinbush 1981; O'Hearn and Molliver 1984; Mamounas et al. 1991). In contrast, the MDMA-induced silver staining is most pronounced in the upper layers, whereas at higher doses it becomes robust in layers III to V. Thus, in contrast to its more restricted regional pattern, silver staining appears to have a more extensive involvement within the frontoparietal cortex than alterations in the immunohistochemical labeling of serotonergic axons. Consistent with these findings, corresponding differences between neurochemical markers of dopamine and silver staining have been described for d-amphetamine (Ryan et al. 1990).

There are several interpretations that could account for the differences in the patterns of alterations revealed by serotonin immunohistochemistry and silver staining. One is that a reduction in the density of immunohistochemically labeled serotonergic processes can in part be accounted for by a loss of transmitter from intact axons, rather than solely by degeneration. A similar argument has been proposed for p-chloro-N-methylamphetamine (Lorez et al. 1976). Another consideration that may account partially for the results obtained with the two methods is that silver staining reveals damage to both serotonergic and nonserotonergic neurons. The contribution of nonserotonergic elements would not be detected by assays selective for assessing serotonin innervation.

There are other interpretations of these differences. One is that fine serotonergic degenerating axons do not take up silver stains. This assertion, although possible, has not been substantiated. However, it does highlight technical limitations of the anatomical methods used to assess MDMA-induced neural

degeneration. There are important limitations to both silver stains and immunohistochemistry. Silver stains do not distinguish between serotonergic and nonserotonergic degenerating axons. Serotonin immunohistochemistry cannot label intact axons that have lost their serotonin as a result of the pharmacological treatments. Thus, neither silver stains nor immunohistochemistry can by themselves discriminate the relative vulnerability of serotonergic and nonserotonergic elements to MDMA. Estimating the relative involvement of different populations of neurons awaits the development of techniques that can assess neural damage comprehensively while simultaneously providing the neurochemical identity of degenerating elements.

Although the precise identity and full complement of neurons vulnerable to MDMA require further characterization, several hypotheses have been put forward to account for the neurotoxicity of MDMA as well as other substituted amphetamines (for reviews, see Fuller 1989; Stone et al. 1991). The majority of these hypotheses are based on the idea that amphetamines are toxic to particular neurochemically defined classes of neurons, possibly through the formation of neurotoxins such as 6-hydroxydopamine and 5,6-dihydroxytryptamine (Commins et al. 1987*b*, 1987*c*; Axt et al. 1990). Our current observation that MK-801, an N-methyl-D-asparate (NMDA) receptor antagonist, blocks MDMA-induced silver staining suggests that there may be several alternate, nonserotonin-specific mechanisms by which substituted amphetamines can cause neural injury. The most obvious possibility is through an excitotoxic mechanism (Sonsalla et al. 1989; Olney et al. 1987). Interestingly, Carlsson has proposed that the neurotoxicity of the amphetamines may result from “uncontrolled positive feedback” from the striatum to the cortex through the thalamus. Such feedback could involve dopamine (cortical projections to the nigra or as presynaptic terminations on dopaminergic nigrostriatal afferents) or serotonin (cortical projections to raphe or as presynaptic terminations on serotonergic afferents to striatum) with the corticofugal projections being glutaminergic. Thus, one possible action of MK-801 could be to restabilize positive feedback, preventing a cascade of overstimulation. This hypothesis is consistent with the observation that MK-801 attenuates amphetamine-induced dopamine release (Weihmuller et al. 1991). It is also consistent with the currently observed incidence of silver staining in cortex, striatum, and thalamus following MDMA administration.

Degeneration blocked by MK-801 could also involve mechanisms other than excitotoxicity. MDMA-induced alteration in thermoregulation may be involved (Miller 1992; Gordon and Rezvani, in press). MK-801 can induce hypothermia that can protect against insults such as hyperthermia (Buchan and Pulsinelli 1990). Interestingly, MDMA-induced hyperthermia is also blocked by 5-HT2 receptor antagonists, and at high doses this effect can be differentiated from long-term reductions in serotonin (Schmidt et al. 1990).

Hyperthermia is not the only peripheral influence that could alter the extent of damage to the brain. Cerebral blood flow is modulated by serotonin (Gilman et al. 1990) and influenced by MK-801 (Roussel et al. 1992). Stress can alter brain tryptophan hydroxylase activity (Singh et al. 1990*a*, 19906; Boadle-Biber et al. 1989) and neocortical dopamine metabolism (Claustre et al. 1986). Because dopamine is involved in the action of substituted amphetamines on serotonin innervation (Stone et al. 1988; Schmidt et al. 1985, 1991), stress may play a role in the observed cortical damage. Indeed, individual variability in stress response (Miller 1992), particularly to multiple toxicant injections, also may play a role in the variability reported for several parameters altered by substituted amphetamines (Seiden 1991; Axt and Molliver 1991; Ryan et al. 1990; O'Dell et al. 1991), including that of silver staining in the striatum observed in the present study.

Finally, it is likely that several of MDMA's actions act in concert to produce silver staining as well as alterations in serotonergic innervation. Hypotheses that focus solely on the unique vulnerability of a particular neurochemically defined class of neurons are not likely to reveal a *more* complete understanding of such a complex phenomenon. Conversely, neural degeneration may not be the only means by which substituted amphetamines produce long-term alterations in neurotransmitter levels in the brain.

In summary, we have made significant steps toward quantifying a historically subjective phenomenon, the staining of degenerating neurons with silver. We have shown how maps of silver staining can be valuable in assessing the dose-dependent nature of toxicant-induced brain damage. With refinement, such maps can help identify components of the brain's complex circuitry that are vulnerable to damage by exposure to toxicants.

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DISCUSSION

Q (Molliver): Are you suggesting that this degeneration after MDMA is due to a neurotoxic effect of the drug?

A: Yes.

Q (Molliver): I feel uncomfortable with that. What is the mortality of your animals, given the huge doses you are giving?

A: We get very little mortality with the MDMA.

Q (Molliver): With dosages that high, you can get hyperthermia, hypertension, ischemia, and breakdown of the blood-brain barrier, in addition to pulmonary hypertension with hypoxia. At doses that high, one wonders whether you are seeing a specific drug effect. It is like the effects of cocaine, d-amphetamine, and methamphetamine, where people found vasculitis and ischemia, etc., with such high doses.

A: Certainly the fact that the doses are so high brings in the possibility about the specificity of the drug, in the sense of how it is being mediated-what kinds of things are kicking in at that kind of dose?

Q (Molliver): That is the dose range that is used to model experimental hypertension.

A: Right. But the time course, I think, what we are seeing is unlikely to be due to those kinds of secondary effects. The fact that we can see it after two doses? So it is within 12 to 16 hours.

Q (Molliver): You can get cerebral infarcts after one dose.

A: Right, but these rats are clearly healthy, by all criteria.

Q (Tilson): Wouldn't you expect the pattern to be more diffuse if that was the case?

A (Molliver): Not necessarily.

Q (Jensen): Aren't things like the hippocampus more susceptible to those kinds of infarcts?

A (Molliver): That's true; the hippocampus is more susceptible to ischemia.

Q (Tilson): How consistent are the changes you see in a population of rats? The slides that you have shown-are those representative of populations of rats?

COMMENT (Landfield): All slides are typical examples. (Laughter)

A: There is a certain degree of variability within a given dose group or in a given survival time. The degree of overlap between different doses is minimal, at least within the ranges that we've used. So that we can distinguish different dose levels, I would say, fairly consistently.

Q (Tilson): I was thinking about a pattern within the dose group, where there is always that area of the cortex, always. . .

A: Yes. Absolutely. In fact, the degree of damage that changes from dose is an increase in size of the area. The area itself never changes.

Q (Molliver): Did you look at the levels of neurotransmitters in the cortex, for example, serotonin?

A: Yes, we did.

COMMENT (Miller): Also, the hyperthermia and heat retention aren't as high when rats are in wire bottom cages,

Q (Molliver): I know, but did you measure temperature?

A (Miller): We have. If they are in plastic cages with wood chip bedding, they go up to 42° core temperature. If they are in wire bottom cages, they usually don't go any higher than 38°.

Q (Molliver): The main effect that I am concerned about is the sympathetic response due to peripheral release of catecholamines. Rats are sensitive to pulmonary hypertension, and the pulmonary vasculature can vasoconstrict.

Q (Jensen): Can they be protected?

A (Molliver): Possibly by an extensive sympathectomy and adrenalectomy. I think that Jim Gibb did that study years ago, and he found that adrenalectomy protected animals against MDA toxicity.

A (Gibb): If you give reserpine beforehand, you don't get the effect because you don't have anything to cause the effect.

COMMENT (Seiden): On the other hand, we've potentiated with reserpine.

COMMENT (Gibb): I am not sure when you gave yours. Did you give yours before? How long before?

COMMENT (Seiden): About 24 hours.

COMMENT (Ricaurte): I think the experiment has been done with AMT [alphamethyl tyrosine], where animals pretreated with AMT and then given a high dose of methamphetamine will not show silver degeneration in the striatum or the somatosensory cortex. In support of your idea, I would just like to reinforce that, because there is no question that the silver methods have a great potential for detecting degeneration. As you have emphasized, a major question is how much of that degeneration is specific in nature so that you could relate it to the catecholamine and monoamine systems and how much is related to nonspecific effects (ischemia, seizures, hyperthermia)'? My suspicion is that some of what has been shown in the cortex is in fact "nonspecific." It may be an interesting phenomenon in and of itself in terms of how it is mediated. But it is not the kind of serotonergic or dopaminergic neurotoxicity that is produced at lower doses. In the approaches that we use, one was with AMT and the other was combining the silver method with the pharmacological approach whereby we would pretreat animals with a dopamine reuptake blocker which we knew protected against the dopamine depletion induced by methamphetamine. We could show that such treatment also prevented the appearance of the silver degeneration in the striatum. My point is that the onus is to really demonstrate the specificity.

A (Jensen): It is true. Every drug does, and the question is what is the specificity and how is it. I should mention that fluoxetine will partially block the degeneration. Significant portions of degeneration are blocked by fluoxetine.

COMMENT (Seiden): I would like to take issue about every drug. With cocaine, in spite of approaching the doses that the animals can barely survive, we don't see neurotoxicity.

COMMENT (Gibb): Lew, that causes hyperthermia, right?

COMMENT (Molliver): It is very transient and is mild.

COMMENT (Gibb): Yes, but those doses that he is talking about cause hyperthermia.

COMMENT (Molliver): I think Karl clearly sees degeneration. I think this is of considerable interest. Perhaps it is even more of interest in terms of the clinical

aspects of drug abuse-induced toxicity seen in the emergency rooms, where the most common situation is vascular-mediated toxicity.

COMMENT (Landfield): As you yourself pointed out, these are new techniques, and you have to determine whether these are temporary pathologies or permanent. I would suggest possibly counting cells in some of these tissues. We all need to begin to quantify just how much degeneration this staining actually reflects.

COMMENT (Jensen): In fact, we are talking about doing an experiment with MPTP [1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine], using standard morphometric methods to count the number of cells in pars compacta and seeing if that doesn't account for a loss in corresponsive cells that we see stained with cupric silver in the early time period. If there were major vascular events at these high doses, I would expect, based on ischemia and stroke models, to see pretty major cerebral gliosis. If you compromise the blood-brain barrier in a fairly major way and let in a lot of lymphokines and astrocyte mitogens, it would leave a fairly broad glial scar; we don't see that, and I will show you that with astrocytes. I think that one other thing that may be of equal consideration is what Dr. Heimer alluded to with some of his examples. When you have this extended damage, you almost have to be concerned about transsynaptic effects as well. The proximate site of the toxicant may be one set of cells, which upon degeneration will result in the damage of other cells. So you may end up with a cascade, particularly with these high doses, that may be dependent upon the presence of compound, but not directly related to the toxicant.

Q (Molliver): Did you see any intercerebral hemorrhages?

A (Jensen): No, we perfuse.

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