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Causes and consequences of the loss of serotonergic presynapses elicited by the consumption of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") and its congeners*

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Summary. The massive and prolonged stimulation of serotonin (5-HT)-release and the increased dopaminergic activity are responsible for the acute psychomimetic and psychostimulatory effects of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") and its congeners. In vulnerable subjects, at high doses or repeated use, and under certain unfavorable conditions (crowding, high ambient temperature), severe, in some cases fatal, averse systemic reactions (hyperthermia, serotonin-syndrome) may occur during the first few hours. Animal experiments revealed the existence of similar differences in vulnerability and similar dose- and context-related influences on a similar sequence of acute responses. The severity of these acute systemic responses is closely related to the severity of the long-term damage to 5-HT axon terminals caused by the administration of substituted amphetamines. Attempts to identify the mechanisms involved in this selective degeneration of 5-HT presynapses brought to light a multitude of different factors and conditions which either attenuate or potentiate the loss of 5-HT terminals caused by MDMA and related amphetamine derivatives. These puzzling observations suggest that the degeneration of 5-HT presynapses represents only the final step in a sequence of events which compromize the ability of 5-HT terminals to maintain their functional and structural integrity. The common feature of all these events is a profound wastage of energy. Substituted amphetamines selectively tax energy metabolism in 5-HT presynapses through their ability to exchange with 5-HT and to dissipate transmembrane ion gradients. The active carrier systems in the vesicular and presynaptic membrane operate at a permanently activated state. The resulting energy deficit can no longer adequately restored by the 5-HT presynapses when their avail-

* This article is dedicated to Prof. Dr. N. Matussek on the occasion of his 75th birthday

ability of substrates for ATP production is additionally reduced by the hyperthermic and other energy consuming reactions which are elicited by the systemic administration of substituted amphetamines. The exhaustion of energy in 5-HT nerve terminals compromizes all energy-requiring endogenous mechanisms involved in the regulation of transmembrane-ion exchange, internal Ca^{++}-homeostasis, prevention of oxidative stress, detoxification, and repair. Above a critical threshold the failure of these self-protective mechanisms will lead to the degeneration of the 5-HT axon terminals.

Based on the role of 5-HT as a global modulatory transmitter-system involved in the stabilization and integration of impulse flow between distributed multifocal neuronal networks, the partial loss of 5-HT presynapses must be expected to impair the ability of these networks to maintain the integrity of signal flow pattern, and increase the likelihood of switching to unstable information processing. Behavioral responding may therefore become more dominated by activities generated in individual networks, and hitherto "buffered" personality traits and predisposition may become manifested as defined psychiatric syndromes in certain predisposed subjects.

Keywords: 3,4 Methylenedioxymethamphetamine, degeneration, ecstasy, MDMA, neurotoxicity, serotonin, substituted amphetamines.

Abbreviations

MDA 3,4-methylenedioxyamphetamine, *5-HT* serotonin, *DA* dopamine, *MDE* 3,4-methylenedioxy-N-ethylamphetamine, *MDMA* 3,4 methylenedioxymethamphetamine, *PCA* *para*-chloroamphetamine.

Introduction

The ring-substituted amphetamine 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy", "XTC", "E") is increasingly being abused as a recreational drug in certain segments of the young generation. MDMA was first synthesized by Merck in 1914, presumably as an anorectic drug. In the mid 80'ies it was beginning to be encountered as a recreational drug in the United States, and in 1987 about 40% of a sample of Stanford undergraduates had used it at least once (Peroutka, 1987). There was a phase of therapeutic enthusiasm for this drug as adjunct to psychotherapy. Allegedly it helped people to get in touch with their feelings, caused a kind of deep harmony in the self and in the relationships to other persons. Nowadays it is a popular recreational, but illicit, drug of choice especially in the techno- and rave-dance culture. In 1994 estimated 30–40% of young people in the U.K. used MDMA and about 7 Mio. tablets were for sale. The attractiveness of this novel drug fashion was not diminished by reports on cases of toxicity and deaths from the exposure to large doses of MDMA (Dowling et al., 1987; Henry et al., 1992). In 1995, seventeen such fatal cases were reported among the estimated 500,000 users of MDMA in Germany. Serious concerns were risen by neurobiologist about the potential long-term damage of serotonergic nerve

endings caused by substituted amphetamines and it is now increasingly recognized that the long-term effects of MDMA are potentially more serious and problematic than its acute toxicity (Green and Goodwin, 1996; Henry, 1996). This view is based on the substantial progress made in recent years in the understanding of the mechanisms of actions of substituted amphetamines, and of the role of the serotonin (5-HT)-system in central information processing.

It is the purpose of this contribution to summarize some of the most interesting findings made in these research areas and to show how, why, and under which circumstances the consumption of substituted amphetamines may damage 5-HT nerve endings. The consequences of such damage will be discussed in relation to the various kinds of long-term behavioral and affective disturbances noticed in psychiatric practice in an increasing number of previous MDMA-consumers.

Mechanisms of actions

MDMA belongs to a group of ring-substituted phenylisopropylamine derivatives (see Fig. 1) which bear structural and pharmacological similarities to both amphetamines and hallucinogens such as mescaline (Nichols, 1986; Shulgin, 1986). The common feature of all these ring-substituted amphetamines is their pronounced stimulatory effect on the release of 5-HT from serotonergic presynapses (Nichols et al., 1982; Berger et al., 1992b). As shown for MDMA, these drugs readily diffuse across membranes and may additionally serve as substrates of the 5-HT transporter. After entering the presynapse either passively or by way of the 5-HT transporter, they exchange with the 5-HT stored in synaptic vesicles and cause 5-HT efflux from the vesicular stores. This cytoplasmatic 5-HT is readily transported through the presynaptic membrane into the extracellular space by the action of the 5-HT transporter (Berger et al., 1992b; Rudnick and Wall, 1992; Gu and Azmitia, 1993). This outward transport is sodium-dependent and blocked by 5-HT reuptake inhibitors (Fuller, 1980; Hekmatpanah and Peroutka, 1990; Gudelsky and Nash, 1996). Procedures which lead to an additional rise of free cytosolic 5-HT levels in the presynapse, such as stimulation of 5-HT synthesis or inhibition of 5-HT degradation, enhance 5-HT release (Iyer et al., 1994). Vice versa, the previous depletion of vesicular 5-HT stores or blockade of 5-HT synthesis attenuates the rise of extracellular 5-HT caused by substituted amphetamines (Brodkin et al., 1993).

A second feature of the acute action of substituted amphetamines is their stimulatory effect on the release of dopamine (DA) from dopaminergic presynapses. They seem to enter dopaminergic presynapses mainly by passive diffusion and cause the release of vesicular DA-stores by a similar mechanism as in 5-HT presynapses (Johnson et al., 1991; Seiden et al., 1993). The outward transport of free cytoplasmatic DA is mediated by the DA transporters of the presynaptic membrane. It is blocked by DA-reuptake inhibitors (Raiteri et al., 1979; Nash and Brodkin, 1991) and attenuated by blockade of DA-synthesis (Stone et al., 1986b; Axt et al., 1990) or previous depletion of

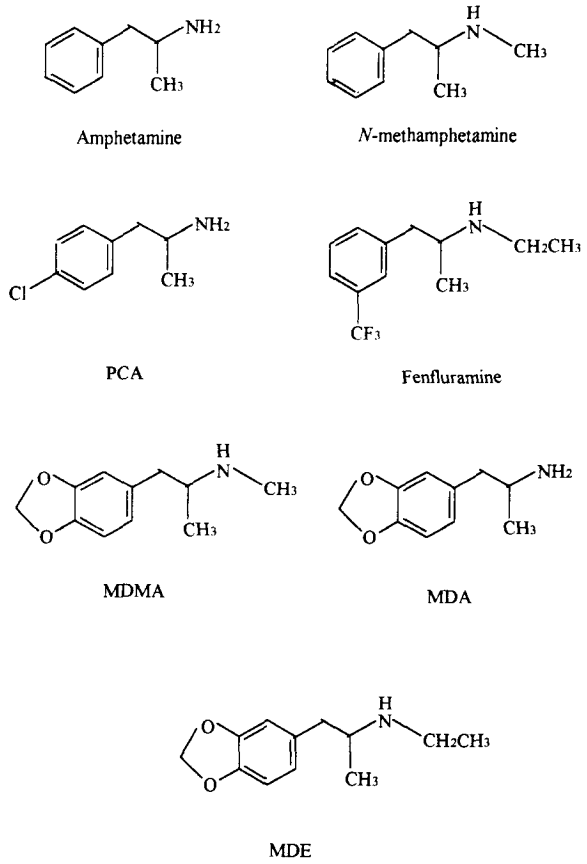

Fig. 1. Chemical structures of amphetamine, N-methamphetamine and their ring substituted derivatives. *PCA* para-chloroamphetamine, *MDA* 3,4 methylenedioxyamphetamine, *MDMA* 3,4 methylenedioxymethamphetamine, *MDE* 3,4-methylenedioxy-N-ethylamphetamine

vesicular DA-stores (Stone et al., 1988). Individual substituted amphetamines differ in their DA-releasing potency and the efficacy of DA-release is dependent on the degree of activation or inhibition of other transmitter systems and/or presynaptic heteroreceptors involved in the regulation of DA-output. The activation of 5-HT_{2A}-receptors by released 5-HT seems to facilitate the amphetamine-induced DA-release (Gudelsky et al., 1994) and the administration of 5-HT_{2A}-antagonists (Nash, 1990; Schmidt et al., 1991) attenuates the effects of substituted amphetamines on DA-release. It is very likely that substituted amphetamines cause the release of norepinephrine from noradrenergic nerve endings by a similar mechanism, but this effect has not yet been investigated. The affinity of substituted amphetamines to monoaminergic receptors is in the micromolar range (Battaglia et al., 1988b; Pierce and Peroutka, 1988) and direct receptor interactions of these drugs seem to make no major contribution to their central actions.

Acute effects

The stimulation of DA-release appears to be responsible for the acute amphetamine like psychostimulatory effects of these drugs whereas the LSD-like hallucinogenic effects, and the subjective feelings of "love, happiness, peace and connection" are probably due to the huge elevation of 5-HT in the extracellular space and the resulting activation of, among others, 5-HT_{2A}-receptors (Pierce and Peroutka, 1989; Wing et al., 1990). At higher doses or in susceptible individuals, substituted amphetamines may elicit a spectrum of acute peripheral responses, such as hyperthermia, tachykardia, hyperventilation, nausea, and others (see Table 1). Most of these adverse side effects are elicited by the massive release of 5-HT and catecholamines from peripheral sites and brain structures endowed with the regulation of blood pressure, respiration, body temperature and feeding. The severity of the hyperthermic response is related to the ambient temperature and influenced by water consumption and by the frequency of drug use (Gordon et al., 1991; Dafters, 1994, 1995). In rats, the MDMA-dosage required to elicit an acute hyperthermia similar to that seen clinically (about 10 mg/kg) is somewhat higher than the estimated typical recreational dose of 1–4 mg/kg consumed by users (Colado et al., 1995). However, the plasma concentrations of MDMA measured one hour after the intraperitoneal administration of 10 mg/kg in rats (about 5 nmol/ml) were almost identical to those reported in patients suffering acute fatal responses to the ingestion of the drug (Dowling et al., 1987; Henry et al., 1992).

Hyperthermia is a major feature in human recreational MDMA users presenting with MDMA-induced toxicity. Body temperatures of up to 43°C have been reported (Henry et al., 1992), and misuse of MDMA under

Table 1. Survey of adverse acute effects and complications reported after intake of MDMA at very high doses or in susceptible subjects (for references see Green et al., 1995)

Acute adverse effects	Acute complications
hyperthermia	fatal hyperthermia
tachycardia	rhabdomyolysis
hypertension	intravenous coagulation
arrhythmia	acute renal failure
hyperventilation	cerebral hemorrhage
nausea	convulsions
vomiting	hepatotoxicity
diarrhea	
myoclonus	
ataxia	
tremor	
myalgia	
trism	
bruxism	

crowded conditions high ambient temperatures, physical activity, and volume depletion seems to exacerbate the acute toxicity problems (for review see Green et al., 1995). MDMA has also been reported to cause hypertensia and arrhythmias (Bedford-Russel, 1992; Henry et al., 1992; Screaton et al., 1992). These vascular problems, together with forced cerebrovascular dilatation and coagulopathy are probably responsible for cases of cerebral hemorrhage in some subjects (Harries and DeSilva, 1992; Gledhill et al., 1993). The hyperthermic effect may be a causal factor in a number of deaths in human recreational MDMA users who have presented for emergency treatment with symptoms including convulsions, disseminated intravenous coagulation, rhabdomyelosis, and acute renal failure (Brown and Osterloh, 1987; Chadwick et al., 1991; Fahal et al., 1992; Henry et al., 1992; Screaton et al., 1992; see also Table 1).

In rats the hyperthermic response to substituted amphetamines is associated with the appearance of hyperactivity and the so-called serotonin behavioral syndrome (Slikker et al., 1989; Spanos and Yamamoto, 1989). This serotonin-syndrome consists of a complex sequence of behavioral changes, including reciprocal forepaw treading, head weaving, piloerection, hind limb adduction, proptosis, ataxia, and unawareness. It is also elicited by other pharmacologic treatments which cause a massive stimulation of the central 5-HT system, e.g. by stimulation of 5-HT synthesis and concomitant inhibition of 5-HT degradation (Graham-Smith, 1971) or by administration of 5-HT-receptor agonists (Green and Heal, 1985). Similar acute behavioral effects of substituted amphetamines were also observed in non-human primates (Hardman et al., 1973), and many of the signs and symptoms of acute MDMA toxicity in men resemble major features of the serotonin syndrome seen in experimental animals (Sternbach, 1991; Ames and Wirsin, 1993; Friedman, 1993).

A direct association of the 5-HT releasing properties of substituted amphetamines with both the behavioral syndrome and the hyperthermia is supported by the major biochemical consequence of all substituted amphetamines, namely a massive release of 5-HT and DA. The loss of up to 80% of the central 5-HT stores during the first few hours after drug administration coincides with the appearance of the acute hyperthermic and behavioral effects triggered through stimulation of 5-HT and DA receptors. The acute 5-HT and DA release, and therefore the severity of the hyperthermic and behavioural responses, is attenuated by the simultaneous administration of 5-HT reuptake inhibitors (Schmidt, 1987; Bradberry et al., 1990; Hekmatpanah and Peroutka, 1990), by the prior depletion of 5-HT stores through 5-HT-releasers or inhibition of 5-HT synthesis (Berger et al., 1992a; Brodkin et al., 1993) or by manipulations which antagonize the release and/or the action of DA (Stone et al., 1988; Schmidt et al., 1985).

In humans, the severity of adverse reactions is not always clearly related to the amount of MDMA taken (Henry et al., 1992). Different individual vulnerabilities seem to exist and, apart from problems of purity and concomitant use of other drugs, repeated exposure may cause sensitization and exacerbate the acute hyperthermic and behavioral responses. The occurrence of undesirable

physical symptoms, including loss of appetite, trismus, nausea, muscle aches, ataxia, sweating, tachycardia, fatigue, and insomnia were reported to increase with successive doses, while the mood enhancing, hallucinogenic effects decreased (Greer and Tolbert, 1986; Peroutka et al., 1988).

Long term effects

As shown by numerous animal studies, the initial release of 5-HT leads to a massive 5-HT depletion during the first few hours after administration of substituted amphetamines. Thereafter, amine levels start to normalize. At higher doses, brain 5-HT content decreases again until the 2–3rd day after drug administration and remains at this lower level for months (Schmidt, 1987; Gibb et al., 1990). The secondary long term reduction of 5-HT content is paralleled by decreased levels of two other biochemical markers of 5-HT presynapses, tryptophan hydroxylase content and 5-HT transporter density (Habert et al., 1985; Zhou et al., 1996). Immunocytochemical studies have demonstrated a severe loss of 5-HT presynapses swollen varicosities, fragmentation and dilatation of 5-HT axons (O'Hearn et al., 1988; Molliver et al., 1990).

The long-term degeneration of serotonergic nerve endings after administration of substituted amphetamines was first noticed in para-chloroamphetamine-treated rats (Sanders-Bush et al., 1972). Subsequent studies demonstrated the degeneration of ascending 5-HT projections after administration of large or repeated doses of methamphetamine (Hotchkiss and Gibb, 1980; Green et al., 1992), 3,4-methylenedioxyamphetamine (Ricaurte et al., 1985; Axt and Molliver, 1991), MDMA (Stone et al., 1986; Battaglia et al., 1987) or fenfluramine (Harvey and McMaster, 1975; Ricaurte et al., 1991). Long-term degenerative effects were also seen in other species, including several species of non-human primates (Ricaurte et al., 1988b; Insel et al., 1989; Wilson et al., 1989). Primates are much more vulnerable to the neurotoxic actions of substituted amphetamines; their 5-HT nerve endings were found to degenerate if only 1/10 of the MDMA-dose required to produce degenerative effects in rats was given (Ricaurte and McCann, 1992). Already the administration of 5mg MDMA/kg body weight caused a substantial degeneration of 5-HT nerve endings in the brains of squirrel monkeys, whether applied subcutaneously or, more importantly, orally (Ricaurte et al., 1988a).

A series of elegant immunocytochemical studies have described in great detail the neurodegenerative effects of substituted amphetamines in the brain of rats and non-human primates (O'Hearn et al., 1988; Molliver et al., 1990; Axt and Molliver, 1991). In rats, these studies have demonstrated the greatest damage to 5-HT axon terminals innervating neocortex, striatum, and thalamus with smaller reductions observed in hippocampus, septum, and amygdala. In neocortex, the loss of 5-HT presynapses was more pronounced in occipital compared to frontal regions. The medial hypothalamus was more severely affected than the lateral hypothalamus. These regional differences were shown to be due to the greater susceptibility of the fine axons projecting

from the dorsal raphe nucleus (Wilson et al., 1989; Battaglia et al., 1991; Mamounas et al., 1991). Also in brain stem, a severe loss of 5-HT terminals has been reported (Harvey et al., 1993). No damage of the serotonergic perikarya in the raphe nuclei was noticed in rats and published reports of "degenerative" effects of MDMA on 5-HT nerve cell bodies in the brain of primates are based on rather questionable histologic evidence (cytoplasmatic inclusions in luxol fast blue /cresyl violet stained sections, Ricaurte and McCann, 1992).

Causes of long term damage to 5-HT projections

Despite the very intense research efforts made during the last two decades, the mechanisms by which substituted amphetamines cause the destruction of 5-HT nerve endings and axons are still not yet completely understood. Most authors agree that the damage caused by ring-substituted amphetamines is rather similar and that this damage is caused by similar mechanisms (Stone et al., 1986; Molliver et al., 1990; Mamounas et al., 1991). Surpisingly, these amphetamine derivatives by themselves are not neurotoxic, at least not at the concentrations reached in the brain after systemic administration of a dose which causes a substantial loss of 5-HT nerve endings. Even direct intracerebral injections failed to produce the degeneration of 5-HT terminals and axons seen after systemic administration (Berger et al., 1990; Paris and Cunningham, 1991). Efforts to identify toxic metabolites have not been successful (McCann and Ricaurte, 1991b; Fuller, 1992; Johnson et al., 1992; Chu et al., 1996). It has therefore been proposed that part of the 5-HT or DA released by the systemic administration of substituted amphetamines may be converted to neurotoxic metabolites, such as 5,7-dihydroxytryptamine (5,7-DHT) or 6-hydroxydopamine (6-OH-DA), and that these endogenously formed neurotoxins cause the damage to 5-HT terminals (Seiden and Vosmer, 1984; Commins et al., 1987). However, the measured levels of endogenously formed 5,7-DHT were much below the critical threshold to cause 5-HT neurotoxic effects (Commins et al., 1987) and earlier reports on the formation of 6-OH-DA (Seiden and Vosmer, 1984) could not be confirmed by the use of more accurate methods (Karoum et al., 1993).

It has nevertheless been hypothesized (and became the currently most widely held belief) that the degeneration of 5-HT terminals seen after systemic administration of substituted amphetamines is due to oxidative damage by dopamine erreously taken up into 5-HT presynapses (Schmidt et al., 1985; Stone et al., 1988; Odell et al., 1991). The proponents of this hypothesis have some difficulties to explain the severe loss of 5-HT afferences in areas of low dopaminergic innervation in the rat, such as hippocampus and cortex and the lack of damage observed after intracerebral administration of these drugs. More importantly, this hypothesis completely ignores the fact that serotonergic (like all other) presynapses are endowed with several protective mechanisms which are very effective in the prevention or repair of oxidative and radical-mediated damage, provided that the efficacy of these mechanisms is not overwhelmed by very high concentrations of these toxins or by a lack of

energy required for their optimal function (Marker et al., 1981; Hothershall et al., 1982; DeVito and Wagner, 1989; Swanson and Choi, 1993). That the exhaustion of endogenous defense mechanisms may indeed represent the most crucial factor in the sequence of events which lead to the degeneration of 5-HT terminals and axons after administration of substituted amphetamines is supported by direct evidence for oxidative stress and radical-mediated damage (Stone et al., 1989; Gudelsky et al., 1994; Sprague and Nichols, 1995) and by the observed protective action of antioxidants and radical scavengers (Colado and Green, 1995; Hirata et al., 1995; Gudelsky, 1996). The inefficiency of endogenous detoxification and repair mechanisms caused by the administration of substituted amphetamines is additionally evidence by the reported destabilization of Ca^{2+} homeostasis and the extended translocation and activation of protein kinase C in MDMA treated raphe cell cultures (Azmitia et al., 1990b; Park and Azmitia, 1991; Kramer et al., 1995). The exhaustion of ATP driven Ca^{++}-pumps, and therefore, large increases of intracellular Ca^{++} are key amplification steps also in many other neurodegenerative processes, including glutamate mediated excitotoxicity (Choi, 1988; Choi and Rothman, 1990; DeErasquin et al., 1990).

The most essential prerequisite for an efficient functioning of the mechanisms involved in the regulation of transmembrane ion exchange, internal Ca^{++}-homeostasis, in the prevention of oxidative stress and lipid peroxidation is energy. Several lines of evidence indicate that the neurotoxic potential of substituted amphetamines is related to their ability to exchange with serotonin and to dissipitate transmembrane ion gradients and thereby tax energy metabolism in 5-HT nerve terminals. Because these drugs prevent the proper storage of 5-HT into the presynaptic vesicles, the active carrier systems in the vesicular and presynaptic membrane operate at a permanently activated state (Rudnik and Wall, 1992). The inefficient transport of the released 5-HT from the extracellular space into the presynapse and from the presynaptic cytosol into the vesicles consumes considerable amounts of ATP required for the restoration and maintenance of ionic gradients. This loss can normally be restored by increased uptake and utilization of substrates for presynaptic energy metabolism, mainly glucose. The single largest energy reserve in the adult brain is glycogen stored in astrocytes. It is rapidly degraded and released in the form of glucose and lactate upon stimulation by, e.g. increased release of serotonin ($5\text{-}HT_2$-receptor-mediated) of catecholamines (β-receptor-mediated) or high levels of extracellular potassium ions associated with generally increased neuronal activity (for reviews see Pentreath et al., 1986; Swanson and Choi, 1993; Eyre et al., 1994). It is therefore not surprising that glycogen phosphorylase in astroglial-rich cell cultures is activated by the addition of 5-HT or MDMA (Poblete and Azmitia, 1995) and that brain glycogen levels are rapidly depleted after systemic administration of substituted amphetamines (see Fig. 2).

Under certain circumstances, the wastage of presynaptic energy resources caused by substituted amphetamines may exceed the capacity of 5-HT terminals to cover these increased ATP demands. If other energy dependent mechanisms involved in the regulation of the functional and structural integ-

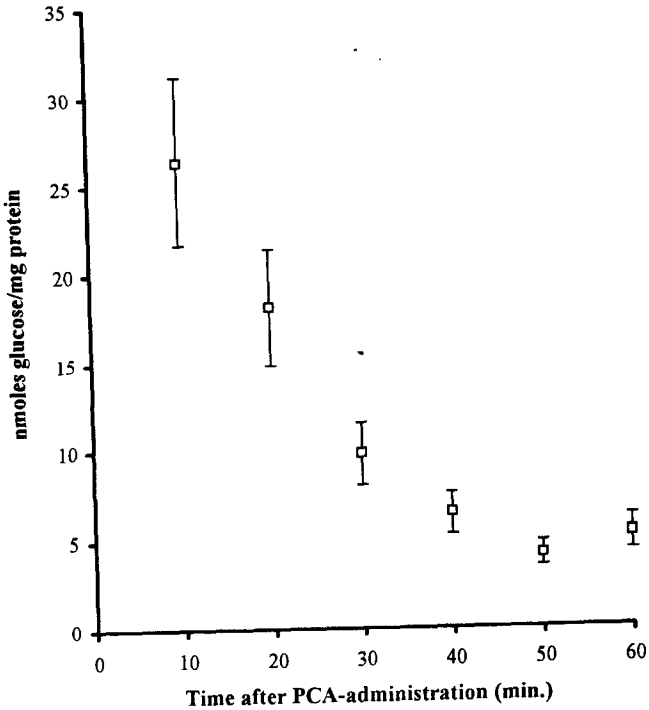

Fig. 2. Effect of acute PAC-administration on the glycogen content in the frontal cortex. Male Wistar rats were given one single dose of PCA (10mg/bw, i.p.) and killed by decapitation at different times thereafter. The glycogen content in the cortex was assayed fluoremetrically by the method of Passoneau and Lauderdale (1974). Values are means ± SD (n = 5) and expressed as nmoles glucose liberated from glycogen by the action of amyloglycosidase per mg protein

rity of the 5-HT nerve terminal are no longer adequately fueled, they will become increasingly inefficient. Intracellular Ca^{++} levels will rise, endogenous proteases will become activated, oxidative stress will cause various kinds of damage, and the synapse and proximal axon segments will degenerate. This fatal sequence of events is not elicited if substituted amphetamines are locally applied by direct intracerebral injections (Berger et al., 1990; Paris and Cunningham, 1991). Obviously, under such conditions, the availability of energy-rich substrates is still sufficient to cover the increased energy demands of 5-HT nerve terminals. Apparently this is no longer the case of the supply of glucose and other substrates for their energy metabolism is additionally compromised by the severe wastage of energy caused by the systemic administration of substituted amphetamines in the periphery. The most energy consuming processes elicited by the systemic administration of substituted amphetamines are hyperthermia and hyperactivity. Both are potentiated by aggregation, by high ambient temperature, by additional external stimulation (loud music, light-shows), and by dehydration, i.e. by the conditions when substituted amphetamines are used at rave and dance parties.

It has been assumed that both the severity of acute systemic side effects and the long-term-damage to 5-HT nerve endings may be potentiated by

these conditions and that individual differences exist in the vulnerability to the acute systemic actions, and therefore to the long-term neurodegenerative effects of substituted amphetamines (for review see Green et al., 1995). We found a remarkable correlation between the severity of the systemic reactions (e.g. the duration of the hyperthermic response) and the loss of 5-HT nerve endings in the brain of two differently susceptible rat strains (see Fig. 3). Also in human subjects, the severity of hyperthermic and other averse reactions is unrelated to the actual dose of MDMA taken (Henry et al., 1992) but may be closely related to the severity of the long-term damage in the brain of those subjects who experienced serve hyperthermic and other energy wasting systemic responses during the first few hours after the consumption of substituted amphetamines.

Several observations in MDMA-treated rats suggest that the energy-wasting, especially the hyperthermic, reactions elicited by the systemic administration of substituted amphetamines play an important permissive role in the long-term degeneration of 5-HT axon terminals (Bowyer et al., 1994; Colado et al., 1995). It has long been noticed that these drugs cause no long-term

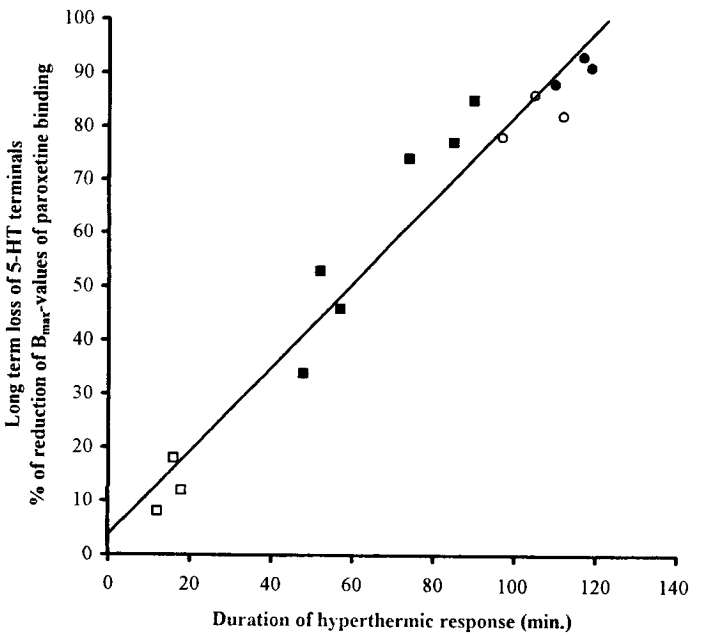

Fig. 3. Relationship between the duration of the hyperthermic response and the severity of the long-term damage to 5-HT presynapses in the frontal cortex of rats caused by the administration of different doses of PCA to rats of two differently vulnerable strains (r = 0.97). The animals of the two differently vulnerable rat stains were treated by PCA and the loss of 5-HT terminals was measured by [^{3}H]-paroxetine binding assay as detailed elsewhere (Zhou et al., 1996). The duration of the hyperthermic response was defined here as the period during which the rectal temperature remained at an elevated level of ≥39.5°C. Squares: Wistar rats, Circles: Sprague-Dawley rats. Open symbols: 4mg PCA/kg body weight. Gray symbols: 6mg PCA/kg body weight. Filled symbols: 8mg PCA/kg body weight

damage to 5-HT presynapses when administered to rats during early postnatal development (Clemens et al., 1978; Lucot et al., 1981; Broening et al., 1994). During the early developmental period, the brain glycogen reserves are particularily high (Brückner and Biesold, 1981) and the energy-wasting hyperthermic response to systemic MDMA administration is much less pronounced (Broening et al., 1994). Apparently, the 5-HT presynapses under such conditions are better protected against the exhaustion of energy and the failure of their endogenous detoxification and repair mechanisms caused by the systemic administration of substituted amphetamines. Another feature of the action of MDMA and its congeners which has been studied in great detail is the attenuation or exacerbation of the long term damage of 5-HT axon terminals in the brain of experimental animals caused by certain pharmacological pretreatments or physiological manipulations. Each of the hitherto proposed hypothesis on the possible mechanisms which may lead to the loss of 5-HT nerve terminals explained some, but not all of these protective or preventive effects. The concept of energy exhaustion proposed here, however, predicts that the long-term damage to 5-HT nerve endings should be less pronounced under conditions

a) When the vesicular 5-HT stores are depleted by prior administration of 5-HT releasers (see Berger et al., 1989, 1992a) or when the uptake of released 5-HT is blocked (see Schmidt, 1987), so that less 5-HT is inefficiently transported and less ATP is consumed for the restoration and maintenance of ionic gradients;
b) when the hyperthermic response is attenuated by drugs which antagonize the rise of body temperature caused by the activation of 5-HT- and DA-receptors involved in central thermoregulation (Schmidt et al., 1990b; Miller and O'Callaghan, 1994), so that more energy-rich substrates can be supplied to the brain;
c) when the amphetamine-like psychostimulatory effects, and therefore the degree of general arousal and hyperactivity are reduced e.g. by pretreatments which prevent the activation of the dopaminergic system, e.g., by lesions of the DA-system (see Schmidt et al., 1985; Stone et al., 1988), by prior DA-depletion or by inhibition of DA-synthesis (see Axt et al., 1990; Brodkin et al., 1993), by administration of DA-antagonists (see Schmidt et al., 1985; Hewitt and Green, 1994), by pharmacologic manipulations of other transmitter systems involved in the regulation of presynaptic DA-output, including administration of 5-HT_2-receptor antagonists (see Schmidt et al., 1991; Gudelsky et al., 1994), of GABA-agonists and/or glutamate (NMDA) antagonists (see Finnegan et al., 1990; Colado et al., 1993; Colado and Green, 1994) or by drugs which cause a general reduction of global energy metabolism in the brain, such as sedativa and anesthetics (see Schmidt et al., 1990a);
d) when the oxidative pressure in 5-HT nerve terminals is reduced, e.g., by prior administration of antioxidants and radical scavengers (see Colado and Green, 1995; Hirata et al., 1995; Gudelsky, 1996); by treatments which reduce the uptake and possible formation of toxic

metabolites in 5-HT presynapses (see Iyer et al., 1994; Sprague and Nichols, 1995) or presumably by any other treatment which improves global energy metabolism in the brain.

Consequences of the loss of 5-HT-afferences

In order to evaluate the consequences of the potential degeneration of 5-HT nerve terminals in the brain of previous MDMA users, a somewhat closer examination of the biological role of the central 5-HT-system is warranted. Information processing in the brain takes place in a serial and parallel manner in multifocal, distributed networks. The activity, and therefore the impact of these local networks on whole brain function is controlled by several globally acting transmitter systems through their extended and partially overlapping projections (Mesulam, 1990). One of these extended transmitter systems involved in the globalisation and harmonization of the activity generated in distributed networks is the 5-HT-system. During daytime, the raphe neurons fire tonically with 3–5 impulses/sec. and affect the neuronal activity in individual neuronal networks throughout the forebrain by the 5-HT released at the terminals of their far reaching and extensively branched axons (Jacobs and Azmitia, 1992). Its endogenous activity is not affected by environmental stimuli, it dimishes during sleep and ceases completely during REM-sleep (Jacobs et al., 1990; Jacobs and Fornal, 1991).

In the cortex, the dense and predominantly non-junctional 5-HT innervation is capable of modulating the activity of vast cellular assemblies (for reviews see Seguela et al., 1989; Spoont, 1992). An imbalance in the innervation density between individual projection fields may have widespread global influences on brain function. In principle, cortical 5-HT activity acts to constrain neuronal information processing. It stabilizes signal propagation through its inhibitory actions on neuronal activity and prevents impingement of the system by exogenous signal sources. Its activity ensures that only signals of sufficient intensity are able to interfere with current information flow.

The ability of the central 5-HT system to act as a global synthesizer and harmonizer of local brain activities appears to be related to the fact that this system develops earlier than the local networks in higher brain regions. Because of its trophic, organizing influences on other, later developing neuronal networks (Lauder, 1990; Rhoades et al., 1990; Azmitia and Whittaker-Azmitia, 1991), it made a major contribution to the establishment and the elaboration of the local neuronal networks which remain under its globalizing, harmonizing, and irrigating influence from early childhood to senescence.

The massive release of 5-HT caused by the action of substituted amphetamines results in a massive enhancement of these harmonizing and stabilizing actions of 5-HT on cortical information processing. This acute effect is perceived by most users as a sudden flash of increased harmony, as feelings of love, happiness, peace, and connection (Cohen, 1995). Such a pharmacologically produced degree of harmony is of cause difficult to achieve under normal

conditions of everyday life with all the disgusting, disturbing, stressing, and ambivalent stimuli from the external world. There is, however, a price to pay for such an artificial opening of serotonergic synapses. And this price is their potential damage.

The loss of 5-HT afferences does not cause very obvious behavioral effects. In experimental animals, rather sophisticated behavioural measurements are required in order to demonstrate the consequences of severe selective lesions to the central 5-HT system (for reviews see Smale et al., 1990; Sirvio et al., 1994). The partial loss of cortical 5-HT presynapses can be expected to impair the ability of distributed neuronal networks to maintain the integrity of the signal flow pattern, and increase the likelihood of switching to unstable information processing. As a consequence of the long-term destruction of this stabilizing and harmonizing serotonergic "buffer-system", behavioral responding may be stronger influenced by certain activities generated in individual networks. Inherited or acquired mental dispositions resulting from a too strong or too week impact of such individual networks on global information processing in the brain must be assumed. The partial loss of serotonergic projections will not necessarily lead to gross behavioral abnormalities. Rather this loss may cause an intensification and accentuation of certain personality features. For this reason, it will be difficult to identify the long-term behavioural consequences of the destruction of 5-HT terminals in the brains of previous MDMA-users in cross sectional epidemiologic studies. These consequences may become more obvious in longitudinal studies which aim at the detection of subtile behavioral changes on an individual basis. Such changes are most likely encountered in subjects which experienced serious averse systemic reactions, e.g. a severe acute hyperthermic response, following the consumption of substituted amphetamines.

Once the damage to the serotonergic projections occurred, some regeneration may occur. This process, however, requires months or even years and will fail to authentically restore the original innervation pattern. Preliminary own results indicate that collateral sprouting of 5-HT axons and formation of new 5-HT presynapses in the cortex is supported by long-term administration of drugs which increase the availability of 5-HT in the extracellular space (see Fig. 4). This regrowth-promoting effect is probably due to a 5-HT-mediated increase in the production and release of astrocytic growth factors which are known to stimulate the outgrowth of 5-HT nerve endings even in the adult brain (Azmitia et al., 1990a; Whittaker-Azmitia et al., 1990). One important argument underlines the need for early and effective treatments which support the regeneration of 5-HT nerve terminals: The loss of serotonergic nerve endings represents only the first step in a sequence of events, which will inevitably lead to the destabilization of these neuronal circuits and synaptic connections in local neuronal networks which are normally stabilized by serotonergic inputs. The severity of these reorganization processes is illustrated by the 30–50% decline in total synaptic density in the cortex of experimental animals seen after lesions of the cortical serotonergic innervation (Okado et al., 1993; Cheng et al., 1994; Azmitia et al., 1995).

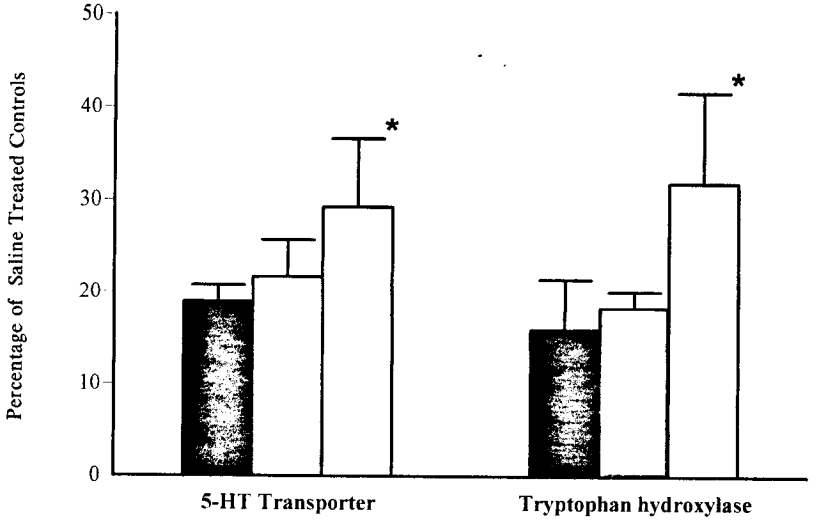

Fig. 4. Effect of long-term antidepressant treatment on the PCA-induced loss of 5-HT presynapses in the frontal cortex of Wistar rats. Rats were pretreated by a single i.p. injection of PCA. One week later they were chronically treated by either amitriptyline or tranylcypromine over a period of 4 weeks (4.5 mg/kg day, added to the drinking water, daily fresh). Rats were killed one week after removal of the antidepressants from their drinking water. The effects of the PCA-treatment and the influence of the chronic administration of antidepressants on the recovery of 5-HT terminals were measured by ^{3}H-paroxetine binding and quantitative enzyme-linked immunoassay for tryptophan hydroxylase apoenzyme as described elsewhere (Zhou et al., 1996). Date are presented in % of values in untreated control animals (B_{max} of [^{3}H]-paroxetine binding = 1011.9 ± 133.3 fmol/mg protein, concentration of tryptophan hydroxylase apoenzyme concentration = 2.64 ± 0.43 ng/protein). Black columns: controls (normal tap water), gray columns: amitriptyline treatment (4.5 mg/kg per day), open columns: tranylcypromine treatment (4.5 mg mg/kg per day)

There must be the usual provision about extrapolating animal findings to man. However, the doses of MDMA used by human subjects recreationally (often more than 3 mg/kg) give rise to serious concerns about MDMA-induced long-term degeneration of 5-HT nerve endings in the human brain. Until now, only a few indirect measurements indicate that previous MDMA users may have lost part of their 5-HT afferences in distant projection fields of the raphe nuclei. The concentrations of the major 5-HT metabolite in the cerebrospinal fluid of regular MDMA users were about 25% lower than in a control group (Recaurte et al., 1990) and they had less non-REM sleep and less total sleep time than controls (Allen et al., 1993). A blunted prolaction-response to L-tryptophan administration has also been observed in heavy MDMA-users (Price et al., 1989). Of particular interest is the multitude of neuropsychiatric syndromes reported in previous MDMA-users (see Table 2). It is very likely that the partial loss of the serotonergic innervation may cause the exaggeration of certain hitherto "buffered" personality traits which, in vulnerable, predisposed subjects may then become manifested as defined psychiatric syndromes. These syndromes have long been linked to serotoner-

Table 2. Survey of long-lasting neuropsychiatric problems reported after use of MDMA (for references see Ricaurte and McCann, 1992; Green et al., 1995; McGuire et al., 1994)

Long-lasting psychiatric problems
panic attacks,
anxiety
insomnia
depression
suicidality
chronic paranoid psychosis
recurrent acute psychosis
memory disturbances

gic dysfunctions. They are often seen in individuals using MDMA repeatedly and usually at high dosage. Some of the reported cases were individuals with prior psychiatric histories (McCann and Ricaurte, 1991a; McGuire et al., 1994). PET and SPECT-imaging techniques for the assessment of, e.g. 5-HT transporter densities, of 5-HT-synthesizing capacities or of stimulated 5-HT release are now available (see for instance Agren and Reibring, 1994; Kuikka et al., 1995; Mann et al., 1996; Meyer et al., 1996). Such measurements in different brain regions of previous MDMA users are urgently needed in order to confirm the severity of long-term effects caused by the misuse of substituted amphetamines on the regional 5-HT innervation density in the human brain and to rule out any doubts about the long-term consequences of this innocent drug fashion: That the price of a short flush of too much "ecstasy" is the irreversible damage to the system which is essentially involved in the generation of feelings of harmony and pleasure.

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References

Agrew H, Reibring L (1994) PET-studies of presynaptic monoamine metabolism in depressed patients and healthy volunteers. Pharmacopsychiat 27: 2–6

Allen RP, McCann UD, Ricaurte GA (1993) Persistent effects of (+)-3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") on human sleep. Sleep 16: 560–564

Ames D, Wirshin WC (1993) Ecstasy, the serotonin syndrome and neuroleptic malignant syndrome – a possible link? JAMA 269: 869

Axt KJ, Molliver ME (1991) Immunocytochemical evidence for methamphetamine-induced serotonergic axon loss in the rat-brain. Synapse 9: 302–313

Axt KJ, Commins DL, Vosmer G, Seiden LS (1990) α-methyl-p-tyrosine pretreatment partially prevents methamphetamine-induced endogenous neurotoxin formation. Brain Res 515: 269–276

Azmitia EC, Whitaker-Azmitia PM (1991) Awakening the sleeping giant; anatomy and plasticity of the brain serotonergic system. J Clin Psychiatry 52: 4–16

Azmitia EC, Frankfurt M, Davila M, Whitaker-Azmitia PM, Zhou F (1990a) Plasticity of fetal and adult CNS serotonergic neurons: role of growth-regulatory factors. Ann NY Acad Sci 600: 342–365

Azmitia EC, Murphy RB, Whitaker-Azmitia EC (1990b) MDMA (Ecstasy) effects on cultured serotonergic neurons: evidence for Ca^{2+}-dependent toxicity linked to release. Brain Res 510: 97–103

Azmitia EC, Rubinstein VJ, Strafaci JA, Pios JC, Whitaker-Azmitia PM (1995) 5-HT_{1A} agonist and dexamethasone reversal of para-chloroamphetamine induced loss of MAP-2 and synaptophysin immunoreactivity in adult rat brain. Brain Res 677: 181–192

Battaglia G, Yeh SY, O'Hearn E, Molliver ME, Kuhar MJ, De Souza EB (1987) 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin termins in rat brain: quantification of neurodegeneration by measurements of [^{3}H]-paroxetine-labelled serotonin uptake sites. J Pharmacol Exp Ther 242: 911–916

Battaglia G, Brooks BP, Kulsakdinun C, De Souza EB (1988) Pharmacologic profile of MDMA (3,4-methylenedioxy-methamphetamine) at various brain recognition sites. Eur J Pharmacol 149: 159–163

Battaglia G, Sharkey J, Kuhar MJ, De Souza EB (1991) Neuroanatomic specificity and time course of alterations in rat brain serotonergic pathways induced by MDMA (3,4-methylenedioxymethamphetamine): assessment using quantitativ autoradiography. Synapse 8: 249–260

Bedford-Russel AR, Schwartz RH, Dawling S (1992) Accidental igestion "ecstasy" (3,4-methylenedioxymethamphetamine). Arch Dis Child 67: 1114–1115

Berger UV, Grzanna R, Molliver ME (1989) Depletion of serotonin using p-chlorophenylalanine (PCPA) and reserpine protects against the neurotoxic effects of p-chloroamphetamine (PCA) in the brain. Exp Neurol 103: 111–115

Berger UV, Grzanna R, Molliver ME (1990) Unlike systemic administration of PCA, direct intracerebral injection does not cause neurotoxicity on 5-HT axons. Exp Neurol 109: 257–268

Berger UV, Grzanna R, Molliver ME (1992a) The neurotoxic effects of p-chloroamphetamine in rat brain are blocked by prior depletetion of serotonin. Brain Res 578: 177–185

Berger UV, Gu XF, Azmitia EC (1992b) The substitued amphetamines 3,4-methylenedioxymethampethamine, methamphetamine, para-chloroamphetamine and fenfluramine induce 5-hydroxytryptamine relase via a common mechanism blocked by fluoxetine and cocaine. Eur J Pharmacol 215: 153–160

Bowyer JV, Davies DL, Schmued L (1994) Further studies of the role of hyperthermia in methamphetamine neurotoxicity. J Pharmacol Exp Ther 286: 1571–1580

Bradberry CW, Sprouse JS, Aghajanian GK, Roth RH (1990) 3,4-MDMA-induced release of endogenous serotonin from the rat dorsal raphe nucleus in vitro: effects of fluoxetine and tryptophan. Neurochem Int 17: 509–513

Brodkin J, Malyala A, Nash JF (1993) Effect of acute monoamine depletion on 3,4-methylenedioxymethamphetamine-induced neurotoxicity. Pharmacol Biochem Behav 45: 647–653

Broening HW, Bacon L, Slikker W jr (1994) Age modulates the long-term but not the acute effects of the serotonergic neurotoxicant 3,4-methylenediamphetamine. J Pharmacol Exp Ther 271: 285–293

Brown C, Osterloh J (1987) Multiple severe complications from recreational ingestion of MDMA ("Ecstasy"). J Am Med Assoc 258: 780–781

Brückner G, Biesold D (1981) Histochemistry of glycogen deposition in perinatal rat brain: importance of radial glial cells. J Neurocytol 10: 749–757

Chadwick IS, Linsley A, Freemont AJ, Doran B, Curry PD (1991) Ecstasy, 3,4-methylenedioxymethamphetamine (MDMA), a fataly associated with coagulopathy and hyperthermia. J Roy Soc Med 84: 371

Cheng L, Hamaguchi K, Ogawa M, Hamada S, Okado N (1994) pCPA reduces both monoaminergic afferents and non-monoaminergic synapses in the cerebral cortex. Neurosci Res 19: 111–115

Choi DW (1988) Calcium mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. Trends Neurosci 11: 465–469

Choi DW, Rothman SM (1990) The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. Annu Rev Neurosci 13: 171–182

Chu T, Kumagain Y, DiStefano EW, Cho AK (1996) Disposition of methylenedioxymethamphetamine and three metabolites in the brains of different rat strains and their possible roles in acute serotonin depletion. Biochem Pharmacol 51: 789–796

Clemens JA, Fuller RW, Perry KW, Sawyer BD (1978) Effects of p-chloroamphetamine on brain serotonin in immature rats. Comm Psychopharmacol 2: 11–16

Cohen RS (1995) Subjective reports on the effects of the MDMA ("ecstasy") experience in humans. Progr Neuropsychopharmacol Biol Psychiatry 19: 1137–1145

Colado MI, Green AR (1994) A study of the mechanism of MDMA ("Ecstasy")-induced neurotoxicity of 5-HT neurones using chlormethiazole, dizocilpine and other protective compunds. Br J Pharmacol 111: 131

Colado MJ, Green AR (1995) The spin trap reagent α-phenyl-N-tert-butyl nitrone prevents "ecstasy"-induced neurodegeneration of 5-hydroxytryptamine neurons. Eur J Pharmacol 280: 343–346

Colado MI, Murray TK, Green AR (1993) 5-HT loss in rat brain following 3,4-methylenedioxymethamphetamine (MDMA), p-chloroamphetamine and fenfluramine administration and effects of chlormethiazole and dizocilpine. Br J Pharmacol 108: 583–589

Colado MI, Williams JL, Green AR (1995) The hyperthermic and neurotoxic effects of "Ecstasy" (MDMA) and 3,4-methylenedioxyamphetamine (MDA) in the Dark Agouti (DA) rat, a model of the CYP2D6 poor metaboliser phenotype. Br J Pharmacol 115: 1281–1289

Commins DL, Axt KJ, Vosmer G, Seiden LS (1987) Endogenously produced 5,6-DHT may mediate the neurotoxic effects of para-chloroamphetamine. Brain Res 419: L253–261

Dafters RI (1994) Effects of ambient temperature on hyperthermia and hyperkinesis induced by 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") in rats. Psychopharmacology 114: 505–508

Dafters RJ (1995) Hyperthermia following MDMA administration in rats: effects of ambient temperature, water consumption, and chronic dosing. Physiol Behav 58: 877–882

De Erasquin G, Manev H, Guidotti A, Costa E, Brooker G (1990) Gangliosides normalize distorted single-cell intracellular free Ca^{++} dynamics after toxic doses of glutamate in cerebellar granule cells. Proc Natl Acad Sci USA 87: 8017–8021

De Vito M, Wagner GC (1989) Methamphetamine-induced neuronal damage: a possible role for free radicals. Neuropharmacology 28: 1145–1150

Dowling GB, McDonough ET, Bost RO (1987) Eve and Ecstasy, a report of five deaths associated with the use of MDEA and MDMA. JAMA 257: 1615–1617

Eyre JA, Stuart AG, Forsyth Jr, Heaviside D, Bartlett K (1994) Glucose export from the brain in man: evidence for a role of astrocytic glycogen as a reservoir of glucose for neural metabolism. Brain Res 635: 349–352

Fahal IH, Sallomi DF, Yaqoob M, Bell GM (1992) Acute renal failure after ecstasy. BMJ 305: 29

Finnegan KT, Skratt JJÖ, Irwin I, Langston JW (1990) The N-methyl-D-aspartate (NMDA) receptor antagonist dextromethorphan prevents the neurotoxic effects of 3,4-methylenedioxymethamphetamine (MDMA) in rats. Neurosci Lett 105: 300–306

Friedman R (1993) Ecstasy, the serotonin syndrome and neuroleptic malignant syndrome – a possible link? JAMA 269: 869–870

Fuller RW (1980) Mechanisms by which uptake inhibitors antagonize p-chloroamphetamine-induced depletion of brain serotonin. Neurochem Res 5: 241–253

Fuller RW (1992) Effects of p-chloroamphetamine on brain serotonin neurons. Neurochem Res 17: 449–456

Gibb JW, Johnson M, Stone D, Hanson GR (1990) MDMA: historical perspectives. Ann NY Acad Sci 600: 601–611

Gledhill JA, Moore DF, Bell D, Henry JA (1993) Subarachnoid haemorrhage associated with MDMA abuse. J Neurol Neurosurg Psychiatry 56: 1036–1037

Gordon CJ, Wilkinson WP, O'Callaghan JP, Miller DB (1991) Effect of 3,4-methylenedioxymethamphetamine on autonomic thermoregulatory responses of the rat. Pharmacol Biochem Behav 38: 339–344

Grahame-Smith DG (1971) Studies in vivo on the relationship between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with monoamine oxidase inhibitor and L-tryptophan. J Neurochem 18: 1053–1066

Green AR, Heal DH (1985) The effects of drugs on serotonin-mediated behavioural models. In: Green AR (ed) Neuropharmacology of serotonin. Oxford University Press, pp 326–365

Green AR, Goodwin GM (1996) Ecstasy and neurodegeneration. Br Med J 312: 1493

Green AR, De Souza RJ, Williams JL, Murray TK, Cross AJ (1992) The neurotoxic effects of methamphetamine on 5-hydroxytryptamine and dopamine in brain: evidence for the protective effect of chlormethiazole. Neuropharmacol 31: 315–321

Green AR, Cross AJ, Goodwin GM (1995) Review of the pharmacology and clinical pharmacology of MDMA. Psychopharmacology 119: 247–260

Greer G, Tolbert R (1986) Subjective reports of the effects of MDMA in a clinical setting. J Psychoact Drugs 18: 319–327

Gu XF, Azmitia EC (1993) Integrative transporter-mediated release from cytoplasmic and vesicular 5-hydroxytryptamine stores in cultured neurons. Eur J Pharmacol 235: 51–57

Gudelsky GA (1996) Effect of ascorbate and cysteine on the 3,4-methylenedioxymethamphetamine-induced depletion of brain serotonin. J Neural Transm 103: 1397–1404

Gudelsky GA, Nash JF (1996) Carrier-mediated release of serotonin by 3,4-methylenedioxymethamphetamine: implications for serotonin-dopamine interactions. J Neurochem 66: 243–249

Gudelksy GA, Yamamoto BK, Nash JF (1994) Potentiation of 3,4-methylenedioxymethamphetamine-induced dopamine release and serotonin neurotoxicity by 5-HT_2 agonists. Eur J Pharmacol 264: 325–330

Habert E, Graham D, Tahraoui L, Claustre Y, Langer SZ (1985) Characterization of [^{3}H]-paroxetine binding to rat cortical membranes. Eur J Pharmacol 118: 107–114

Hardman HF, Haavik CO, Seevers MH (1973) Relationship of the strsucture of mescaline and seven analogs to toxicity and behaviour in five species of laboratory animals. Toxicol Appl Pharmacol 25: 299–309

Harries DP, DeSilva R (1992) Ecstasy and intracerebral haemorrhage. Scot Med J 37: 150–152

Harvey JA, McMaster SE (1975) Fenfluramine: evidence for a neurotoxic action in midbrain and a long term depletion of serotonin. Pharmacol Commun 1: 217–228

Harvey JA, McMaster SE, Romano AG (1993) Methylenedioxyamphetamine: neurotoxic effects of serotonergic projections to brainstem nuclei in the rat. Brain Res 619: 1–14

Hekmatpanah CR, Peroutka SK (1990) 5-Hydroxytryptamine uptake blockers attenuate the 5-hydroxytryptamine releasing effect of 3,4-methylenedioxymethamphetamine and related agents. Eur J Pharmacol 177: 95–98

Henry JA (1996) Ecstasy and serotonin depletion. Lancet 347: 833

Henry JA, Jeffreys KJ, Dawling KS (1992) Toxicity and deaths from 3,4-methylenedioxyamphetamine ("ecstasy"). Lancet 340: 384–387

Hewitt KE, Green AR (1994) Chlormethiazole, dizocilpine and haloperidol prevent the degeneration of serotonergic nerve terminals induced by administration of MDMA ("Ecstasy"). Neuropharmacology 33: 1589–1595

Hirata H, Ladenheim B, Rothman RB, Epstein C, Cated JL (1995) Methamphetamine-induced serotonin neurotoxicity is mediated by superoxide radicals. Brain Res 677: 345–347

Hotchkiss A, Gibb JW (1980) Long term effects of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase in rat brain. J Pharmacol Exp Ther 214: 257–262

Hothershall JS, Greenbaum AL, McLean P (1982) The functional significance of the pentose phosphate pathway in synaptosomes: protection against peroxidative damage by catecholamines and oxidants. J Neurochem 39: 1325–1332

Insel TR, Battaglia G, Johanssen J, Marra S, De Souza EB (1989) 3,4-Methylenedioxymethamphetamine ("Ecstasy") selectively destroys brain serotonin nerve terminals rhesus monkeys. J Pharmacol Exp Ther 249: 713–720

Iyer RN, Sprouse JS, Aghajanian GK, Roth RH, Bradberry CW (1994) Tryptophan pretreatment augmentation of p-chloroamphetamine-incuded serotonine and dopamine release and reduction of long-term neurotoxicity. Biochem Pharmacol 48: 1501–1508

Jacobs BL, Fornal CA (1991) Activity of brain serotonergic neurons in the behaving animal. Pharmacol Rev 43: 563–578

Jacobs BL, Azmitia E (1992) Structure and function of the brain serotonin system. Physiol Rev 72: 165–229

Jacobs BL, Wilkinson LO, Fornal CA (1990) The role of brain serotonin. A neurophysiologic perspective. Neuropsychopharmacology 3: 473–479

Johnson MP, Elayan I, Hanson GR, Foltz RL, Gibb JW, Lim HK (1992) Effects of 3,4-dihydroxymethamphetamine and 2,4,5-trihydroxymethamphetamine, on central serotonergic and dopaminergic systems. J Pharmacol Exp Ther 261: 447–453

Johnson MP, Conarty PF, Nichols DE (1993) [^{3}H]-Monoamine releasing and uptake inhibition properties of 3,4-methylenedioxymethamphetamine and p-chloroamphetamine analogues. Eur J Pharmacol 200: 9–16

Kapur S, Meyer J, Wilson AA, Houle S, Brown GM (1994) Modulation of cortical neuronal activity by a serotonergic agent: a PET study in humans. Brain Res 646: 292–294

Karoum F, Chrapusta SJ, Egan MF, Wyatt RJ (1993) Absence of 6-hydroxydopamine in the rat brain after treatment with stimulants and other dopaminergic agents: a mass fragmentographic study. J Neurochem 61: 1369–1375

Kramer K, Poblete JCP, Azmitia EC (1995) 3,4-Methylenedioxymethamphetamine ("Ecstasy") promotes the translocation of protein kinase C (PKC): requirement of viable serotonin nerve terminals. Brain Res 680: 1–8

Kuikka JT, Tiihonen J, Bergström KA, Karhu J, Hartikainen P, Viinamäki H, Länsimies E, Lehtonen J, Hakola P (1985) Imaging of serotonin and dopamine transporters in the living human brain. Eur J Nucl Med 22: 346–350

Lauder JM (1990) Ontogeny of the serotonergic system in the rat: serotonin as developmental signal. Ann NY Acad Sci 600: 287–314

Lucot JB, Horwitz J, Seiden LS (1981) The effects of p-chloramphetamine administration on locomotor activity and serotonin in neonatal and adult rats. J Pharmacol Exp Ther 217: 738–744

Mamounas LA, Müllen CA, Ohearn E, Molliver ME (1991) Dual serotoninergic projections to forebrain in the rat – morphologically distinct 5-HT axon terminals exhibit differential vulnerability to neurotoxic amphetamine derivatives. J Comp Neurol 314: 558–586

Mann JJ, Malone KM, Diel DJ, Perel J, Cooper TB, Mintum MA (1996) Demonstration in vivo of reduced serotonin responsitivity in the brain of untreated depressed patients. Am J Psychiatry 153: 174–182

Marker H, Weiss C, Silides DJ, Cohen G (1981) Coupling of dopamine oxidation to glutathione oxidation via the generation of hydrogen peroxide in rat brain homogenates. J Neurochem 36: 589–593

McCann UD, Ricaurte GA (1991a) Lasting neuropsychiatric sequelae of (+)-melthylenedioxymethamphetamine ("Ecstasy") in recreational users. J Clin Psychopharmacol 11: 302–305

McCann UD, Ricaurte GA (1991b) Major metabolites of 3,4-methylenedioxymethamphetamine do not mediate its toxic effects on brain serotonin neurons. Brain Res 545: 279–282

McGuire PK, Cope H, Fahy TA (1994) Diversity of psychopathology associated with use of 3,4-methylenedioxymethamphetamine ("Ecstasy"). Br J Psychiatry 165: 391–395

Mesulam MM (1990) Large-scale neurocognitive networks and distributed processing for attention, language and memory. Ann Neurol 28: 597–613

Meyer JH, Kapur S, Wilson AA, DaSilva JN, Houle S, Brown GM (1996) Neuromodulation of frontal and temporal cortex by intravenous d-fenfluramine: An $[^{15}O]H_2O$ PET study in humans. Neurosci Lett 207: 25–28

Miller DB, O'Callaghan JP (1994) Environment and stress-induced alterations in body temperature affect of the neurotoxicity of substituted amphetamines in the C57BL/6J mouse. J Pharmacol Exp Ther 270: 752–760

Molliver ME, Berger UV, Mamounas LA, Molliver DL, O'Hearn E, Wilson MA (1990) Neurotoxicity of MDMA and related compunds: anatomic studies. Ann NY Acad Sci 600: 640–661

Nash JF (1990) Ketanserin pretreatment blocks MDMA-induced dopamine release in the striatum as measured by in vivo microdialysis. Life Sci 47: 2401–2408

Nash JF, Brodkin J (1991) Microdialysis studies on 3,4-methylenedioxymethamphetamine induced dopamine release: effect of dopamine uptake inhibitors. J Pharmacol Exp Ther 259: 820–825

Nichols DE (1986) Differences between the mechanisms of action of MDMA, MBDB, and the classic hallucinogens. Identification of a novel therapeutic class: entactogens. J Psychoact Drugs 18: 305–313

Nichols DE, Lloyd DH, Hoffman AJ, Nichols MB, Yim GKW (1982) Effects of certain hallucinogenic amphetamine analogues on the release of [^{3}H]serotonin from rat brain synaptosomes. J Med Chem 25: 530–535

O'Hearn E, Battaglia G, De Souza EB, Kuhar MJ, Molliver ME (1988) Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. J Neurosci 8: 2788–2803

Odell SJ, Weihmuller FB, Marshall JF (1991) Multiple methamphetamine injections induce marked increases in extracellular striatal dopamine which correlate with subsequent neurotoxicity. Brain Res 564: 256–260

Okado N, Cheng L, Tanatsugu Y, Hamada S, Hamaguchi K (1993) Synaptic loss following removal of serotonergic fibers in newly hatched and adult chickens. J Neurobiol 24: 687–698

Paris JM, 'Cunningham KA (1992) Lack of serotonin neurotoxicity after intraraphe microinjection of (+)-3,4methylenedioxymethamphetamine. Brain Res Bull 28: 115–119

Park WP, Azmitia EC (1991) MDMA (ecstasy) and nimodipine effects on $^{45}Ca^{++}$-uptake into rat brain synaptosomes. Ann NY Acad Sci 635: 438–442

Passoneau JV, Lauderdale VR (1974) A comparison of three methods of glycogen measurement in tissues. Anal Biochem 60: 405–412

Pentreath VW, Seal LH, Morrison JH, Magistretti PJ (1986) Transmitter mediated regulation of energy metabolism in nervous tissue at the cellular level. Neurochem Int 9: 1–10

Peroutka SJ (1987) Incidence of recreational use of 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) on an undergraduate campus. N Engl J Med 317: 1542–1543

Peroutka SJ, Newman H, Harris H (1988) Subjective effects of 3,4-methylenedioxymethamphetamine in recreational users. Neuropsychopharmacology 1: 273–278

Pierce PA, Peroutka SJ (1988) Ring-substituted amphetamine interactions with neurotransmitter receptor binding sites in human cortex. Neurosci Lett 92: 208–212

Pierce PA, Peroutka SJ (1989) Hallucinogenic drug interactions with neurotransmitter receptor binding sites in human cortex. Psychopharmacol 97: 118–122

Poblete JC, Azmitia EC (1995) Activation of glycogen phosphorylase by serotonin 3,4-methylenedioxymethamphetamine in astroglial-rich primary cultures: involvement of the 5-HT_{2A}-receptor. Brain Res 680: 9–15

Price LP, Ricaurte GA, Krystal JH, Heninger GR (1989) Neuroendocrine and mood response to L-tryptophan in 3,4-methylenedioxymethamphetamine (MDMA) users. Arch Gen Psychiatry 46: 20–22

Rhoades RW, Bennett Clarke CA, Chiaia NL, White FA, Macdonald GJ, Haring JM, Jacquin MF (1990) Development and lesion induced reorganization of the cortical representation of the rat's body surface as revealed by immunocytochemistry for serotonin. J Comp Neurol 293: 190–207

Ricaurte GA, McCann UD (1992) Neurotoxic amphetamine analogues: effects in monkeys and implications for humans. Ann NY Acad Sci 684: 371–382

Ricaurte G, Bryan G, Strauss L, Seiden L, Schuster C (1985) Hallucinogenic amphetamine selectiveley destroys brain serotonin terminals. Science 229: 986–988

Ricaurte GA, Delanney LE, Irwin I, Langston JW (1988a) Toxic effects of MDMA on central serotonergic neurons in the primate: importance of route and frequency of drug adminsitration. Brain Res 446: 165–168

Ricaurte GA, Forno LS, Wilson MA, De Lanney LE, Irwin I, Molliver ME, Langston JW (1988b) MDMA selectively damages central serotonergic neurons in the primate. JAMA 260: 51–55

Ricaurte GA, Finnegan KT, Irwin I, Langston JW (1990) Aminergic metabolites in cerebrospinal fluid of humans previously exposed to MDMA: preliminary observations. Ann NY Acad Sci 600: 699–710

Ricaurte GA, Molliver ME, Martello MB, Katz JL, Wilson MA, Martello AL (1991) Dexfenfluramine neurotoxicity in brains of non-human primates. Lancet 338: 1487–1488

Rudnick G, Wall SC (1992) The molecular mechanisms of ecstasy [3,4-methylenedioxyamphetamine (MDMA)]. Serotonin transporters are targets for MDMA-induced serotonin release. Proc Natl Acad Sci USA 89: 1817–1821

Sanders-Bush E, Bushing JA, Sulser F (1972) p-Chloroamphetamine-inhibition of cerebral tryptophan hydroxylase. Biochem Pharmacol 21: 1501–1510

Schmidt CJ (1987) Neurotoxicity of the psychedelic amphetamine, methylenedioxmethylamphetamine. J Pharmacol Exp Ther 240: 1–7

Schmidt CJ, Ritter JK, Sonsalla PK, Hanson GR, Gibb JW (1985) Role of dopamine in the neurotoxic effects of methamphetamine. J Pharmacol Exp Ther 233: 539–544

Schmidt CJ, Black CK, Abbate GM, Taylor VL (1990a) Chloral hydrate anesthesia antagonizes the neurotoxicity of MDMA. Eur J Pharmacol 191: 213–218

Schmidt CJ, Black CK, Abbate GM, Taylor VL (1990b) MDMA-induced hyperthermia and neurotoxicity are independently mediated by 5-HT_2-receptors. Brain Res 529: 85–90

Schmidt CJ, Taylor VL, Abbate GM, Nieduzak TR (1991) 5-HT_2-antagonists steroselectively prevent the neurotoxicity of 3,4-methylenedioxymethamphetamine, p-chloroamphetamine or methamphetamine to rats. Eur J Pharmacol 203: 41–49

Screaton GR, Singer M, Cairns HS, Thrasher A, Sarner M, Cohen SL (1992) Hyperpryrexia and rhabdomyolysis after MdMA ("Ecstasy") abuse. Lancet 339: 677–678

Seguela P, Watkins KC, Descarries L (1989) Ultrastructural relatinship of serotonin axon terminals in the cerebral cortex of the adult rat. J Comp Neurol 289: 129–142

Seiden JS, Vosmer G (1984) Formation of 6-hydroxydopamine in candate nucleus of rat brain after a single large dose of methamphetamine. Pharmacol Biochem Behav 21: 29–31

Seiden LS, Sabol KE, Ricaurte G (1993) Amphetamine effects on catecholamine systems and behavior. Annu Rev Pharmacol 32: 639–677

Shulgin AT (1986) The background and chemistry of MDMA. J Psychoact Drugs 18: 291–304

Sirvio J, Riekkinen P jr, Jakala P, Riekkinen PJ (1994) Experimental studies on the role of serotonin in cognition. Prog Neurobiol 43: 363–379

Slikker W jr, Holson RR, Ali SF, Kolta MG, Paule MG, Scallet AC, McMillan DE, Bailey JR, Hong JS, Scalzo FM (1989) Behavioural and neurochemical effects of orally adminisstered MDMA in the rodent and nonhuman primate. Neurotoxicology 10: 529–542

Smale L, Michels KM, Morre RY, Morin LP (1990) Destruction of the hamster serotonergic system by 5,7-DHT: effects on circadian rhythm phase, intrainment and response to triazolam. Brain Res 515: 9–19

Spanos LJ, Yamamoto BK (1989) Acute and subchronic effects of methylenedioxymethamphetamine [(+)-MDMA] on locomotion and serotonin syndrome behaviour in the rat. Pharmacol Biochem Behav 32: 835–840

Spoont MR (1992) Modulatory role of serotonin in neural information processing: implications for human psychopathology. Psychol Bull 112: 330–350

Sprague J, Nichols DE (1995) The monoamine oxidase B inhibitor L-deprenyl protects against 3,4-methylenedioxymethamphetamine-induced lipid peroxidation and long-term serotonergic deficits. J Pharmacol Exp Ther 273: 667–673

Sternbach H (1991) The serotonin syndrome. Am Psychiat 148: 705–713

Stone DM, Stahl DC, Hanson GR, Gibb JW (1986) The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxymamphetamine (MDA) on monoaminergic systems in the rat brain. Eur J Pharmacol 128: 41–48

Stone DM, Johnson M, Hanson GR, Gibb JW (1988) Role of endogenous dopamine in the central serotonergid deficits induced by 3,4-methylenedioxymethamphetamine. J Pharmacol Exp Ther 247: 79–87

Stone DM, Hanson GR, Gibb JW (1989) In vitro reactivation of rat cortical tryptophan hydroxylase following in vivo inactivation by MDMA. J Neurochem 53: 572–581

Swanson RA, Choi DW (1993) Glial glycogen stores affect neuronal survival during glucose deprivation in vitro. J Cereb Blood Flow Metab 13: 162–169

Whitaker-Azmitia PM, Murphy R, Azmitia EC (1990) Stimulation of astroglial $5HT_{1A}$ receptors releases the serotonergic growth factor, protein S100, and alters astroglial morphology. Brain Res 528: 155–158

Wilson MA, Ricaurte GA, Molliver ME (1989) Distinct morphologic classes of serotonergic axons in primates exhibit differential vulnerability to the psychotropic drug 3,4-methylenedioxymethamphetamine. Neuroscience 28: 121–137

Wing LL, Tapson GS, Geyer MA (1995) 5-HT-2 mediation of acute behavioral-effects of hallucinogens in rats. Psychopharmacology 100: 417–425