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Neuropharmacology and Neurotoxicity of 3,4-Methylenedioxymethamphetamine

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With the continuing and increasing popularity of the amphetamine analog 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) as a drug of abuse, concern also has increased regarding the long-term psychological and neurochemical effects of this drug. The acute psychological effects of MDMA include a mild sense of euphoria and sense of well being and increased ability to interact with others ***(1)*** which contribute to the drug's popularity. Recent studies in laboratory animals and humans indicate that repeated exposure to MDMA elicits long-term changes in neurochemistry and behavior that are viewed as resulting from a selective neurotoxicity of 5-hydroxytryptamine (5-HT)-containing axon terminals.

1. Acute Neurochemical and Physiological Effects of MDMA

MDMA exerts a pronounced stimulatory effect on the release of 5-HT in the brain, as evidenced under in vitro conditions from rat brain slices and synaptosomes ***(2,3)***, as well as in vivo ***(4,5)***. The MDMA-induced release of 5-HT is most likely a carrier-mediated process that involves the interaction of MDMA with the 5-HT uptake site ***(5,6)***.

It also is well documented that MDMA increases dopamine release in vitro ***(3)*** and in vivo ***(7,8)***. The MDMA-induced release of dopamine involves both carrier-mediated and impulse-dependent processes ***(9,10)***. The contribution of impulse-dependent processes to MDMA-induced dopamine release appears to involve 5-HT neuronal systems, inasmuch as the magnitude of MDMA-induced dopamine release is modulated by 5-HT_2 receptors ***(10–13)***.

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Recently, MDMA also has been shown to increase the release of acetylcholine in striatal slices and in the cortex and hippocampus in vivo ***(14–16)***. Although the MDMA-induced release of acetylcholine in the striatum involves histaminergic mechanisms ***(14)***, the neurotransmitter interactions underlying the ability of MDMA to enhance cholinergic function in the cortex and hippocampus are unknown at present.

The excessive release of dopamine and 5-HT induced by MDMA is thought to mediate many of the physiological and behavioral effects of the drug. MDMA produces a 5-HT_2 receptor-dependent increase in the serum concentrations of the hormones prolactin and corticosterone ***(17)***. MDMA also produces a marked increase in core body temperature, and evidence supports the view that activation of 5-HT_2 receptors contributes to this response ***(17,18)***. Motor function also is enhanced by MDMA. A characteristic 5-HT behavioral syndrome is produced by MDMA ***(19,20)***, and low doses of MDMA induce hyperlocomotion that is mediated, in part, by activation of 5-$HT_{1B/1D}$ receptors ***(21)***.

Recently, the acute effects of MDMA on social function in rats have been investigated. Morley and McGregor ***(22)*** report that MDMA can elicit both anxiogenic and anxiolytic effects that are dependent on the test situation employed. MDMA elicits anxiogenic effects in the elevated plus maze and the emergence test. Anxiolytic effects of acute MDMA administration are evident in the reduction of aggressive behavior and increase in the duration of social interaction ***(22)***.

2. Long-Term Effects of MDMA: 5-HT Neurotoxicity

The single or repeated administration of MDMA consistently has been shown to result in long-term reductions in (1) 5-HT concentrations in multiple brain regions of the rat and nonhuman primate ***(23,24)***, (2) the density of 5-HT uptake sites ***(25)***, (3) the activity of tryptophan hydroxylase ***(26)***, and (4) the density of fine axons of 5-HT neurons ***(27)***. The monoamine-depleting effect of MDMA is selective for 5-HT. Dopamine and norepinephrine concentrations are unaffected by MDMA; an exception is the mouse, in which MDMA depletes dopamine ***(28,29)***. In nonhuman primates and humans exposed to MDMA, data from positron emission tomography and single photon emission computed tomography studies are indicative of MDMA-induced reductions in the density of 5-HT transporters ***(30,31)***.

The effect of MDMA on brain concentrations of 5-HT is biphasic and can be divided into early and late or long-lasting phases. An early, reversible phase of 5-HT depletion occurs within 3–6 h after its administration, after which 5-HT concentrations return to normal values ***(23)***. A long-lasting depletion of 5-HT occurs 2–3 d after drug treatment, and this depletion of 5-HT is evident

in most brain regions containing 5-HT terminals ***(32)***. There is only a partial recovery, at best, to control concentrations of 5-HT after depletion produced by MDMA. In fact, 5-HT concentrations remain depleted in most brain regions up to 1 yr following MDMA administration ***(32,33)***. The fact that 5-HT cell bodies are spared by MDMA may allow for the regeneration of "pruned" 5-HT axons. Indeed, the regeneration of 5-HT axon terminals has been reported following a "neurotoxic" regimen of MDMA, although the pattern of reinnervation is abnormal ***(34)***.

On the basis of the persistence of MDMA effects on 5-HT terminals and the accompanying functional changes (*see* **Subheading 7.**), there is a general concensus that MDMA induces selective 5-HT neurotoxicity, that is, degeneration of 5-HT axon terminals. Nevertheless, there is a lack of consistent histopathological or cytochemical changes that usually accompany neurotoxicity in MDMA-treated rats. Specifically, MDMA induces little increase in silver staining for degenerating neurons ***(35,36)***, and there is little induction of glial fibrillary acidic protein ***(37)***. However, consistent with the view that MDMA produces neurotoxicity, presumably within 5-HT axon terminals, is the finding that MDMA treatment results in a reduction of anterograde axonal transport of labeled material to forebrain regions containing 5-HT innervation ***(38)***. In addition, MDMA promotes the cleavage of the cytoskeletal protein tau in the hippocampus ***(39)***. These findings support the conclusion that MDMA produces structural brain damage in the rodent brain that accompanies the long-term depletion of brain 5-HT.

3. Role of Dopamine in MDMA-Induced Neurotoxicity

The excessive release of dopamine elicited by MDMA is proposed to contribute, in part, to the 5-HT neurotoxicity produced by this drug. A correlation exists between the extent of dopamine release and extent of long-term 5-HT depletion induced by MDMA and structurally related compounds ***(8)***. Moreover, attenuation of the MDMA-induced release of dopamine by the lesioning of neurons ***(40,41)***, treatment with dopamine uptake inhibitors ***(42,43)***, or inhibition of dopamine synthesis ***(26)*** affords protection against MDMA-induced 5-HT neurotoxicity. Furthermore, elimination of dopamine transporters in the striatum with the use of antisense oligonucleotides also prevents the MDMA-induced depletion of striatal 5-HT ***(44)***. Conversely, facilitation of dopamine release with 3,4-dihydroxy-L-phenylalanine (L-DOPA) or 5-HT_2 agonists results in an augmentation of the long-term depletion of 5-HT induced by MDMA ***(12,45)***. The ontogeny of MDMA-induced 5-HT neurotoxicity also appears to be dependent on the ability of MDMA to release dopamine ***(41)***.

The contribution of excessive extracellular dopamine to the process of MDMA neurotoxicity is predicated on the fact that dopamine itself is cytotoxic ***(46,47)***. Along these lines, dopamine, present in excessive synaptic concentrations, may be taken up by an activated 5-HT transporter to initiate dopamine-dependent oxidative processes within the 5-HT terminal ***(48)***.

4. Role of 5-HT and Its Transporter in MDMA-Induced Neurotoxicity

Although a sustained activation of the dopamine transporter and increase in the release of dopamine appear necessary for the expression of MDMA-induced neurotoxicity, it is not sufficient to explain the pattern of neurotoxicity exhibited selectively by MDMA and not by other amphetamines. The acute action of MDMA on 5-HT terminals themselves appears to contribute to the selective depletion of 5-HT produced by this agent. Amphetamine, which increases dopamine release but has little effect on 5-HT release, or 5-methoxy-6-methyl-2-aminoindane (MMAI), which selectively enhances 5-HT release, given alone do not produce 5-HT toxicity ***(49)***. However, the coadministration of MMAI and amphetamine does produce 5-HT depletion. Thus, a concomitant increase in the extracellular concentration of dopamine and activation of the 5-HT transporter appear necessary for 5-HT neurotoxicity.

It can be envisioned that activation of the 5-HT transporter by MDMA renders 5-HT terminals vulnerable to further initiators of toxicity. Indeed, MDMA-induced 5-HT neurotoxicity is prevented in animals in which the 5-HT transporter is inhibited by fluoxetine ***(50,51)***. Actions of MDMA on the 5-HT transporter may be responsible for the generation of reactive oxygen species, i.e., free radicals, or for an increase in intracellular calcium concentrations ***(52)***, both of which could contribute to the process of toxicity.

The direct administration of MDMA into the brain does not result in 5-HT neurotoxicity ***(53–56)***. This has led to the hypothesis that a neurotoxc metabolite of MDMA is formed peripherally. 5-(Glutathion-*S*-yl)-α-methyldopamine is one reactive metabolite of MDMA that has been proposed to mediate its toxicity ***(57,58)***. MDMA undergoes demethylenation to form *N*-methyl-α-methyldopamine. This catechol may undergo further oxidation to form a reactive quinone that reacts readily with glutathione (GSH) to form thioether conjugates of α-methyldopamine. GSH and *N*-acetylcysteine conjugates of α-methyldopamine produce selective 5-HT neurotoxicity when injected directly into the brain ***(57)***. Moreover, inhibition of the breakdown of thioether conjugates of α-methyldopamine augments MDMA-induced 5-HT neurotoxicity, whereas the administration of GSH to reduce the entry of these thioether conjugates into the brain diminishes MDMA-induced neurotoxicity ***(50,59)***.

Thus, MDMA neurotoxicity may result from the peripheral formation and subsequent actions of thioether conjugates of *N*-methyl-α-methyldopamine. The mechanism by which these reactive metabolites of MDMA induce neurotoxicity is unclear but may involve an interaction of the metabolite with the 5-HT transporter and the redox cycling of these compounds to generate reactive oxygen species.

5. Role of Hyperthermia in MDMA-Induced Neurotoxicity

Considerable attention has been given to the role of environmental and/or core body temperature in the long-term neurotoxic effects of MDMA on 5-HT terminals. Maintenance of rats at a cool ambient temperature attenuates the MDMA-induced depletion of brain 5-HT ***(60,61)***. In addition, the magnitude of the thermal response to MDMA is correlated with the extent of MDMA-induced 5-HT neurotoxicity ***(62)***. Importantly, many drugs (e.g., dizolcipine, ketanserin, haloperidol) that afford protection against MDMA-induced 5-HT neurotoxicity attenuate MDMA-induced hyperthermia ***(63,64)***. Thus, it is not possible to distinguish between the roles of specific neurotransmitter receptors and diminished hyperthermia in the neuroprotective actions of these drugs.

Although hyperthermia may contribute to the extent of MDMA toxicity, it is neither necessary nor sufficient for the expression of this toxicity. Thus, MDMA is capable of producing 5-HT neurotoxicity in the absence of drug-induced hyperthermia ***(63)***, as well as under conditions in which MDMA by itself evokes hypothermia ***(62)***. In addition, although MDMA is capable of producing hyperthermia in rats of postnatal age 21 d, there is no long-term depletion of brain 5-HT produced by MDMA in rats at this age ***(41)***.

6. Role of Oxidative and Bioenergetic Stress in MDMA Neurotoxicity

Increasing evidence suggests that MDMA-induced 5-HT neurotoxicity results from increased free radical formation and the subsequent induction of a state of oxidative stress. Support for a free radical hypothesis of MDMA toxicity is based on findings that (1) MDMA increases the formation of free radicals, (2) MDMA produces cellular damage that often accompanies free radical formation, and (3) free radical scavengers and/or antioxidants attenuate MDMA-induced 5-HT neurotoxicity.

Although free radicals are short-lived reactive species, in the presence salicylic acid, a stable adduct, that is, 2,3-dihydroxybenzoic acid (DHBA), is formed that can be quantified analytically. MDMA produces a rapid and sustained increase in the extracellular concentration of 2,3-DHBA in brain ***(43,51,***

65,***66)***. The MDMA-induced generation of hydroxyl radicals appears to be dependent on the activation of both the dopamine and 5-HT transporter ***(43***,***51)***.

There are several potential sources for free radicals generated by MDMA. Dopamine may undergo enzymatic or nonenzymatic oxidation to form superoxide radical and hydrogen peroxide. The importance of superoxide radicals in MDMA neurotoxicity is evident in transgenic mice that overexpress superoxide dismutase, which is responsible for the degradation of superoxide radicals. These transgenic mice are resistant to MDMA-induced neurotoxicity ***(67)***. Alternatively, metabolites of MDMA may serve as sources of free radicals. Quinone thioethers described in the preceding section have the capability of redox cycling to produce reactive oxygen species.

Reactive species that contribute to MDMA toxicity may not be limited to oxygen-based radicals but also may include reactive nitrogen species, for example, nitric oxide and peroxynitrite. Inhibitors of nitric oxide synthase (NOS) provide protection against MDMA-induced dopamine depletion in the mouse ***(68)***, as well as 5-HT depletion in the rat ***(69***,***70)***. However, it has not been possible to ascribe the neuroprotective effects of these drugs specifically to the inhibition of NOS, inasmuch as NOS inhibitors, for the most part, markedly attenuate MDMA-induced hyperthermia ***(69)***. However, the NOS inhibitor *S*-methyl-L-thiocitrulline attenuates MDMA-induced dopamine toxicity in the mouse without modifying MDMA-induced hyperthermia ***(68)***. *S*-Methyl-L-citrulline also attenuates the long-term depletion of 5-HT, as well as dopamine, in the striatum of the rat following the intrastriatal administration of MDMA and malonate (Gudelsky, *unpublished observations*).

The exact mechanism whereby MDMA increases the formation of reactive nitrogen species is unknown, but it is unlikely to involve an increase in the release of glutamate ***(71)*** or glutamate receptor stimulation ***(68)***. Conceivably, treatment with MDMA may produce an increase in intracellular calcium through impairment of cellular energetics ***(61)***, disruption of mitochondrial function ***(72)***, or activation of protein kinase C ***(52)*** that ultimately results in the activation of NOS. Regardless of the nature of the reactive oxygen or nitrogen species, the importance of the 5-HT transporter itself in the generation of reactive oxygen or nitrogen species is underscored by the finding that MDMA-induced free radical formation is absent in rats treated with fluoxetine ***(51)*** or in rats in which 5-HT terminals have been disrupted by fenfluramine ***(65)***.

The administration of MDMA also results in cellular damage or changes consistent with the induction of oxidative stress. MDMA increases the formation of malondialdehyde-related substances that are indicative of free radical-induced lipid peroxidation ***(73***,***74)***. MDMA also increases the formation of nitro-tyrosine residues (Yamamoto, *unpublished observations*) that is consistent with nitric oxide- or peroxynitrite-induced protein nitration. Finally, a

neurotoxic regimen of MDMA depletes brain concentrations of the endogenous antioxidants vitamin E and ascorbic acid ***(75)***.

The contribution of free radical-induced damage in the process of MDMA neurotoxicity is inferred from the findings that the administration of antioxidants ***(50,59,75)*** or spin trap agents ***(76,77)*** prevents the MDMA-induced depletion of brain 5-HT. Furthermore, as discussed previously, overexpression of superoxide dismutase renders transgenic mice resistant to MDMA-induced dopamine toxicity ***(67)***.

In addition to a role of oxidative stress in MDMA-induced neurotoxicity, alterations in energy metabolism also may contribute to the process of neurotoxicty induced by psychostimulant drugs. Methamphetamine reduces brain concentrations of ATP ***(78)*** and increases the extracellular concentration of lactate ***(79)***. In addition, the administration of energy substrates attenuates dopamine neurotoxicity elicited by methamphetamine ***(79,80)***. These findings suggest that psychostimulants may acutely impair mitochondrial function. Indeed, methamphetamine and MDMA acutely inhibit the activity of the mitochondrial enzyme cytochrome oxidase ***(72)***. Furthermore, the combined administration of methamphetamine and malonate, a complex II inhibitor of mitochondrial function, synergize to deplete striatal dopamine concentrations ***(81,82)***. The intrastriatal administration of malonate and MDMA, neither of which alone depletes tissue 5-HT concentrations, together produces significant reductions in striatal 5-HT and dopamine concentrations ***(55)***. These data suggest a role for bioenergetic stress in the long-term effects of MDMA on 5-HT, and possibly dopamine, nerve terminals.

MDMA, as well as methamphetamine and parachloroamphetamine, appear to disrupt cellular energetics by further promoting glycogenolysis ***(61,83)***. MDMA and methamphetamine produce a transient decrease in brain glycogen concentrations that may involve the 5-HT_2 receptor-dependent activation of glycogen phosphorylase ***(84)***. MDMA also produces a sustained elevation in the extracellular concentration of glucose in the brain ***(61)***. The increased extracellular concentration of glucose may be indicative of increaesd regional cerebral blood flow and glucose utilization. This ability of MDMA to promote glycogenolysis is associated with the hyperthermia produced by MDMA ***(61)***. Thus, the involvement of hyperthermia in the process by which MDMA depletes energy stores may be the same mechanism through which hyperthermia exacerbates MDMA-induced 5-HT neurotoxicity.

7. Functional Consequences of MDMA Neurotoxicity

Although the long-term effects of MDMA on 5-HT axon terminals have been well documented, the potential functional consequences associated with MDMA-induced 5-HT depletion have been investigated only recently. It is

known that the administration of a 5-HT depleting regimen of MDMA results in diminished behavioral, thermal, neurochemical, and neuroendocrinological responses to a subsequent administration of MDMA or other 5-HT releasing drugs ***(20,85–87)***. These diminished responses appear to be due to a reduced amount of evoked 5-HT release ***(20,88)*** in animals treated previously with a neurotoxic regimen of MDMA.

However, alterations in 5-HT receptor sensitivity induced by high-dose MDMA administration also may underlie abnormal responses to 5-HT agonists or releasing agents ***(89)***. The repeated administration of MDMA increases the density of 5-HT_{1A} receptors in the frontal cortex and augments the hypothermic response produced by the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin hydrobromide (8-OH-DPAT) ***(90)***. Prior MDMA treatment also results in an increased serum prolactin response to fenfluramine ***(87)***.

In addition to impaired or abnormal responses evoked by pharmacological agents in MDMA-treated rats, numerous physiological responses also are rendered abnormal by prior, repeated MDMA administration. For example, rats treated with a 5-HT depleting regimen of MDMA exhibit reduced diurnal and nocturnal locomotor activity ***(91)***. Furthermore, thermoregulation in rats is disrupted by the repeated administration of MDMA. This is evidenced by exaggerated hyperthermia in response to an elevated environmental temperature ***(92,93)***. Neurotransmitter responses to stress also are altered in rats given MDMA repeatedly. Restraint stress results in increased extracellular concentrations of 5-HT and dopamine in the frontal cortex and hippocampus; these responses are diminished in MDMA-treated rats ***(94)***.

The repeated administration of MDMA also disrupts cycles of sleep/wakefulness in the rat. According to Jones ***(95)***, 5-HT facilitates the transition from slow-wave sleep to REM sleep and inhibits wakefulness. Therefore, it follows that a depletion of 5-HT may increase wakefulness. Consistent with this hypothesis, **Fig. 1** shows that rats pretreated with neurotoxic doses of MDMA exhibit increased bout lengths of wakefulness and increased overall time spent in wakefulness but do not exhibit any significant changes in REM or slow wave sleep (**Fig. 2**).

Behaviors related to anxiety and learning and memory also are abnormal in MDMA-treated animals, although some of these data are conflicting. Morley et al. ***(96)*** report that rats given a neurotoxic regimen of MDMA exhibit greater anxiety-related behaviors, as assessed in an elevated plus maze and social interaction and emergence tests. In contrast, Mechan et al. ***(97)*** conclude that MDMA-treated rats display a reduction in anxiety-related behaviors.

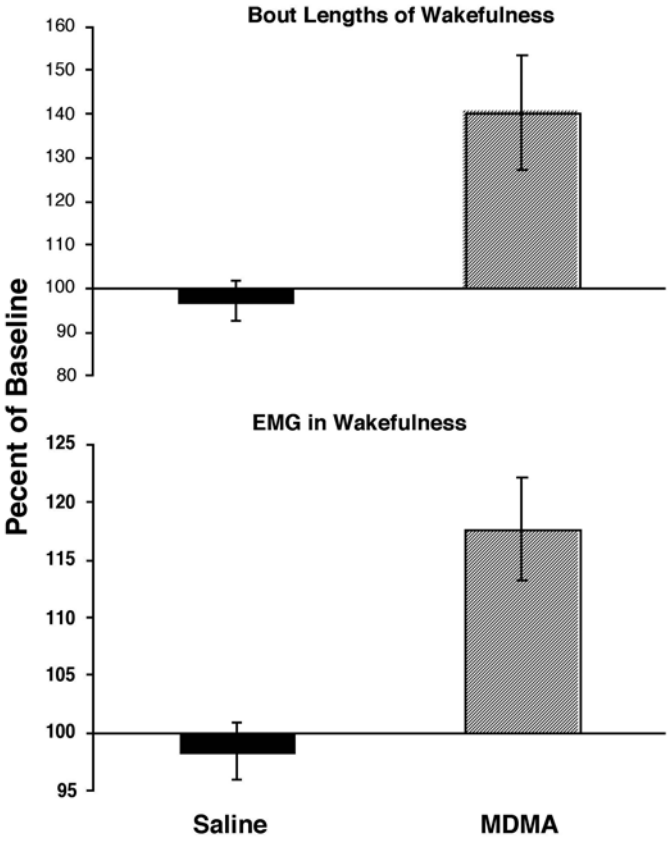

Fig. 1. Baseline EEG and neck EMG recordings were taken for 2 d prior to injections of saline or MDMA (10 mg/kg, i.p. every 2 h for a total of four injections). The data presented are those from recordings obtained 7 d after MDMA treatment and are expressed as percent of the baseline recordings taken prior to MDMA treatment.

Differences in the strain of rat used in these studies may account for differences in behavior.

Importantly, reduction in social interaction in MDMA-treated rats may not be related to depletion of brain 5-HT. Social interaction is reduced in rats treated with a moderate dosage regimen of MDMA that does not produce 5-HT neurotoxicity ***(96)*** and in rats treated on postnatal d 39 ***(98)***, at which time rats are insensitive to 5-HT neurotoxicity induced by MDMA ***(41**,**62)***. Thus,

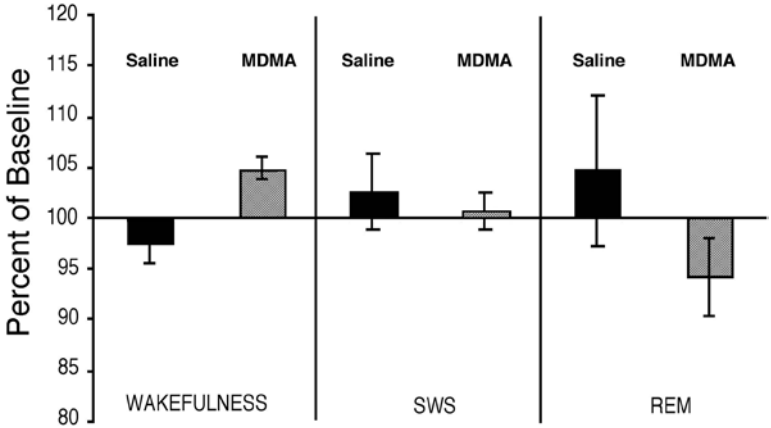

Fig. 2. Treatment of rats and recordings are the same as described in the legend for Fig. 1. The duration of time spent in wakefulness, slow-wave sleep (SWS), and REM sleep (REM) is plotted as a percentage of values obtained prior to drug treatment.

MDMA may induce long-term alterations in behavior that are unrelated to the long-term depletion of brain 5-HT.

Treatment of adult or neonatal rats with MDMA also results in impairments in learning and memory, and these effects also may be unrelated to long-term deficits in brain 5-HT. In adult rats, MDMA treatment results in deficits in object recognition ***(96)***. The administration of MDMA on postnatal d 11–20 results in deficits in tests of sequential learning and spatial learning and memory when rats are tested as adults, and these deficits are evident in the absence of significant depletions in brain 5-HT ***(99)***.

8. Summary

The existing data indicate that MDMA produces long-term deficits in markers of 5-HT axon terminals in the rodent brain. Increased cleavage of the cytoskeletal protein tau, impairment of axonal transport, and functional consequences associated with a 5-HT depleting regimen of MDMA support the view that MDMA induces structural brain damage, that is, axonal degeneration. A confluence of oxidative stress and bioenergetic stress induced by MDMA is hypothesized to underlie the process of MDMA neurotoxicity (**Fig. 3**). The actions of MDMA on the 5-HT transporter to promote free radical formation and/or intracellular calcium may synergize with MDMA-induced disturbances in cellular energetics and hyperthermia to effect selective toxicity to 5-HT axon terminals.

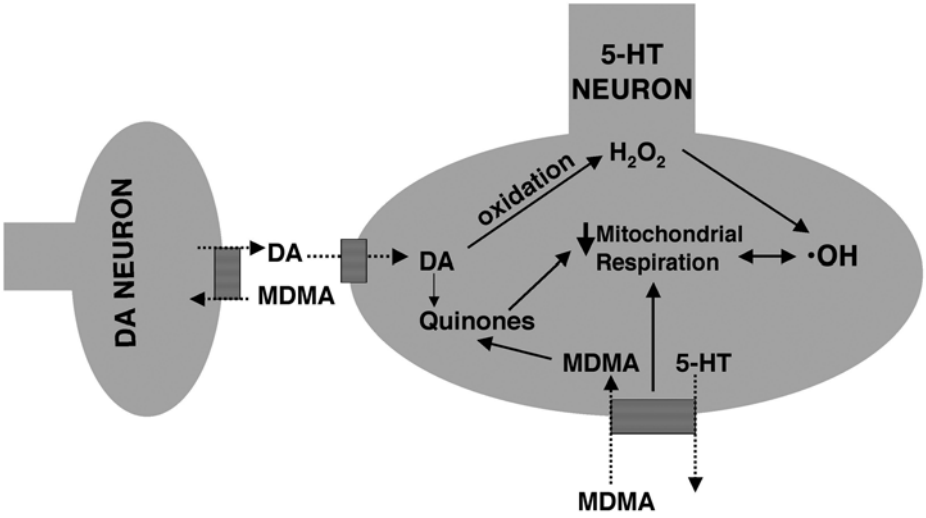

Fig. 3. Hypothetical model of the mechanism of MDMA-induced 5-HT neurotoxicity. The convergence of oxidative stress and energetic stress in the mechanism of MDMA neurotoxicity is depicted in which (1) MDMA (or a toxic metabolite)-induced activation of the 5-HT transporter facilitates free radical formation and energy depletion which act in concert to (2) promote mitochondrial impairment and (3) further generate free radicals and the cellular toxicity associated with these processes.

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