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# THE EFFECTS OF METHYLENEDIOXYMETHAMPHETAMINE (MDMA, "ECSTASY") ON MONOAMINERGIC NEUROTRANSMISSION IN THE CENTRAL NERVOUS SYSTEM

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**Abstract**—Methylenedioxymethamphetamine (MDMA, Ecstasy) is a popular recreationally used drug among young people in Europe and North America. The recent surge in use of MDMA and increasing concerns about possible toxic effects of the drug have inspired a great deal of research into the mechanisms by which the drug may affect the central nervous system. This paper reviews studies on the neurochemical, behavioral and neurophysiological effects of MDMA, with emphasis on MDMA effects in regions of the brain that have been implicated in reward.

Experiments in awake, behaving laboratory animals have demonstrated that single injections of MDMA increase extracellular levels of the neurotransmitters dopamine (DA) and serotonin (5HT) in the nucleus accumbens and in several other brain regions that are important for reward. Most of the behavioral and electrophysiological changes that have been reported to date for single doses of MDMA appear to be mediated by this MDMA-induced increase in extracellular DA and 5HT. As an example, MDMA-induced hyperthermia and locomotor hyperactivity in laboratory animals can be blocked by administering drugs that prevent MDMA-induced 5HT release and can be attenuated by administering 5HT receptor antagonists, whereas effects of MDMA on delayed reinforcement tasks appear to be mediated by MDMA-induced increases in extracellular DA. Similarly, the effects of MDMA on neuronal excitability in the nucleus accumbens and in several other brain regions can be prevented by administering drugs that block MDMA-induced 5HT release and can be attenuated by depleting brain DA levels or by administering either DA $D_1$ receptor antagonists or 5HT receptor antagonists.

In addition to the acute effects of MDMA, it is now well established that repeated or high-dose administration of MDMA is neurotoxic to a subpopulation of 5HT-containing axons that project to the forebrain in laboratory animals. Recent studies have shown that this neurotoxic effect of MDMA is associated with long-duration changes in both DA and 5HT neurotransmission in the nucleus accumbens. Whether these long-duration changes in neurotransmission might be related to reports of depression and other psychopathologies by some frequent users of MDMA remains to be determined. Methylenedioxymethamphetamine has been found to increase extracellular levels of norepinephrine and to alter brain levels of several neuropeptides as well as altering levels of DA and 5HT. Much additional research is required to understand the multiple ways in which this complex drug may alter neurotransmission in the brain, both acutely and in the long term. Copyright © 1996 Elsevier Science Ltd.

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## ABBREVIATIONS

| | |
|---|---|
| AMPT | α-Methyl-*p*-tyrosine |
| CCK | Cholecystokinin |
| CP | Caudate/putamen |
| CPBG | 1-(m-Chlorophenyl)-biguanide |
| CuZnSOD | Copper–zinc superoxide dismutase |
| DA | Dopamine |
| DOI | 1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane |
| EPSPs | Excitatory postsynaptic potentials |
| FLX | Fluoxetine |
| GABA | γ-Aminobutyric acid |
| HPLC | High-pressure liquid chromatography |
| 6OHDA | 6-Hydroxydopamine |
| 8-OHDPAT | 8-Hydroxy-(2-di-*n*-propylamine)tetralin |
| 5HIAA | 5-Hydroxyindolacetic acid |
| 5HT | 5-Hydroxytryptamine |
| IPSPs | Inhibitory postsynaptic potentials |
| KET | Ketanserin |
| mPFC | Medial prefrontal cortex |
| MDMA | Methylenedioxymethamphetamine |
| 2M5HT | 2-Methyl-5-hydroxytryptamine |
| MTSG | Methysergide |
| NaCl | Sodium chloride |
| NE | Norepinephrine |
| NMDA | *N*-methyl-D-aspartate |
| PCPA | *p*-Chlorophenylalanine |
| TH | Tyrosine hydroxylase |
| VTA | Ventral tegmental area |
| WAY | WAY100135 |

## 1. INTRODUCTION

Methylenedioxymethamphetamine (MDMA, "Ecstasy") is a legally restricted amphetamine derivative (Fig. 1) that has become increasingly popular in Europe and North America over the last 10 years because of its euphoria-inducing and mild stimulant properties (Cregg and Tracey, 1993; Cuomo *et al.*, 1994; Green *et al.*, 1995; McKenna and Peroutka, 1990; Steele *et al.*, 1994). MDMA (Ecstasy) is used primarily by young people in large dance and music settings, but sometimes it is used in small social gatherings (Green *et al.*, 1995; Steele *et al.*, 1994). Although MDMA often is described as a relatively harmless drug (for example, note the entries on the MDMA home page on the Internet), acute toxic reactions including malignant hyperthermia, convulsions, hepatitis and death have been attributed to MDMA use by young people (Dykhuizen *et al.*, 1995; Green *et al.*, 1995; Khakoo *et al.*, 1995; O'Connor, 1994; Randall, 1992).

Adding to concerns about possible dangers to humans, repeated injections of low doses of MDMA produce long-lasting damage to a selective population of serotonin-containing axons that project to the forebrain in laboratory rats and monkeys (Battaglia *et al.*, 1991; Mamounas *et al.*, 1991; O'Hearn *et al.*, 1988; Ricaurte *et al.*, 1985; Ricaurte *et al.*, 1988). The doses that produce this damage in monkeys are close to those that are used by humans and there is suggestive evidence that people who have repeatedly used MDMA also may suffer damage to serotonin neurons in the central nervous system (McCann *et al.*, 1994; Ricaurte *et al.*, 1990; Ricaurte and McCann, 1992). High doses of MDMA cause damage to forebrain dopamine-containing axons as well as serotonin-containing axons in laboratory rats, and appear to damage selectively only dopamine-containing axons in mice (Cadet *et al.*, 1995; Logan *et al.*, 1988; Stone *et al.*, 1987), raising the possibility that dopaminergic neurons may also be at risk in humans that repeatedly ingest MDMA. Clinical reports of psychopathology including panic attacks, depression, flashbacks and psychosis that occur days to months following the last MDMA ingestion suggest that MDMA may, indeed, produce long-term changes in neurotransmission in humans as well as laboratory animals (Creighton *et al.*, 1991; McCann and Ricaurte, 1991; McGuire *et al.*, 1994; Pallanti and Mazzi, 1992; Schifano and Magni, 1994).

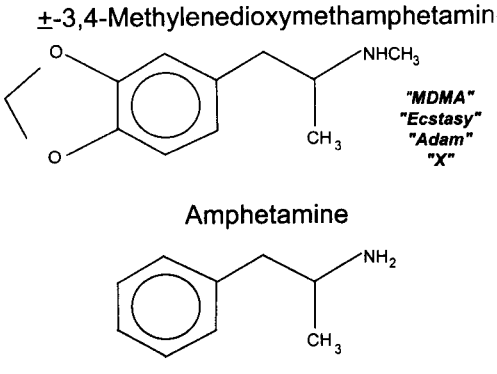


Fig. 1. Chemical structures for MDMA and amphetamine. Popular names for MDMA are Ecstasy, Adam and X.

The recent surge in popularity of illicit use of MDMA and the increasing reports of toxic reactions in humans have inspired a great deal of research on the mechanisms by which MDMA might affect central nervous system functioning. It is now well established that administration of single doses of MDMA to laboratory animals induces acute increases in extracellular levels of the monoamines serotonin (5HT), dopamine (DA) and norepinephrine (NE) in several brain regions (Berger *et al.*, 1992; Fitzgerald and Reid, 1990; Gough *et al.*, 1991; Johnson *et al.*, 1986; Nash and Brodkin, 1991; Nichols *et al.*, 1982; Schmidt *et al.*, 1987; Yamamoto and Spanos, 1988). This observation and the evidence that multiple doses of MDMA may be neurotoxic to serotonin-containing and perhaps dopamine-containing axons suggest that MDMA may produce complex and widespread changes in neurotransmission in the brain that may vary depending upon dose and frequency of exposure to MDMA. The purpose of this paper is to review recent laboratory findings about the neurochemical, behavioral and neurophysiological effects of MDMA and to discuss the extent to which the mood-altering effects of MDMA might be attributable to alterations in monoaminergic

neurotransmission in brain regions that are considered to be part of the neural circuitry mediating the rewarding properties of abused drugs.

## 2. ACUTE MDMA, NEUROCHEMICAL CHANGES

### 2.1. Monoamine Release and Re-Uptake *In Vitro*

Nichols *et al.* (1982) first demonstrated that bath application of MDMA induced release of [$^3$H]5HT from synaptosomes prepared from whole rat brain. This observation was confirmed subsequently by several laboratories and extended to DA release (Schmidt *et al.*, 1987; McKenna *et al.*, 1991), although MDMA is a more potent releaser of 5HT than DA *in vitro*. MDMA released 5HT from striatal slices at concentrations about 10-fold lower than those that were required to stimulate DA release (Schmidt *et al.*, 1987). MDMA appears to interact with the 5HT transporter to cause 5HT release because MDMA-induced 5HT release from brain synaptosomes, hippocampal and brainstem slices, membrane vesicles and fetal 5HT neurons in culture can be blocked by fluoxetine or imipramine which are inhibitors of the 5HT transporter (Berger *et al.*, 1992; Fitzgerald and Reid, 1990; Gu and Azmitia, 1993; Hekmatpanah and Peroutka, 1990; McKenna *et al.*, 1991; Rudnick and Wall, 1992; Sprouse *et al.*, 1989). The carrier-mediated release of 5HT and DA from brain slices by MDMA is $Ca^{++}$ independent because release of 5HT is not inhibited by removal of $Ca^{++}$ from the superfusion solution (Schmidt *et al.*, 1987). In addition to stimulating 5HT and DA release, MDMA has been shown to induce NE release from hippocampal slices (Fitzgerald and Reid, 1990). Presumably MDMA interacts with the NE transporter to release NE because NE release was blocked by desmethylimipramine, a specific inhibitor of the NE transporter (Fitzgerald and Reid, 1990). Since MDMA does not appear to accumulate within synaptosomes (Wang *et al.*, 1987), it is thought to induce monoamine release by interacting with the monoamine carriers to reverse the direction of neurotransmitter flow (Hekmatpanah and Peroutka, 1990).

In addition to stimulating 5HT, DA and NE release, MDMA can also increase extracellular levels of all three monoamines by inhibiting re-uptake (Berger *et al.*, 1992; Johnson *et al.*, 1991a; Steele *et al.*, 1987) and by delaying metabolism through inhibition of monoamine oxidase (Gu and Azmitia, 1993; Kokotos Leonardi and Azmitia, 1994). Ecstasy also causes an acute stimulation of striatal dopamine synthesis that is mediated by $5HT_2$ receptors (Schmidt *et al.*, 1991). These observations suggest that a major mechanism by which MDMA may acutely affect neuronal excitability in the brain is by increasing extracellular levels of serotonin and catecholamines and thereby indirectly activating 5HT, DA and NE receptors. However, changes in neuropeptide neurotransmission also may mediate some of the effects of MDMA. Single injections of low doses of MDMA have been shown to increase both neurotensin-like immunoreactivity and dynorphin-like immunoreactivity measured 18 hr post-injection in homogenates of neostriatum, nucleus accumbens and substantia nigra (Johnson *et al.*, 1991b). These changes appeared to be mediated by both dopaminergic and glutaminergic systems because they could be blocked by the dopamine $D_1$ receptor antagonist SCH 23390 and by the *N*-methyl-D-aspartate (NMDA) antagonist MK-801 (Johnson *et al.*, 1991b).

### 2.2. Extracellular Monoamine Levels *In Vivo*

Advances in microdialysis and in *in vivo* voltammetry techniques have allowed investigators to examine directly the effects of MDMA administration on extracellular levels of monoamines in awake, behaving animals. These studies have revealed that MDMA produces a marked increase in extracellular DA in regions of the brain that are innervated richly by DA-containing axon terminals. Most dialysis studies of MDMA to date have been conducted in laboratory rats with the dialysis probe inserted in the striatum (caudate nucleus and putamen), a structure that is part of the classical "extrapyramidal" motor system, but that also may play a role in cognitive and motivated behaviors. Single systemic injections of MDMA increased extracellular levels of DA in the striatum of awake, behaving rats as measured by high-pressure liquid chromatography (HPLC) analysis of dialysate samples (Gough *et al.*, 1991; Schmidt *et al.*, 1994; Yamamoto *et al.*, 1995). Systemic injection of MDMA also increased extracellular levels of 5HT in the striatum, but the 5HT increase was much less than that of DA (Gough *et al.*, 1991). Pretreatment of the rats with specific DA uptake blockers prevented the increase in extracellular DA that was otherwise produced by infusion of MDMA directly into the striatum via the dialysis probe (Nash and Brodkin, 1991). This suggests that MDMA increases DA in part via a DA transporter-mediated mechanism which is independent of its effects on 5HT release (Nash and Brodkin, 1991). However, systemic injections of $5HT_2$ receptor antagonists or infusion of the antagonists directly into the striatum through the dialysis probe attenuated MDMA-induced DA release (Nash, 1990; Schmidt *et al.*, 1994; Yamamoto *et al.*, 1995), indicating that DA efflux produced by MDMA is regulated partially by 5HT receptors.

The nucleus accumbens of the forebrain is a brain region that has been particularly implicated in the rewarding properties of abused drugs (Koob, 1992; Wise and Bozarth, 1984). Drugs including opiates, alcohol, amphetamine and cocaine have the common property of increasing extracellular concentrations of DA (and also of 5HT) in this nucleus in freely moving rats (Di Chiara and Imperato, 1988; Hernandez *et al.*, 1987; Yoshimoto *et al.*, 1991). Similarly to the other abused drugs, systemic injection of MDMA increased extracellular DA in the nucleus accumbens measured by *in vivo* voltammetry (Yamamoto and Spanos, 1988). A recent study from our laboratory confirmed and extended this finding by demonstrating that MDMA infused directly into the nucleus accumbens through the dialysis probe significantly increased extracellular levels of both DA and 5HT in awake, behaving rats (White *et al.*, 1994). Thus, MDMA can

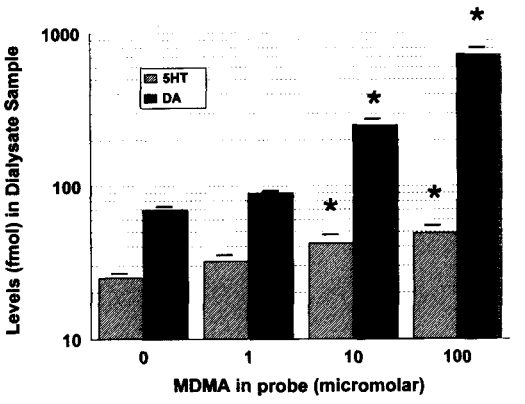


Fig. 2. MDMA infused through a dialysis probe into the nucleus accumbens of awake rats produced a dose-dependent increase in levels of 5HT and DA in dialysate samples. The histograms indicate the mean monoamine concentration in 20 min dialysis samples taken 60–80 min following the onset of MDMA infusion and the bars indicate the SEM. The numbers of animals tested with each dose ranged from 6 to 14. The 10 and 100 μM doses of MDMA produced statistically significant increases in 5HT and DA levels above the basal (0 dose) level. Note that the $y$-axis is a log scale. The maximal 5HT increase produced by MDMA was about double the pre-MDMA basal level, whereas the maximal DA increase was about 10-fold higher than the basal level. *Significant increase ($p < 0.01$) from basal levels, paired $t$-test, two-tailed with Bonferonni adjustments for multiple comparisons (modified from White *et al.*, 1994).

act directly upon terminals within the nucleus accumbens to increase extracellular DA and 5HT. As was reported previously for the striatum (Gough *et al.*, 1991), the magnitude of increase was much greater for DA than for 5HT (Fig. 2). However, the DA release may have been mediated at least partially by MDMA-induced 5HT release because it is well established that infusion of 5HT or 5HT agonists directly into the nucleus accumbens or the striatum through the dialysis probe increases extracellular levels of DA measured in dialysate samples (Benloucif and Galloway, 1991; Blandina *et al.*, 1989; Galloway *et al.*, 1993; Jacocks and Cox, 1992; Parsons and Justice, 1993a).

## 3. REPEATED MDMA, NEUROCHEMICAL CHANGES

Repeated systemic administration of MDMA to laboratory rats, guinea-pigs or monkeys produces long-lasting decreases in neurochemical and histological indices of serotonin function in the forebrain. MDMA reduces forebrain tissue levels of 5HT and its metabolite 5-hydroxyindolacetic acid (5HIAA) and depresses the activity of tryptophan hydroxylase, the synthetic enzyme for 5HT (Schmidt *et al.*, 1987; Stone *et al.*, 1988). These deficits can persist from weeks to more than a year following multiple doses of MDMA depending upon the brain area, the species studied and the doses of MDMA used (Commins *et al.*, 1987; Fischer *et al.*, 1995; Ricaurte and McCann, 1992; Scanzello *et al.*, 1993). Uptake sites for 5HT in the cerebral cortex, caudate nucleus, hippocampus, nucleus accumbens, olfactory tubercle and many thalamic nuclei also are reduced markedly as measured by quantitative *in vitro* autoradiography of [$^3$H]-paroxetine binding in rats after repeated systemic injections of MDMA (Battaglia *et al.*, 1991). The MDMA-induced decrease in 5HT uptake sites occurred in most brain regions within 24 hr of the last dose and persisted for at least 2 weeks. The decrease in uptake sites appeared to be specific for the serotonin system in the rat because [$^3$H] mazindol binding (which labels catecholamine uptake sites) was unaffected by multiple injections of MDMA at doses lower than 20 mg/kg (Battaglia *et al.*, 1991). However, repeated injections of MDMA at doses of 40 mg/kg or higher decrease NE and DA levels in guinea-pigs (Commins *et al.*, 1987). Single microinjections of MDMA directly into the dorsal or median raphe nuclei which contain the 5HT cell bodies that send projections to the forebrain do not change serotonin or catecholamine levels in the hippocampus or striatum of rats measured 2 weeks post-injection (Paris and Cunningham, 1992). This suggests that MDMA may act at the axon terminals to produce damage. However, effects of repeated microinjections of MDMA into the raphe nuclei have not been examined.

Neurotoxic effects of MDMA in primates are more pronounced than in rats (Ricaurte and McCann, 1992). Serotonin depletion and decreases in 5HT uptake sites in the cerebral cortex of monkeys occur at lower doses of repeated MDMA than for rats, the maximal effect of MDMA on 5HT depletion is greater in monkey than rat, and the depletion of 5HT levels and 5HT uptake sites persists for longer periods of time post-injection in monkeys. In most rats, 5HT and 5HIAA levels and paroxetine-labeled 5HT uptake sites showed nearly complete recovery by 32 weeks (Scanzello *et al.*, 1993), but decreases in these 5HT markers persisted at 18 months in neocortex, hippocampus and striatum of monkeys and may be permanent (Ricaurte and McCann, 1992; Fischer *et al.*, 1995). Some neuronal cell bodies in the dorsal raphe nucleus appear to be damaged in squirrel monkeys following repeated MDMA administration, whereas the cell bodies are spared in rats, perhaps allowing for more successful axonal sprouting and reinnervation (Ricaurte and McCann, 1992). In both rats and monkeys, the hypothalamus becomes abnormally hyperinnervated by serotonin terminals by 52–72 weeks following repeated injections of MDMA (Fischer *et al.*, 1995).

Immunohistochemical studies indicate that repeated injections of MDMA appear to cause selective degeneration of fine-diameter serotoninergic axons with small varicosities that arise from the dorsal raphe nucleus and project to the forebrain. Forebrain serotonin-containing axons with large round varicosities and small intervaricose segments ("beaded" fibers) that arise predominantly from the median raphe nuclei appear to be spared by MDMA (O'Hearn *et al.*, 1988; Mamounas *et al.*, 1991; Wilson *et al.*, 1989). Histological evidence of serotonin nerve terminal destruction includes extreme swelling and abnormally shaped and fragmented 5HT-immunoreactive axon terminals at 1–3 days after multiple MDMA injections followed by a persistent reduction

in the density of fine-diameter 5HT-containing fibers in the forebrain that lasts for many weeks in rats and may be permanent in some brain regions in monkeys (Wilson *et al.*, 1993; Fischer *et al.*, 1995).

Although the precise mechanisms by which MDMA causes damage to 5HT axons are not known, DA appears to play a major role in the neurotoxic effects of MDMA. Acute depletion of DA by pretreatment with α-methyl-*p*-tyrosine or reserpine attenuated MDMA-induced DA efflux in the striatum and prevented subsequent decreases of 5HT uptake sites and tryptophan hydroxylase activity (Brodkin *et al.*, 1993; Stone *et al.*, 1988). $5HT_2$ receptor antagonists also prevent MDMA-induced acute stimulation of DA synthesis and prevent long-lasting deficits in forebrain 5HT concentrations (Schmidt *et al.*, 1991). It has been proposed by Schmidt *et al.* (1991) that in the absence of $5HT_2$-mediated enhanced synthesis of DA, the DA neuron cannot sustain the carrier-mediated DA release which is essential for the development of MDMA-induced neurotoxicity. Similarly, $5HT_2$ receptor agonists administered together with MDMA, potentiate the MDMA-induced DA release and increase the toxic effect of MDMA on 5HT levels measured 7 days post-administration (Gudelsky *et al.*, 1994).

Sprague and Nichols (1995) suggest that deamination of excessive DA that has been taken up by 5HT terminals generates hydrogen peroxide that may lead to membrane lipid peroxidation and perhaps other oxidative insults, resulting in 5HT terminal degeneration after MDMA administration. Deprenyl, a monoamine oxidase-B inhibitor protects against MDMA-induced lipid peroxidation and long-term decreases in 5HT, 5HIAA levels and 5HT uptake sites. The MDMA-induced depletion of 5HT from the terminals may increase vulnerability to excess DA accumulation after DA has been released by MDMA. 5HT release and depletion alone do not lead to serotonin terminal degeneration. Instead, repeated exposure to MDMA may produce the following sequence of events as proposed by Sprague and Nichols (1995): MDMA depletes 5HT from serotonin neurons which renders them vulnerable to the toxic process; MDMA dramatically increases DA synthesis and release; the DA from the increased extracellular pool is transported into depleted 5HT terminals by the 5HT uptake carrier where it is deaminated by MAO-B to generate hydrogen peroxide; this hydrogen peroxide then leads to lipid peroxidation and perhaps other oxidative insults and selective 5HT axonal degeneration. Recent studies in transgenic mice suggest that oxidative insults may, indeed, mediate MDMA-induced neuronal toxicity (Cadet *et al.*, 1995). In mice, MDMA is neurotoxic to DA rather than 5HT axons (Logan *et al.*, 1988; Stone *et al.*, 1987), and Cadet *et al.* (1995) confirmed this observation by showing that in non-transgenic mice, a single high dose of MDMA produced DA depletion in the striatum at 24 days and 2 weeks. However, in homozygous transgenic mice that carried the complete sequence of the human copper–zinc superoxide dismutase (CuZnSOD) gene, MDMA produced no DA depletion at either time point. The increased cytosolic levels of the antioxidant CuZnSOD appeared to protect the DA axons of the transgenic mice against damage by oxygen-based free radicals (Cadet *et al.*, 1995).

Excitatory amino acids also have been implicated in MDMA-induced neurotoxicity because systemic injections of dizocilpine and other excitatory amino acid antagonists protect against the neurotoxic effects of systemic injection of MDMA on 5HT terminals in the neocortex and hippocampus (Colado *et al.*, 1993; Farfel *et al.*, 1992). However, systemic injections of MDMA, unlike injections of methamphetamine, do not increase glutamate in the striatum of awake, freely moving rats as measured by *in vivo* microdialysis (Nash and Yamamoto, 1992). Therefore, forebrain depletion of 5HT content is probably not due to an excitotoxic effect of excess extracellular glutamate released by MDMA. The excitatory amino acid antagonists may prevent MDMA-induced neurotoxicity by preventing MDMA-induced hyperthermia (Farfel and Seiden, 1995). Dizocilpine, which ordinarily protects against both MDMA-induced hyperthermia and MDMA-induced 5HT neurotoxicity, does not protect 5HT terminals in the forebrain from damage by MDMA when the temperature of the rats is artificially maintained above 38.4°C, (Farfel and Seiden, 1995). If MDMA-induced hyperthermia is prevented by maintaining animals at a reduced ambient temperature, the decrease in 5HT and 5HIAA is prevented (Schmidt *et al.*, 1990). However, 5HT uptake blockers prevent the MDMA-induced toxicity without preventing the hyperthermia. Therefore, the hyperthermia produced by MDMA and the 5HT release produced by MDMA appear to contribute independently to the neurotoxicity (Schmidt *et al.*, 1990). A recent study of MDMA effects on cultured glial cells suggests that MDMA-induced neurodegeneration may also be a consequence of MDMA-induced depletion of energy stores in the glial cells that surround the 5HT-containing axon terminals (Poblete and Azmitia, 1995).

In addition to neurotoxic actions on 5HT axons (and DA axons in mice), repeated injections of MDMA have been reported to have several other effects including a transient down-regulation of $5HT_2$ receptors in the forebrain (Scheffel *et al.*, 1992); a decrease in glucocorticoid and mineralocorticoid receptor mRNA expression and an increase in $5HT_{2C}$ receptor mRNA expression in the hippocampus (Yau *et al.*, 1994); a significant increase in glucose utilization in subregions of the hippocampus despite profound reductions in 5HT uptake sites in the hippocampus (Sharkey *et al.*, 1991); and a significant decrease in preprocholecystokinin mRNA levels in the substantia nigra (Wotherspoon *et al.*, 1994). Whether any or all of these changes may be secondary to the neurotoxic effects of MDMA on serotonin neurotransmission is not yet known.

## 4. BEHAVIORAL EFFECTS OF MDMA

Ecstasy is rewarding to laboratory animals as well as humans; monkeys will press levers to self-administer MDMA (Beardsley *et al.*, 1986; Lamb and Griffiths, 1987). In addition to its rewarding properties, MDMA produces locomotor hyperactiv-

ity, hyperthermia, head-weaving and other components of the "serotonin syndrome" in laboratory animals (Green *et al.*, 1995). Systemic injection of single or multiple doses of MDMA increases locomotor activity in laboratory rats (Gold *et al.*, 1988; Matthews *et al.*, 1989; McNamara *et al.*, 1995). Hyperactivity also can be induced by infusing MDMA directly into the nucleus accumbens (Callaway and Geyer, 1992). The hyperactivity produced by systemic injection of MDMA appears to be mediated at least partially by MDMA-induced 5HT release because it is attenuated by pretreatment with the 5HT uptake blocker fluoxetine which prevents the uptake carrier-mediated release of 5HT by MDMA (Callaway *et al.*, 1990; Hekmatpanah and Peroutka, 1990). The $5HT_{1B}$ receptor agonist RU 24969, like MDMA, produces locomotor hyperactivity in rats, whereas agonists for $5HT_{1A}$ and $5HT_{2A,2C}$ receptors decrease locomotion (Rempel *et al.*, 1993). These observations suggest that MDMA stimulates locomotor behavior by releasing 5HT which then acts on $5HT_{1B}$ receptors to produce the locomotor hyperactivity. The hyperthermia that follows MDMA injection can be blocked by the $5HT_{2A,2C}$ receptor antagonist ketanserin (Nash *et al.*, 1988).Other behavioral effects that have been reported for MDMA are similar to those produced by amphetamine and appear to be mediated by DA release. For example, MDMA administration decreased reinforcement rate and increased response rate of rats performing on a differential-reinforcement-of-low-rate task in a manner that was typical of amphetamine and other psychomotor stimulants (Li *et al.*, 1989). After repeated injections of MDMA that resulted in forebrain depletion of 5HT, subsequent injections of MDMA produced even higher rates of responding (and therefore decreased reinforcement rates) than did the first dose of MDMA. Thus, although MDMA can increase extracellular levels of 5HT as well as DA, the high rate of responding that occurred during this behavioral task appeared to be mediated by DA (Li *et al.*, 1989). Sympathetic stimulation effects of MDMA (increased heart rate and blood pressure) may be mediated by MDMA-induced monoamine release in both the central and the peripheral nervous system, and perhaps also by direct effects of MDMA on $\alpha_2$-adrenergic receptors (Green *et al.*, 1995). Although the exact mechanisms by which MDMA produces its many behavioral effects are not known, it is likely that they are mediated by MDMA-induced increases in extracellular levels of 5HT, DA and NE in the nervous system.

## 5. ACUTE MDMA EFFECTS ON NEURONAL EXCITABILITY IN BRAIN REGIONS IMPLICATED IN THE REWARDING PROPERTIES OF ABUSED DRUGS

There is some evidence to suggest that MDMA might have direct postsynaptic effects on neuronal excitability as well as indirect effects that are mediated by monoamine release. MDMA binds with similar, relatively high affinities to $5HT_2$ receptors, $\alpha_2$-adrenoreceptors and M-1 muscarinic cholinergic receptors as to 5HT uptake sites in homogenates of frontal cortex and striatum (Battaglia *et al.*, 1988). Since $5HT_2$ receptors, $\alpha_2$-adrenoreceptors and M-1 muscarinic receptors are located postsynaptically as well as presynaptically in several brain regions, the potential exists for MDMA to have direct postsynaptic actions on neurons that contain these receptors. As evidence for direct effects of MDMA on $5HT_2$ receptors, MDMA has been shown to weakly stimulate (compared to 5HT) [3H] inositol monophosphate accumulation in cultured fibroblasts that express the cloned $5HT_{2A}$ receptor and to stimulate more strongly accumulation in fibroblasts expressing the cloned $5HT_{2C}$ receptor (Nash *et al.*, 1994). In contrast to the serotonergic system, MDMA has relatively low affinity for dopamine $D_1$ and $D_2$ receptors, suggesting that MDMA-induced effects that are mediated by DA are indirect (Battaglia *et al.*, 1988). Indeed, most electrophysiological studies to date indicate that MDMA alters central nervous system neuronal excitability primarily by increasing extracellular levels of monoamines that are released from presynaptic terminals. Interestingly, MDMA has been demonstrated to affect neuronal firing by increasing extracellular levels of monoamines in several brain regions that are implicated in mood and in the rewarding effects of abused drugs.

### 5.1. The Mesocorticolimbic Dopamine Pathway

The mesocorticolimbic dopamine pathway has been implicated widely in drug reinforcement (Dackis and Gold, 1985; Di Chiara and Imperato, 1988; Koob, 1992; Wise and Bozarth, 1984). The DA-containing cells in this pathway originate mainly in the ventral tegmental area (VTA) of the midbrain and project to the nucleus accumbens, olfactory tubercle, frontal cortex, amygdala and septal area (Bjorklund and Lindvall, 1984; Oades and Halliday, 1987). However, some of the dopaminergic projections to the lateral regions of the nucleus accumbens arise from the medial region of the substantia nigra as well (Brog *et al.*, 1993). The DA projections from the VTA to the nucleus accumbens appear to be essential for the rewarding effects of abused drugs. Destruction of DA terminals in the nucleus accumbens or of DA cell bodies in the VTA by injecting the neurotoxin 6-hydroxydopamine markedly attenuates intravenous self-administration of cocaine and amphetamine in laboratory animals (Gawin, 1991; Roberts *et al.*, 1980), and kainic acid lesions of the nucleus accumbens disrupt both cocaine and heroin self-administration (Zito *et al.*, 1985). Like cocaine, amphetamine, opiates and several other abused drugs (Di Chiara and Imperato, 1988; Yoshimoto *et al.*, 1991), MDMA increases extracellular levels of DA in the nucleus accumbens (White *et al.*, 1994; Yamamoto and Spanos, 1988) and it is very likely that the DA projection to the nucleus accumbens is as essential for the rewarding properties of MDMA as it is for cocaine and amphetamine.

The role that 5HT terminals in the nucleus accumbens might play in the rewarding effects of abused drugs is less clear than that of DA. However, many abused drugs increase extracellular levels of 5HT in the nucleus accumbens (White *et al.*, 1994;

Yoshimoto *et al.*, 1991) and 5HT itself increases release of DA in the nucleus accumbens (Benloucif and Galloway, 1991; Parsons and Justice, 1993a). The serotonergic input to the nucleus accumbens is of moderate density, with the shell region being more densely innervated than the core (Steinbusch, 1981). Most of the 5HT terminals in the nucleus accumbens arise from cell bodies in the dorsal raphe nucleus of the midbrain, but some innervation arises from the median raphe nucleus as well (Azmitia and Segal, 1978; Lorens and Guldberg, 1974; Vertes, 1991).

In addition to the monoaminergic input from the VTA and the dorsal raphe nucleus, the nucleus accumbens gets excitatory amino acid (glutamate and/or aspartate) input from such limbic system associated areas as the medial prefrontal cortex, the hippocampus, the amygdala and the midline thalamic nuclei (Fuller *et al.*, 1987; Groenewegen *et al.*, 1990; see Kalivas, 1993 for review). Over the past decade, several electrophysiological studies have examined the effects of MDMA on neuronal excitability in some of the brain regions that are part of the mesocorticolimbic system and these studies are reviewed below.

### 5.2. MDMA Effects in the Medial Prefrontal Cortex

The medial prefrontal cortex (mPFC) which provides a major excitatory drive to the nucleus accumbens (Brog *et al.*, 1993; Fuller *et al.*, 1987; Walaas, 1981; Wright and Groenewegen, 1995) and to the VTA and adjacent substantia nigra of the midbrain (Carter, 1982; Christie *et al.*, 1989) is richly innervated by both 5HT- and DA-containing axon terminals (Steinbusch, 1981; Ungerstedt, 1971). Pan and Wang 1991a); Pan and Wang, 1991b) demonstrated that acute systemic injection of MDMA inhibited spontaneous firing recorded extracellularly from cells in the mPFC of anesthetized rats. The inhibition induced by MDMA appeared to be mediated at least in part by 5HT because it was partially reversed by systemic injections of the 5HT antagonists granisetron and metergoline. The MDMA-induced inhibition did not appear to be mediated by DA or other catecholamines because it was attenuated by pretreating rats with the 5HT synthesis inhibitor *p*-chlorophenylalanine (PCPA) but not by pretreatment with the catecholamine synthesis inhibitor α-methyl-*p*-tyrosine (AMPT). Furthermore, systemic injection of the 5HT precursor 5-hydroxytryptophan but not the DA precursor L-DOPA restored the inhibitory effect of MDMA in PCPA-pretreated rats. The MDMA-induced inhibition was also prevented by fluoxetine, an inhibitor of the 5HT transporter which prevents MDMA-induced 5HT release, but not by GBR 12909, a specific blocker of DA uptake. These observations by Pan and Wang 1991a), 1991b) suggest that the excitatory drive from the mPFC to the nucleus accumbens would be reduced following systemic administration of MDMA and that this effect of MDMA is mediated primarily indirectly by MDMA-induced increases in extracellular 5HT. Similarly, the excitatory drive from the mPFC to the VTA (the source of dopaminergic afferents to the nucleus accumbens) would also presumably be reduced following administration of MDMA. However, because MDMA and the 5HT antagonists were administered systemically by Pan and Wang 1991a), 1991b), it is not known whether the MDMA was acting on presynaptic terminals within the mPFC to produce the inhibition or was acting in some other brain region that projects to the mPFC.

### 5.3. MDMA Effects in the Hippocampus

The hippocampus also provides an excitatory drive to the nucleus accumbens (Boeijinga *et al.*, 1990; Fuller *et al.*, 1987; Pennartz and Kitai, 1991; Yim and Mogenson, 1988). Effects of MDMA on excitability of hippocampal cells *in vivo* or in brain slice preparations have not yet been studied. However, bath application of MDMA produced a dose-dependent increase in inward current recorded from cultured rat hippocampal neurons (Premkumar and Ahern, 1995). The inward current was associated with a decrease in the conductance of a barium-sensitive resting $K^+$ channel and with increased neuronal excitability. The blockade of the resting potassium channel did not appear to be mediated by MDMA-induced 5HT release within the culture preparation because direct application of 5HT to the cultured hippocampal cells activated $K^+$ conductance and decreased the excitability of the cells (Premkumar and Gage, 1994). Whether MDMA may have similar effects on hippocampal cells *in vivo* is not known. However, a $K^+$ channel conductance increase produced by 5HT released from presynaptic terminals *in vivo* by MDMA may override the $K^+$ conductance decrease that was observed in the cultured neurons. The MDMA-induced DA release may have a predominantly inhibitory effect in the hippocampus. DA application to guinea-pig hippocampal neurons in brain slices hyperpolarized the resting membrane potential and increased the amplitude and the duration of the spike after-hyperpolarization (Suppes *et al.*, 1985), both of which would lead to decreased firing rates of the cells. Furthermore, Hsu (1996) has reported recently that DA decreased excitatory postsynaptic potentials (EPSPs) that were evoked by stimulation of the Schaffer Collateral–Commissural pathway in hippocampal slices. The inhibition of the EPSPs was dose-dependent and appeared to be mediated by presynaptic DA $D_2$ receptors. Studies of MDMA effects on hippocampal neuronal excitability in brain slices and *in vivo* would be useful for determining whether MDMA is likely to reduce excitatory afferent input to the nucleus accumbens from the hippocampus as well as from the prefrontal cortex.

### 5.4. MDMA Effects on 5HT Neurons in the Dorsal Raphe Nucleus

Most of the 5HT-containing axons that project to the forebrain arise from the dorsal raphe nucleus and the median raphe nucleus of the brainstem (Azmitia and Segal, 1978; Steinbusch, 1981). The median raphe nucleus gives rise to 5HT fibers with large spherical varicosities while the 5HT fibers arising from dorsal raphe cells are very fine and have small varicosities

(Kosofsky and Molliver, 1987). The fine fibers that project from cells in the dorsal raphe nucleus are the ones that are most vulnerable to damage by repeated administration of MDMA (Wilson *et al.*, 1989). The dorsal raphe nucleus is also the source of most of the 5HT-containing fibers within the nucleus accumbens (Vertes, 1991). Sprouse *et al.* (1989), Sprouse *et al.* (1990)) and Bradberry *et al.* (1991) examined the effects of acute bath application of MDMA on extracellularly recorded spontaneous firing of physiologically identified serotonergic cells in the dorsal raphe nucleus in a midbrain slice preparation. Serotonin cells in the raphe nucleus are inhibited by 5HT acting at autoreceptors located on the somatodendritic regions of the cells (Aghajanian and VanderMaelen, 1982). Bath application of MDMA inhibited firing of the cells in a concentration dependent manner, presumably by releasing 5HT from terminals in the slice. Dialysis studies conducted in parallel with the recording studies indicated that application of MDMA to the bath medium caused the release of 5HT with a time course that corresponded to the decrease in dorsal raphe cell firing rate (Sprouse *et al.*, 1989). The selective 5HT uptake inhibitor fluoxetine which prevented MDMA-induced 5HT release in the midbrain slice (Bradberry *et al.*, 1991) also blocked the MDMA-induced inhibition of 5HT cell firing in the raphe nucleus in the slice preparation. In contrast, the selective NE uptake inhibitor desipramine did not alter the MDMA-induced inhibition of firing. Furthermore, pretreatment of the slices with the 5HT precursor L-tryptophan lowered the dose of MDMA required to inhibit neuronal firing and increased the amount of 5HT released from the slice by MDMA (Bradberry *et al.*, 1991; Sprouse *et al.*, 1990). This set of experiments strongly suggests that MDMA can act directly within the dorsal raphe nucleus to release 5HT which in turn, inhibits firing of the serotonin cells in the nucleus. Intracellular studies have not yet been conducted in the raphe nuclei to determine whether MDMA may have any direct effects on the excitability of the serotonin cells that are not mediated by MDMA-induced release of 5HT or another neurotransmitter.

If MDMA also inhibits the firing of serotonergic cells in the dorsal raphe nucleus *in vivo*, it would be expected to decrease impulse-mediated release of 5HT from terminals in the nucleus accumbens and from terminals in the VTA that are the origin of the DA-containing fibers in the nucleus accumbens. However, direct stimulation of 5HT and DA release from terminals in the nucleus accumbens by MDMA may override any MDMA-induced inhibitory effects on firing rates of the dorsal raphe nucleus cells. Indeed, *in vivo* dialysis results indicate that both systemic and local administrations of MDMA increase extracellular levels of 5HT and DA in the nucleus accumbens and adjacent striatum (Gough *et al.*, 1991; White *et al.*, 1994) despite any effects that it may have on firing rates of the serotonergic cells.

### 5.5. MDMA Effects on DA Neurons in the Ventral Tegmental Area

The dopaminergic cells in the VTA and substantia nigra that project to the nucleus accumbens and the striatum can be distinguished from non-dopaminergic cells in the same brain regions by their characteristic slow firing rates, irregular firing patterns and long-duration biphasic or triphasic action potential waveforms (Aghajanian and Bunney, 1977; Bunney *et al.*, 1973; Grace and Bunney, 1983). Spontaneous firing of these DA-containing cells is inhibited by application of DA which acts directly on $D_2$ autoreceptors to hyperpolarize the cells (Aghajanian and Bunney, 1977; Grace and Bunney, 1983; Johnson and North, 1992) and indirectly by acting on $D_1$ receptors on non-dopaminergic cells to stimulate release of the inhibitory neurotransmitter GABA (Cameron and Williams, 1993). Acute systemic injections of MDMA also partially inhibit spontaneous firing of DA cells in the VTA and the substantia nigra of anesthetized rats (Kelland *et al.*, 1989; Matthews *et al.*, 1989) and the MDMA-induced inhibition is attenuated significantly following

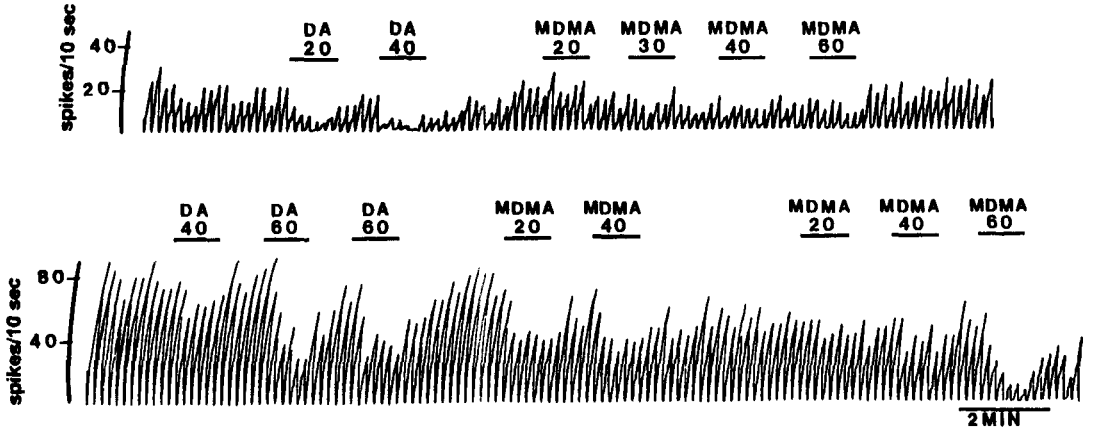


Fig. 3. Effects of microiontophoretically applied DA and MDMA on spontaneous firing of two physiologically identified dopaminergic cells in the VTA. The oscillograph pen that recorded cummulative spikes was reset every 10 sec. Lines above the records indicate periods of drug ejection and numbers indicate ejection current (nA).

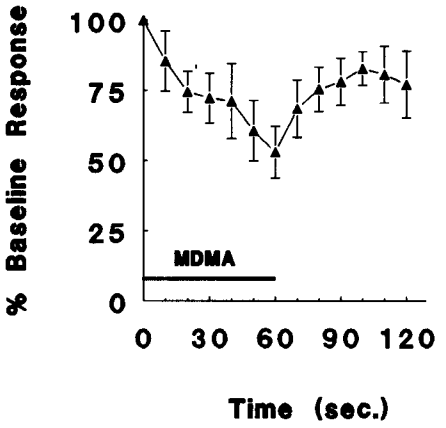


Fig. 4. The time course of the effect of MDMA (60 nA, 60 sec) on spontaneous firing of 18 dopaminergic cells in the VTA is plotted (means and SEM). The firing rate during each 10 sec period during and following MDMA application is expressed as a percentage of the baseline firing rate averaged over a 2 min period just prior to MDMA application.

depletion of either 5HT or DA (Kelland *et al.*, 1989). Our laboratory recently has extended these observations by demonstrating that MDMA applied locally by microiontophoresis also partially inhibits spontaneous firing of electrophysiologically identified DA cells in the VTA of chloral-hydrate anesthetized rats (Fig. 3). Records from a DA cell that fired spontaneously at about 3 Hz (top) and a cell that fired at about 8 Hz (bottom) are shown. Microiontophoretic application of MDMA (20–60 nA, 60 sec) inhibited the spontaneous firing rate of both cells, as did microiontophoretic application of DA. The amount of inhibition produced by MDMA ejection varied both within and between cells (Fig. 3). The time course for the effect of MDMA (60 nA, 60 sec) on 18 dopaminergic cells in the VTA is plotted in Fig. 4. The inhibitory effect of MDMA was statistically significant ($p < 0.01$) but failed to suppress entirely the firing of most cells as was found previously with systemic injections of MDMA (Matthews *et al.*, 1989). The inhibition developed slowly following onset of the MDMA ejection period and the firing rate slowly recovered to pre-injection baseline levels within 2–3 min following offset of MDMA application for all cells. Microiontophoretic application of the $D_2$ antagonist sulpiride (30 nA) beginning 2 min before and continuing for 2 min after the offset of MDMA ejection (60 nA, 120 sec) significantly attenuated the inhibitory effect of MDMA (Fig. 5).

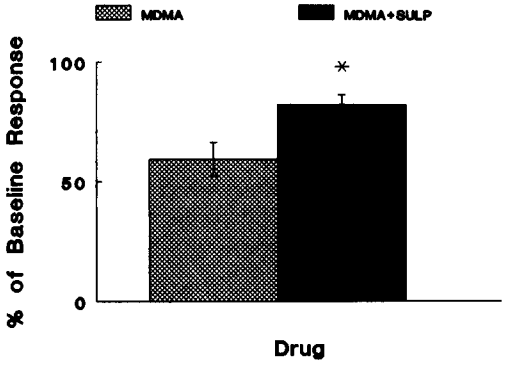


Fig. 5. The DA $D_2$ receptor antagonist sulpiride (SULP) attenuated significantly the inhibitory effect of MDMA on spontaneous firing of dopaminergic cells in the VTA. Means and SEM from nine cells that were tested with MDMA (60 nA, 120 sec) and then retested with the same dose of MDMA applied during ejection of SULP (30 nA, 6 min). *Significant difference between MDMA and MDMA + SULP, $p < 0.05$, paired $t$-test, two-tailed.

These results strongly suggest that MDMA can act locally within the VTA to partially inhibit spontaneous firing of the dopaminergic cells in the VTA by increasing extracellular concentrations of DA. Systemic administration of MDMA is known to increase extracellular levels of DA in the adjacent substantia nigra (Yamamoto *et al.*, 1995) and very likely does so in the VTA as well. The DA-mediated inhibitory effects of MDMA on dopaminergic neurons in the VTA may be counteracted partially by 5HT-mediated excitatory effects. The VTA is richly innervated by 5HT-containing terminals, some of which make synaptic contact with the DA cells (Herve *et al.*, 1987) and MDMA probably induces an increase in extracellular levels of 5HT in the VTA as it does in other brain regions that contain 5HT terminals. Microiontophoresis of 5HT into the substantia nigra of anesthetized rats or electrical stimulation of the dorsal raphe nucleus cells that send 5HT projections to the substantia nigra inhibit the firing of dopaminergic cells in that region (Crossman *et al.*, 1974; Dray *et al.*, 1976; Kelland *et al.*, 1990). However, there is evidence that 5HT may also have excitatory effects on the dopaminergic cells. Microinfusion of 5HT into the VTA increases DA efflux in the nucleus accumbens, suggesting that 5HT does increase the firing of these dopaminergic cells that project to the accumbens (Guan and McBride, 1989). One mechanism by which 5HT appears to increase the excitability of dopaminergic cells in the VTA is by interacting with $5HT_{1B}$ receptors to inhibit presynaptic release of GABA (Johnson *et al.*, 1992). However, 5HT also may stimulate somatodendritic DA release from the dopaminergic cells in the VTA (as it does from DA terminals in the nucleus accumbens) which could, in turn, inhibit the firing of the cells by acting on the $D_2$ autoreceptors. In brain slices, bath application of serotonin was found to enhance DA-induced inhibition of VTA dopaminergic neurons whether 5HT increased or decreased spontaneous firing of the cells when applied alone (Brodie and Bunney, 1994). Although 5HT and DA have complex effects in the VTA, the predominant effect of MDMA on spontaneous firing of the dopaminergic cells is inhibitory. The mechanisms by which MDMA decreases spontaneous firing of the dopaminergic cells in the VTA are not known. However, cocaine, which also has a predominantly inhibitory effect on spontaneous firing of dopaminergic cells, has been demonstrated to both hyperpolarize the dopaminergic cells by blocking DA uptake (Lacey *et al.*, 1990) and to inhibit GABA release from presynaptic terminals by blocking 5HT uptake (Cameron and Williams, 1994). MDMA

probably has effects in the VTA that are at least as complex because, like cocaine, it blocks reuptake of 5HT and DA, but in addition, it also stimulates release of DA and 5HT from presynaptic terminals (see Section 2).

### 5.6. MDMA Effects in the Nucleus Accumbens

As discussed previously (Section 2), drugs with euphoric properties increase extracellular DA in the nucleus accumbens whether the drugs are central nervous system stimulants such as cocaine, amphetamine and MDMA (Di Chiara and Imperato, 1988; Hernandez *et al.*, 1987; Kalivas and Duffy, 1990; White *et al.*, 1994; Yamamoto and Spanos, 1988) or central nervous system depressants such as opiates and alcohol (Di Chiara and Imperato, 1988; Yoshimoto *et al.*, 1991). Food reward also increases extracelluar DA in the nucleus accumbens (Hernandez and Hoebel, 1988) and some neurons within the nucleus accumbens of awake, behaving animals fire specifically preceding or just after reward delivery or when the animals view a stimulus associated with reward (Apicella *et al.*, 1991; Rolls and Williams, 1988; Schultz *et al.*, 1992). In addition to firing in association with food or water reward, some neurons in the nucleus accumbens alter firing patterns in cocaine self-administering rats just prior to the time when the animal will press a lever to obtain intravenous cocaine or for a few minutes just after a lever press delivered cocaine (Carelli and Deadwyler, 1994; Chang *et al.*, 1994). These findings combined with early observations that lesions in the nucleus accumbens disrupted cocaine and heroin self-administration (Zito *et al.*, 1985) indicate that the nucleus accumbens is an essential component of brain pathways that mediate reward as proposed more than 10 years ago by Roberts *et al.* (1980) and Wise and Bozarth (1984).

No studies of MDMA effects on neuronal activity in the nucleus accumbens of awake, behaving animals have been conducted to date, but our laboratory recently has examined the effects of MDMA on neuronal firing in the nucleus accumbens of anesthetized rats using extracellular unit recording combined with microiontophoresis (Fig. 6). The nucleus accumbens neurons were driven by cycled pulses of glutamate because most nucleus accumbens neurons are silent in anesthetized rats (White and Wang, 1986; Woodruff *et al.*, 1976). The effects of MDMA on glutamate-evoked firing were compared to the effects of DA and 5HT which are known to be released in the nucleus accumbens by MDMA (White *et al.*, 1994). In addition, DA and 5HT depleting drugs and receptor antagonists were employed to test whether MDMA effects on nucleus accumbens excitability might be mediated by DA and/or 5HT. Both 5HT and DA previously have been demonstrated to inhibit glutamate-evoked firing of most nucleus accumbens cells (White, 1986; White and Wang, 1986). Since many neurochemical and anatomical differences have been described for the core and shell regions of the nucleus accumbens (Brog *et al.*, 1993; Deutsch and Cameron, 1992; Groenewegen and Russchen, 1984; Heimer *et al.*, 1991; Meredith *et al.*, 1992; Pennartz *et al.*, 1994; Zahm and Heimer, 1993), effects of MDMA in these two regions of the accumbens are discussed separately.

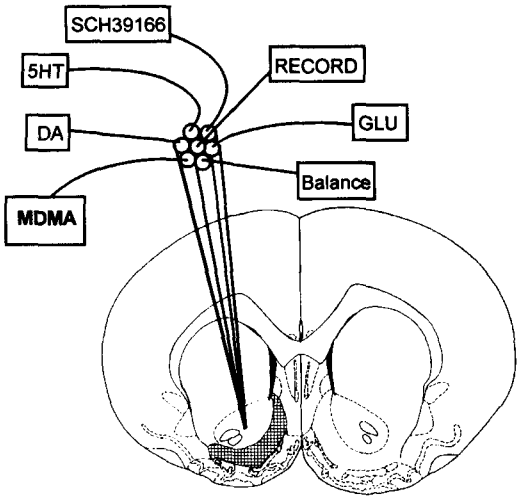


Fig. 6. Single unit recording from the nucleus accumbens core combined with microiontophoresis using a multi-barrel glass microelectrode. The hatched area medial to and ventral to the nucleus accumbens core indicates the nucleus accumbens shell. The micropipettes contained NaCl for recording and for automatic current balance, dye to mark the recording site, glutamate (GLU) to drive the quiescent cells, MDMA, and some combination of 5HT, DA, agonists and antagonists for specific subtypes of 5HT and DA receptors or a specific 5HT uptake blocker. The drawing of the brain section is modified from Paxinos and Watson (1986).

#### 5.6.1. *Nucleus Accumbens Core*

Microiontophoretic application of MDMA produced a dose-dependent inhibition of glutamate-evoked firing in nearly every cell that has been tested in the core region of the nucleus accumbens (Obradovic *et al.*, 1996; White *et al.*, 1994). Examples of this inhibition are illustrated in Fig. 7. The inhibition was not mimicked by ejection of $Na^+$ ions from a NaCl control solution, but was similar to inhibition produced by DA and 5HT ejection. Simultaneous application of MDMA potentiated the inhibition of glutamate-evoked firing that was produced by low ejection currents of DA or 5HT (White *et al.*, 1994). Dose (current)–response curves indicated that the inhibitory effect of MDMA was statistically significant compared to the effect of equivalent currents applied to the saline control solution for MDMA doses of 30 nA, 60 sec and higher (Obradovic *et al.*, 1996). Applications of 5HT or the selective $5HT_{2A,2C}$ receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (Martin and Humphrey, 1994; Van Wijngaarden *et al.*, 1990) also significantly inhibited glutamate-evoked firing of the nucleus accumbens cells with a dose–response relationship that was not different from MDMA (Fig. 8). A selective agonist for $5HT_{1A}$ receptors, 8-hydroxy-(2-di-*n*-propylamine) tetralin (8-OHDPAT) and relatively selective agonists for $5HT_3$ receptors, 2-methyl-5-hydroxytryptamine

(2M5HT) and 1-(m-chlorphenyl)-biguanide (CPBG) also were effective inhibitors of glutamate-evoked firing. An agonist with high affinity for $5HT_{1B}$ receptors, CGS12066B also inhibited glutamate-evoked firing of nucleus accumbens cells (Obradovic *et al.*, 1996), but there is no highly selective agonist for this receptor subtype currently available (Van Wijngaarden *et al.*, 1990). These results suggest that 5HT released by MDMA may interact with a variety of 5HT receptor subtypes to depress excitation of nucleus accumbens cells by excitatory amino acids. Blockade of MDMA-induced 5HT release by application of the selective 5HT uptake inhibitor fluoxetine (FLX) prevented inhibition of glutamate-evoked firing by MDMA, as did application of the $5HT_{2A,2C}$ receptor antagonist ketanserin (KET) and the selective $5HT_{1A}$ receptor antagonist WAY100135 (Fig. 9), providing further evidence that MDMA inhibits neuronal firing in the nucleus accumbens at least in part by increasing extracellular levels of 5HT.

The MDMA-induced inhibition of glutamate-evoked firing in the nucleus accumbens also is partially dependent upon MDMA-induced increases in extracellular DA. Pretreatment of rats with the 5HT synthesis inhibitor PCPA did not prevent MDMA-induced inhibition of glutamate-evoked firing in the nucleus accumbens. However, pretreatment with both PCPA and the catecholamine synthesis inhibitor AMPT did significantly attenuate the inhibitory effect of MDMA (White *et al.*, 1994). The selective DA $D_1$ receptor antagonist SCH39166 (Chipkin *et al.*, 1988), but not the $D_2$ receptor antagonist sulpiride, also significantly attenuated the inhibitory effect of MDMA on neuronal firing in the nucleus accumbens (White *et al.*, 1994). Furthermore, depletion of DA by combined pretreatment with the neurotoxin 6-hydroxydopamine (6OHDA) and the

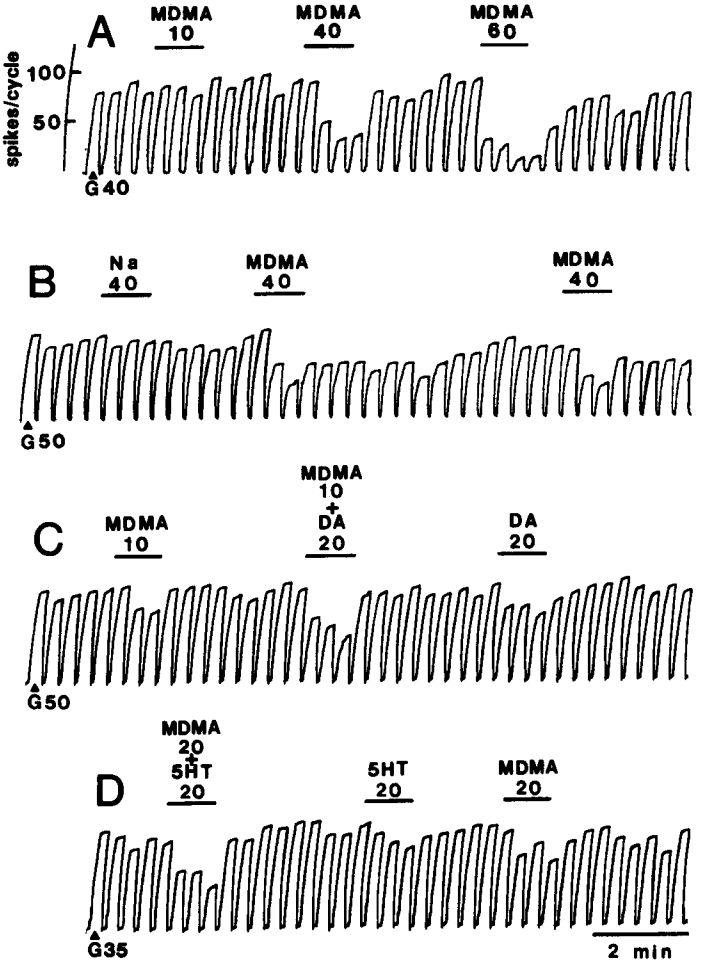


Fig. 7. Effects of MDMA, 5HT and DA on glutamate-evoked firing of four different nucleus accumbens cells. The cells were driven by cycled pulses of glutamate and the oscillograph pen that recorded cummulative spikes was reset at the onset of each glutamate pulse. The height of each histogram indicates the total number of spikes that occurred in each glutamate-application cycle. Lines above each record indicate periods of drug ejection and numbers indicate ejection currents (nA). The Na in (B) represents sodium (and hydrogen) ions ejected from an acidic saline control solution. (A) and (B) MDMA produced a dose-dependent inhibition of glutamate-evoked firing. (C) and (D) MDMA also potentiated the inhibitory effects of low doses of DA and 5HT (White *et al.*, 1994).

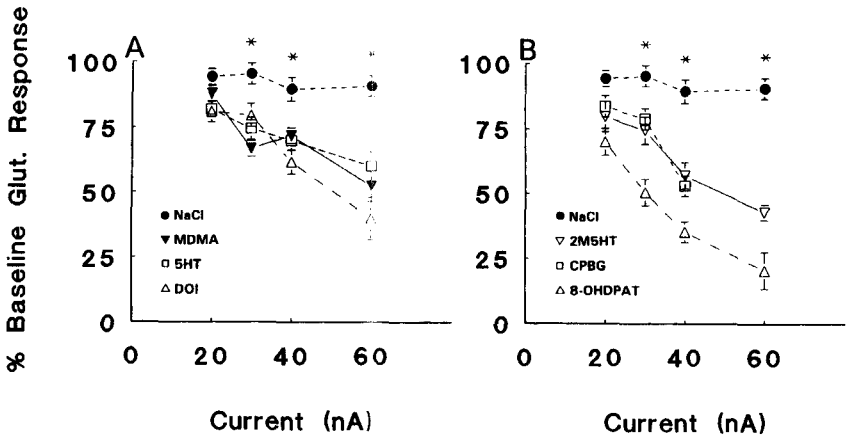


Fig. 8. MDMA, 5HT and several 5HT agonists that are relatively selective for specific 5HT receptor subtypes all significantly inhibited glutamate-evoked firing of cells in the nucleus accumbens core compared to the effects of equivalent currents applied to an acidic saline (NaCl) control solution. For each ejection current tested (20, 30, 40 and 60 nA), the decrease in firing during the 60 sec ejection period is expressed as a percentage of the baseline firing rate that occurred in the 2 min period immediately preceding drug ejection. Means and SEM are plotted and the NaCl results are shown on both the left- and the right-hand sides for ease of comparison with the other drugs. The MDMA was tested on 57 cells in 28 rats, 5HT on 44 cells in nine rats, DOI on 35 cells in 10 rats, 2M5HT on 13 cells in five rats, CPBG on 27 cells in seven rats and 8-OHDPAT on 18 cells in six rats. *Significant differences ($p < 0.01$) between NaCl and all the other drugs, two-way repeated measures analysis of variance with *post hoc* comparisons of means (Obradovic *et al.*, 1996).

synthesis inhibitor AMPT significantly attenuated the inhibitory effect of MDMA applied with all but the highest ejection currents (Fig. 10). The inhibitory effects of 5HT, however, were unaltered in the same cells in which the inhibitory effects of MDMA were attenuated (Fig. 10). Taken together, the ability of fluoxetine, $5HT_{2A,2C}$ and $5HT_{1A}$ receptor antagonists, the $D_1$ receptor antagonist, and DA depletion to attenuate MDMA-induced inhibition in the nucleus accumbens strongly suggests that both MDMA-induced DA release and an intermediate step of MDMA-induced 5HT release are the means by which MDMA alters neuronal activity in this nucleus.

Anatomical and electrophysiological studies suggest that complex mechanisms underlie alterations in nucleus accumbens neuronal excitability that are produced by MDMA-induced release of 5HT and DA. Ultrastructural analysis showed that a large proportion of 5HT terminals in the nucleus accumbens make direct synaptic contact with dendrites in the nucleus (Van Bockstaele and Pickel, 1993). In addition, many 5HT terminals are in apposition to tyrosine hydroxylase (TH)-containing terminals (presumably dopaminergic), and many are in apposition to unlabeled (presumably non-dopaminergic) terminals. Thus, the anatomical framework is present for 5HT to affect directly postsynaptic cells in the nucleus accumbens and to modulate (or be modulated by) presynaptic release of DA and other neurotransmitters. Nearly every known 5HT receptor subtype is present within the nucleus accumbens including high densities of $5HT_4$ receptors and high levels of mRNA for $5HT_6$ receptors (Jakeman *et al.*, 1994; Ruat *et al.*, 1993); high to intermediate densities of $5HT_{2A}$ (formerly named $5HT_2$), $5HT_{2C}$ (formerly $5HT_{1C}$) and $5HT_{1B}$ receptors (Bruinvels *et al.*, 1994; Mengod *et al.*, 1990a, Mengod *et al.*, 1990b; Pazos and Palacios, 1985; Pazos *et al.*, 1985; Pompeiano *et al.*, 1994; Voigt *et al.*, 1991); moderate to low densities of $5HT_3$ receptors (Barnes *et al.*, 1990; Kilpatrick *et al.*, 1987) and low or sparse densities of $5HT_{1A}$ receptors (Khawaja, 1995). Thus, 5HT that is released by MDMA has the potential to have multiple direct and indirect effects on nucleus accumbens neuronal excitability. For example, GABAergic cells are a major constituent of both the shell and the core of the nucleus accumbens (Mugnaini and Oertel, 1985) and are both the projection neurons of the nucleus and constitute a population of interneurons that synapse within the nucleus. Excitability of these cells may be modified by direct, postsynaptic effects of 5HT or by indirect 5HT modulation of presynaptic terminal release of GABA from projection neuron axon collaterals or interneurons, DA from mesoaccumbens terminals, and/or other neurotransmitters from presynaptic terminals of interneurons within the accumbens or from presynaptic terminals of other afferent neurons.

The inhibitory effects of 5HT on glutamate-evoked firing of nucleus accumbens cells that are illustrated in Fig. 7 and Fig. 8 are compatible with previous observations that electrical stimulation of the dorsal raphe nucleus produced depression of neuronal firing in the anatomically similar striatum (Miller *et al.*, 1975; Olpe and Koella, 1977). The raphe-evoked depression was prevented by pharmacological depletion of brain 5HT, suggesting that the inhibition was mediated by 5HT release. However, it is not known whether the effects of 5HT released by raphe stimulation or 5HT applied by iontophoresis are direct effects on the post-synaptic cells or are mediated by presynaptic modulation of the release of

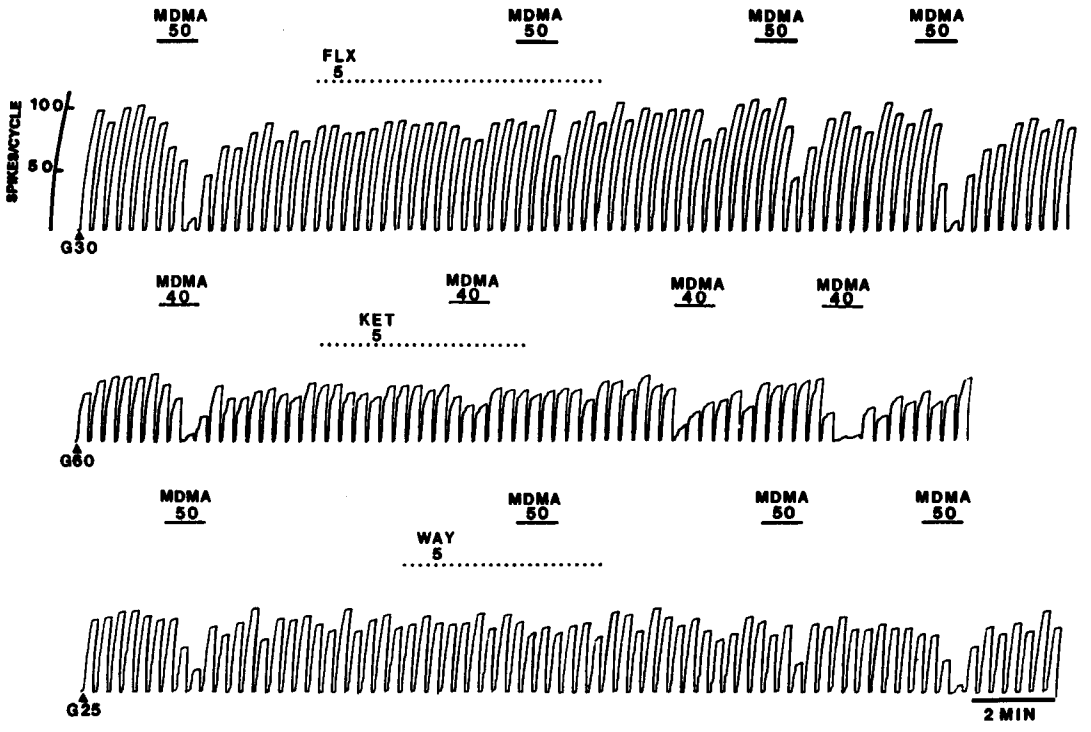


Fig. 9. The inhibitory effect of MDMA on glutamate-evoked firing of cells in the nucleus accumbens core was markedly attenuated by the 5HT uptake blocker fluoxetine (FLX, top record) which prevents MDMA-induced 5HT release, by the $5HT_{2A,2C}$ receptor antagonist ketanserin (KET, middle record) and by the $5HT_{1A}$ receptor antagonist WAY100135 (WAY, bottom record). Note that the inhibitory effect of MDMA recovered by 5–10 min after offset of the uptake blocker and the antagonists. Ejection periods for FLX and the 5HT receptor antagonists are indicated by dotted lines above the records, ejection periods for MDMA are indicated by solid lines and ejection currents (nA) are indicated by the numbers (modified from Obradovic *et al.*, 1996).

other transmitters. In brain slices, 5HT produced a direct (tetrodotoxin insensitive) depolarization of accumbens cells that was blocked by the relatively selective $5HT_2$ antagonists ketanserin and mianserin (North and Uchimura, 1989). Selective $5HT_{1A}$ and $5HT_3$ agonists did not affect accumbens cell membrane potentials in this *in vitro* study. These observations suggest that 5HT may have quite different and perhaps opposite direct effects on postsynaptic membrane potentials and indirect effects on terminals that are presynaptic to the cells. Nicola *et al.* (1996) demonstrated recently that bath application of 5HT reduced the amplitude of electrically evoked excitatory postsynaptic potentials (EPSPs) in nucleus accumbens cells in brain slices. Further studies to determine whether this is a presynaptic effect of 5HT, what neurotransmitter might mediate the effect if it is presynaptic, and whether 5HT may also alter IPSPs in the accumbens have not yet been reported.

The potential mechanisms by which MDMA-induced DA release might alter neuronal excitability in the nucleus accumbens are as complex as those of 5HT. The GABAergic medium spiny neurons are most likely the exclusive output route of the accumbens (Chang and Kitai, 1985) and are likely to be the neurons that are most often examined in unit recording studies. The nucleus accumbens also contains a population of GABAergic interneurons, and GABA-containing neurons are the most numerous cells in the nucleus (Mugnaini and Oertel (1985). Ultrastructural studies revealed that terminals in the nucleus accumbens that are positive for TH-like immunoreactivity (which are presumably DA-containing terminals) make synaptic contacts with GABA-immunoreactive dendrites and somata indicating that DA can have direct postsynaptic actions in the accumbens, perhaps on both GABAergic projection neurons and GABAergic interneurons (Pickel *et al.*, 1988). In addition, terminals that were positive for TH-like immunoreactivity were found in apposition to presynaptic terminals in the accumbens, some of which were also positive for TH-like immunoreactivity and some of which were non-dopaminergic (Zahm and Brog, 1992). Thus DA is anatomically situated in locations that would allow for modulation of presynaptic release of transmitters as well as for direct postsynaptic effects. As a further complication, DA routinely escapes from the synaptic cleft during dopaminergic synaptic transmission in the nucleus accumbens and so could act in a paracrine fashion to alter neuronal excitability at a distance from the synaptic terminal (Garris *et al.*, 1994). The presence of multiple DA receptor subtypes in the nucleus accumbens (Bardo and Hammer, 1991; Booze and Wallace, 1995; Mansour *et al.*, 1992; Yung

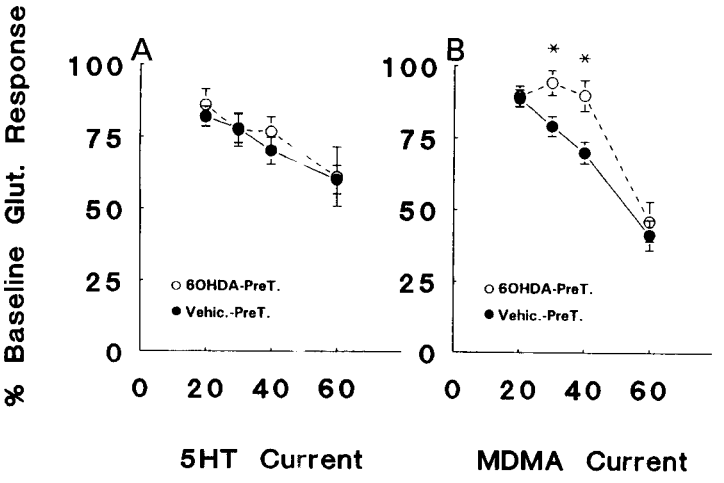


Fig. 10. Depletion of DA by pretreatment with the neurotoxin 6-hydroxydopamine and the synthesis inhibitor AMPT (6OHDA-PreT group) did not alter inhibition of glutamate-evoked firing produced by 5HT (A), but reduced significantly the inhibition produced by MDMA (B) compared to effects on cells in vehicle pretreated control animals (Vehic.-PreT group). For the six rats in the 6OHDA-PreT group, MDMA was tested on 23 cells and 5HT was tested on 15 of the 23 cells. For the six rats in the Vehic.-PreT group, MDMA was tested on 27 cells and 5HT was tested on 25 of these 27 cells. *Significant differences ($p < 0.01$) between the 6OHDA-PreT and the Vehic.-PreT. groups, two-way repeated measures analysis of variance with *post hoc* comparisons of means (modified from Obradovic *et al.*, 1996).

*et al.*, 1995) provides yet more possibilities for complex actions of DA in the nucleus.

Woodruff *et al.* (1976) was the first to demonstrate that microiontophoretic application of DA inhibits spontaneous and excitatory amino acid-evoked firing of most neurons in the nucleus accumbens *in vivo*. This finding has been reproduced many times (i.e. Akaike *et al.*, 1983; Henry and White, 1991; White and Wang, 1984, White and Wang, 1986; White *et al.*, 1995a, White *et al.*, 1995b) and appears to be physiologically relevant because electrical stimulation of the VTA inhibits glutamate-evoked and synaptically evoked firing of nucleus accumbens cells, and the inhibition is blocked by selective $D_1$ antagonists (Yim and Mogenson, 1982; Hara *et al.*, 1989). In contrast to these inhibitory findings, intracellular recording from nucleus accumbens cells *in vivo* indicated that DA application produced a consistent small depolarization of the cells even though it did not increase spontaneous firing (Yim and Mogenson, 1988). However, despite this depolarizing effect, DA strongly inhibited the postsynaptic depolarization of accumbens cells that was evoked by amygdala stimulation (Yim and Mogenson, 1988). Recordings from accumbens cells in guinea-pig tissue slices revealed both hyperpolarizing ($D_1$ receptor-mediated) and depolarizing ($D_2$ receptor-mediated) tetrodotoxin insensitive effects of DA on membrane potentials, and the majority of cells responded to DA with an initial hyperpolarization followed by depolarization (Uchimura *et al.*, 1986; Higashi *et al.*, 1989). Pennartz *et al.* (1992) found that DA attenuated both excitatory and inhibitory synaptic inputs to nucleus accumbens shell cells in rat brain slices by acting at presynaptic $D_1$ receptors. However, direct effects of DA on membrane potential and input resistance were variable and did not correlate with changes in the postsynaptic potentials. Whether this discrepancy in DA direct postsynaptic effects on membrane potentials can be attributed to differences in recording locations within the nucleus accumbens or to species differences has not been resolved. Nicola *et al.* (1996) also found that DA acted at presynaptic $D_1$ receptors to reduce EPSPs elicited in nucleus accumbens cells by stimulation of cortical afferents in rat brain slices. Similarly to Pennartz *et al.* (1992), DA reduced the size of the EPSPs without affecting the resting membrane potential or input resistance. Cocaine and amphetamine also reduced the excitatory synaptic responses elicited by stimulation of cortical afferents, and although the mechanism by which cocaine acted was not tested, amphetamine appeared to act via stimulation of a presynaptic $D_1$ receptor (Nicola *et al.*, 1996). The response to amphetamine appeared to be mediated by DA release because it was reduced following depletion of DA by the neurotoxin 6-hydroxydopamine. The identity of the neurotransmitter that was affected by DA was not established. However, DA did not appear to be activating GABA release because the EPSPs were depressed by DA to the same extent whether or not a GABA receptor antagonist was present. Although similar studies have not been conducted with MDMA, it is very likely that MDMA reduces firing of nucleus accumbens cells *in vivo* by releasing DA and 5HT that act at least in part on presynaptic terminals to reduce tonic excitatory synaptic transmission.

### 5.6.2. *Nucleus Accumbens Shell*

There are several differences in DA and 5HT distribution in the shell and core subregions of the nucleus accumbens. Concentrations of both DA and 5HT are significantly greater in the shell than in the core, reflecting differences in the density of terminals that contain these monoamines (Deutsch and

Cameron, 1992; Steinbusch, 1981; Voorn *et al.*, 1989). The morphology of the 5HT-containing fiber terminals also differs in these two subdivisions of the nucleus accumbens. Most of the 5HT-containing fibers in the core region are very fine in diameter with small varicosities (Van Bockstaele and Pickel, 1993). In contrast, a large number of fibers in the shell region have thicker diameter with large varicosities. Many more of the DA-containing terminals in the medial shell than in the core region of the nucleus accumbens contain co-localized cholecystokinin (CCK); and CCK receptors are more densely concentrated in the medial shell than in the core (Hokfelt *et al.*, 1980; Zarbin *et al.*, 1983). White and Wang (1984) demonstrated that the anatomical difference in CCK terminal and receptor distribution is complimented by a functional difference. Cells located in the dorsomedial nucleus accumbens (shell region) were excited by significantly lower doses of CCK applied by microiontophoresis than were cells located in the lateral (core) region. Part of the effect of CCK on neuronal excitability in the nucleus accumbens may be mediated by DA release. Bath application of CCK inhibits DA release from slices of the anterior nucleus accumbens and stimulates DA release from slices of the posterior nucleus accumbens (Marshall *et al.*, 1991). Whether diverse effects of CCK on DA release might be observed in shell vs core regions of the nucleus accumbens is not known. Pennartz *et al.* (1992) found that DA decreased postsynaptic responses that were recorded from cells in the shell region but not the core region of the nucleus accumbens *in vitro*, further suggesting that the neurochemical differences between the core and the shell might reflect functional differences in the effectiveness of the neurotransmitters.

Since the effects of MDMA on neuronal excitability in the nucleus accumbens appear to be mediated largely by MDMA-induced increases in extracellular DA and 5HT (White *et al.*, 1994; Obradovic *et al.*, 1996), MDMA might also be expected to have differential effects in the two subregions of the nucleus. This hypothesis was tested recently in our laboratory (White *et al.*, 1995b). Effects of microiontophoretically applied MDMA on glutamate-evoked firing were compared for cells in the nucleus accumbens core and shell. Responses of the nucleus accumbens cells also were compared to responses of cells in the caudate/putamen (CP, striatum) because the core region of the nucleus accumbens has input–output connections and neurotransmitter distributions that are more analogous to those of the striatum than to the shell of the nucleus accumbens (Heimer *et al.*, 1991; Zahm and Heimer, 1993). Similarly to nucleus accumbens cells, glutamate-evoked firing of most cells in the CP is suppressed by microiontophoretic application of DA, DA $D_1$ receptor agonists and 5HT (Hu and Wang, 1988). Applications of MDMA (20–60 nA, 60 sec) produced a dose-dependent inhibition of glutamate-evoked responding in the core, the shell and the CP (Fig. 11). The inhibition produced by the highest dose of MDMA (60 nA, 60 sec) significantly inhibited cells in all three regions compared to the effect of an equivalent current applied to a NaCl control solution. However, cells in the shell region of the nucleus accumbens were significantly less inhibited than cells in the core region by all but the highest dose (60 nA, 60 sec) of MDMA tested (Fig. 11, left). Responses to MDMA did not significantly differ between cells in the core and cells in the CP (Fig. 11, right-hand side).

Microiontophoretic application of DA, the $D_1$ agonist SKF 38393, 5HT and the $5HT_{2A,2C}$ agonist DOI all produced equivalent inhibition of glutamate-evoked firing in the nucleus accumbens core and shell

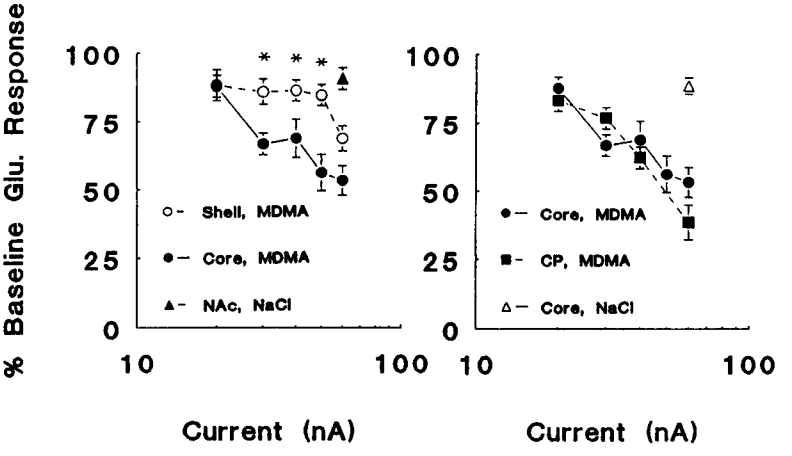


Fig. 11. The MDMA (applied at 30, 40, and 50 nA for 60 sec, but not at 20 or 60 nA) was significantly less inhibitory on glutamate-evoked firing of cells in the shell region of the nucleus accumbens than on cells in the core region of the accumbens or in the striatum (CP). The data from the cells in the nucleus accumbens core are plotted on both the left- and the right-hand sides for ease of comparison to cells in the other regions. Effects of the highest ejection current (60 nA, 60 sec) applied to an acidic saline control solution (NaCl) are indicated by the triangles (filled triangle represents cells from the core and shell regions combined; open triangle represents cells from the core only). Note that the *x*-axis is a log scale. *Significant differences ($p < 0.01$) between effects of MDMA on cells in the shell and cells in the core, two-way repeated measures analysis of variance with *post hoc* comparisons of means.

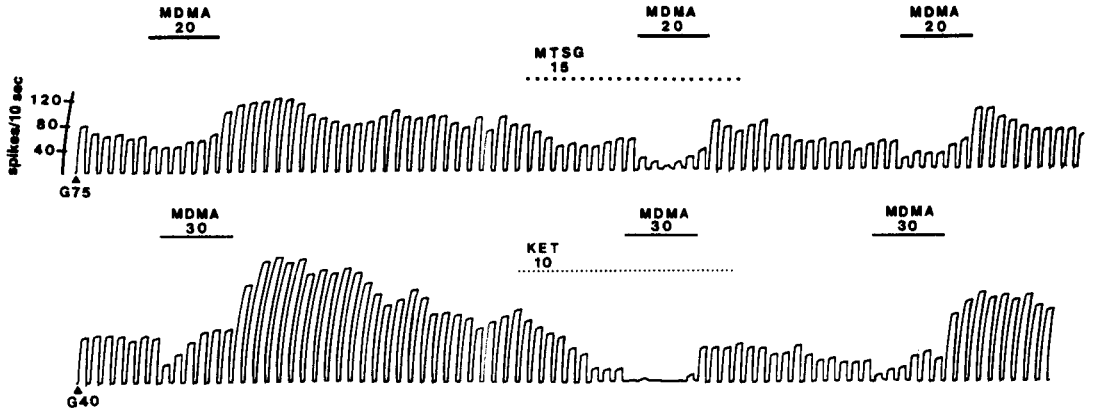


Fig. 12. MDMA produced a long-lasting facilitation of glutamate-evoked firing of motoneurons in the hypoglossal nucleus that was sometimes preceded by a short-lasting inhibition. The 5HT receptor antagonists methysergide (MTSG) and ketanserin (KET) attenuated the excitatory effect of MDMA and enhanced the inhibitory effect. The motoneurons were driven by cycled pulses of glutamate (G) and the oscillograph pen that recorded cumulative action potentials was reset at the onset of each glutamate cycle. The height of each histogram represents the total number of spikes that occurred during the glutamate cycle. The dotted lines above the records indicate periods of MTSG and KET application and the solid lines indicate periods of MDMA application. Numbers indicate ejection currents (nA).

(White *et al.*, 1995b), as has been reported previously for DA (Hu and White, 1994). Therefore, the difference in shell and core sensitivity to the effects of MDMA cannot be attributed to differences in sensitivity of cells in the two regions to DA and 5HT. The most likely explanation for the differential effect of MDMA in the two regions is that MDMA may increase extracellular levels of 5HT and/or DA to a greater extent in the core region than in the shell, even though 5HT and DA terminals are denser in the shell than in the core. Although there is no direct evidence in support of this mechanism to date, there is suggestive evidence. MDMA is selectively toxic to the fine-diameter 5HT-containing fibers that predominate in the core region (Mamounas *et al.*, 1991; Van Bockstaele and Pickel, 1993; Wilson *et al.*, 1989) and this neurotoxicity depends upon initial 5HT depletion from the terminals (Sprague *et al.*, 1994). The larger diameter, beaded fibers in the shell may be spared from the toxic effects of MDMA because MDMA may not release 5HT as readily from these fibers as from the fibers in the core. The MDMA-induced inhibition of glutamate-evoked firing in the core is known to be dependent upon 5HT release, because fluoxetine, which blocks 5HT release, also blocks MDMA-induced inhibition (Fig. 9). If MDMA is, indeed, less effective at releasing 5HT from terminals in the shell, less 5HT would be available to stimulate DA release, and the combination of less release of both of these monoamines would be expected to lead to less inhibition of glutamate-evoked firing in the shell region. As additional evidence that this may occur, Pierce and Kalivas (1995) reported data that showed that a high dose of amphetamine (30 μM) applied through a dialysis probe in the medial region of the accumbens shell of saline-treated rats increased extracellular DA less than an equivalent dose of amphetamine infused into the core region of the nucleus accumbens.

The specific cell types from which recordings were made in the studies discussed above are not known. Since the medium spiny GABAergic neurons are the most numerous cell type in the accumbens, it is likely that many recordings were from those cells. However, the acetylcholine-containing interneurons have large cell bodies (Meredith *et al.*, 1989) and so may have been sampled disproportionately in the recording studies even though they are relatively few in number in the nucleus accumbens. Further studies to determine whether MDMA has differential effects on activity of identified projection cells in the shell and core would clarify whether MDMA has a greater inhibitory effect on the output pathways from the core that are more classically "basal ganglia-like" pathways (Zahm and Heimer, 1993) than on output pathways from the shell which appear to be more "limbic-like".

### 5.7. MDMA Effects on Motoneurons in the Hypoglossal Nucleus

In addition to euphoric properties, MDMA is a sympathetic nervous system stimulant which also stimulates somatic motor tone. Following ingestion of MDMA, human subjects experience jaw clenching, increased deep tendon reflexes and gait instability as well as increased heart rate and blood pressure (Steele *et al.*, 1994). It is likely that both the vasomotor and the somatomotor effects of MDMA also are mediated by increases in extracellular levels of monoamines. Our laboratory recently examined the effect of microiontophoretic application of MDMA on glutamate-evoked firing of motoneurons in the hypoglossal nucleus of anesthetized rats. MDMA produced a slowly developing, long-lasting increase in glutamate-evoked firing of the motoneurons that was preceded in some cells by short-lasting inhibition (Fig. 12), effects that are mimicked by 5HT or NE application to facial or spinal motoneurons (McCall and Aghajanian, 1979; White and Neuman, 1980).

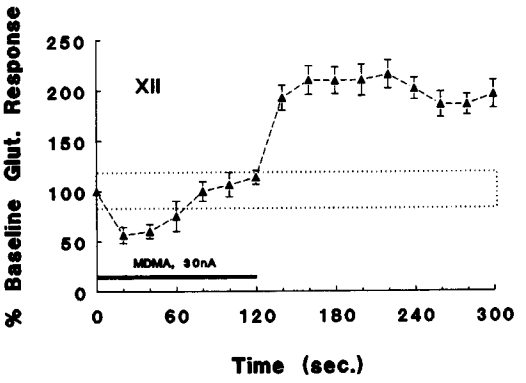


Fig. 13. The time course of MDMA (30 nA, 120 sec) effects on glutamate-evoked firing of 22 hypoglossal motoneurons is shown. The dotted rectangle indicates the highest and lowest changes from baseline firing that were produced by application of an equivalent ejection current to an acidic saline control solution. The MDMA produced a brief inhibition followed by a very long-lasting facilitation of glutamate-evoked firing. The firing rates did not return to pre-MDMA baseline rates until 5–10 min after ejection offset.

The excitatory effect of MDMA was inhibited markedly by the non-specific 5HT antagonist methysergide (MTSG) and by the selective $5HT_{2A,2C}$ receptor antagonist ketanserin (KET). Both MTSG and KET (to a greater extent) partially inhibited glutamate-evoked firing when applied alone (perhaps by blocking the excitatory effects of tonically released 5HT) and increased the initial inhibitory effects of MDMA (Fig. 12). This 5HT antagonist-induced inhibition of the excitation produced by MDMA and "unmasking" of the inhibition produced by MDMA mimicked the effects that these antagonists have on 5HT-mediated excitation and inhibition of glutamate-evoked firing of spinal motoneurons (Jackson and White, 1990), suggesting that the MDMA effects were mediated in part by increasing extracellular levels of 5HT. Effects of MDMA (30 nA, 120 sec) on 22 hypoglossal motoneurons are shown in Fig. 13. MDMA produced a short-lasting but statistically significant inhibition of glutamate-evoked firing that was followed by a statistically significant increase in firing that did not return to baseline until 5–10 min after MDMA ejection offset.

It is well established that 5HT, NE and DA all have excitatory effects on motoneurons in the brainstem and the spinal cord (Bayliss *et al.*, 1995; Katakura and Chandler, 1990; Kubin *et al.*, 1992; Larkman and Kelly, 1992; Parkis *et al.*, 1995; Smith *et al.*, 1995; VanderMaelen and Aghajanian, 1980; Wang and Dun, 1990; White and Fung, 1989; White *et al.*, 1991) and neuronal terminals that contain these monoamines are found in both the ventral horn of the spinal cord and in cranial motor nuclei in the brainstem. Therefore, MDMA ingestion by humans may increase deep tendon reflexes and muscle tone by increasing extracellular levels of all three of these amines. Effects of MDMA on blood pressure and heart rate may be mediated similarly by monoamines released from terminals in the intermediolateral nucleus of the spinal cord (the location of

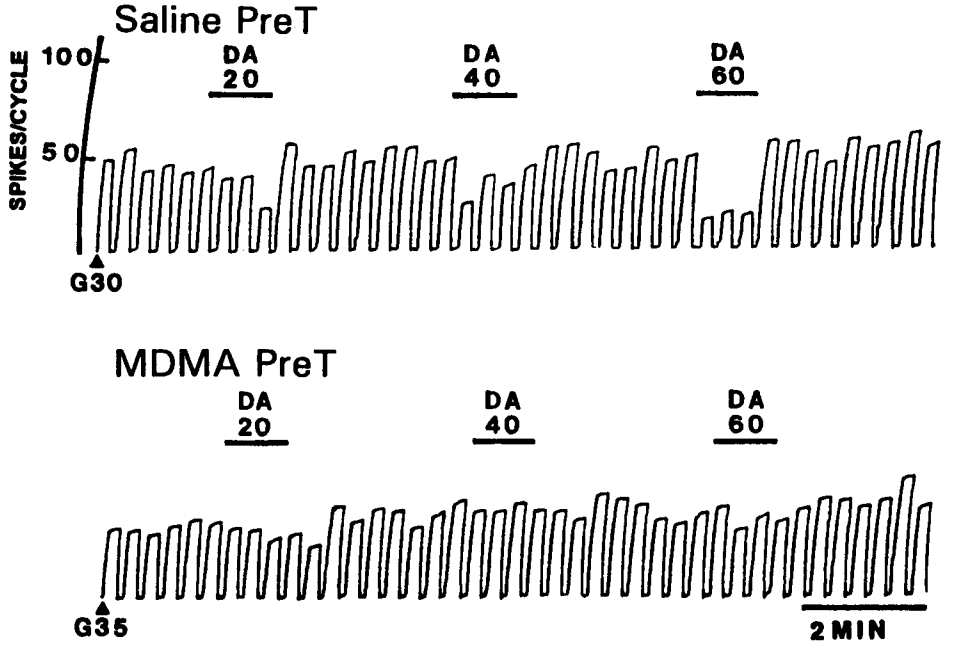


Fig. 14. Effects of DA application on glutamate-evoked firing of cells in the nucleus accumbens core of rats tested 14 days after the last injection of saline (Saline PreT) or MDMA (MDMA PreT). Lines above the records indicate periods of DA ejection and numbers indicate current intensity (nA). Cells were driven by cycled pulses of glutamate (G), and onset of each glutamate application reset the curvilinear oscillograph pen that recorded cumulative spikes. The peak of each histogram indicates the number of spikes that occurred during each glutamate cycle.

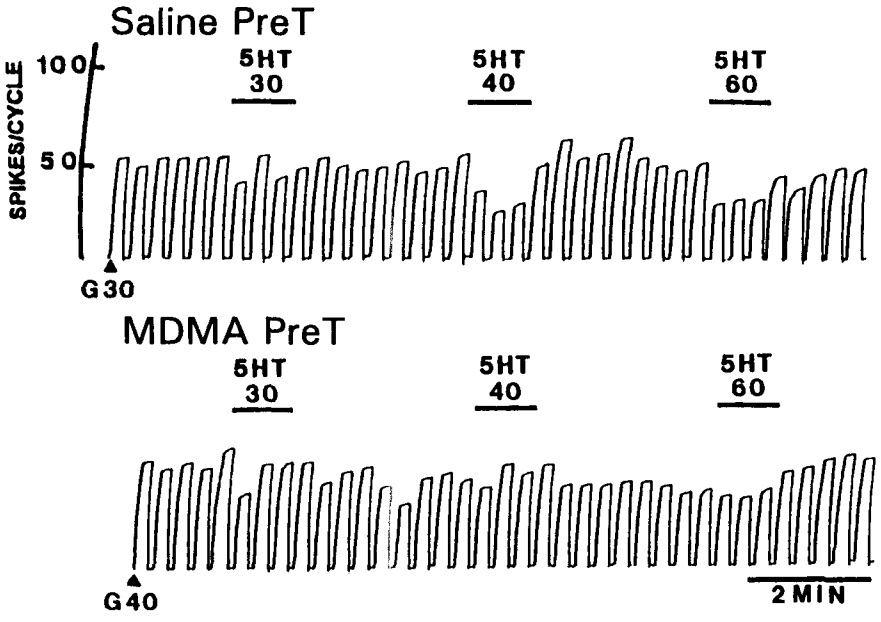


Fig. 15. Effects of 5HT on a nucleus accumbens core cell in a saline-pretreated rat (Saline PreT) and in an MDMA-pretreated (MDMA PreT) are shown. Lines and numbers are as described for Fig. 14.

sympathetic preganglionic neurons that are excited by both NE and 5HT), or by catecholamines released peripherally. Effects of MDMA on excitability of visceromotor neurons in the spinal cord or brainstem have not yet been tested.

## 6. REPEATED MDMA EXPOSURE, EFFECTS ON NEURONAL FIRING IN THE NUCLEUS ACCUMBENS

The neurotoxic effects that repeated MDMA has on 5HT-containing axons in the forebrain of laboratory animals are long-lasting. Levels of 5HT are reduced and 5HT immunoreactive fibers are diminished for several months or longer after the last administration of MDMA in some brain regions, including the striatum and nucleus accumbens (Fischer *et al.*, 1995; Scanzello *et al.*, 1993). It is highly likely, therefore, that toxic doses of MDMA would produce long-term changes in serotonergic and perhaps dopaminergic neurotransmission in the nucleus accumbens. These long-term changes may underlie some of the psychopathologies that are reported by human patients long after the last ingestion of MDMA (Steele *et al.*, 1994). Our laboratory (Obradovic *et al.*, 1995) has investigated whether responses of cells in the nucleus accumbens core to microiontophoretic application of DA or 5HT are altered 9–15 days after the last adminis-

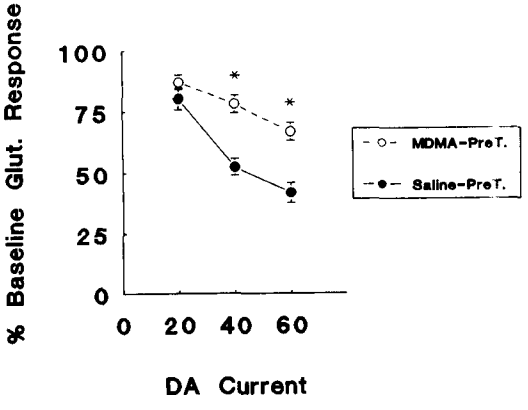


Fig. 16. Dose (current)–response curves for DA effects on glutamate-evoked firing of 33 nucleus accumbens core cells from rats in the MDMA-PreT group and 28 cells from rats in the Saline-PreT group are plotted. *Significant differences ($p < 0.01$) between the cells from the two pretreatment groups, two-way repeated measures analysis of variance with *post hoc* comparisons of means.

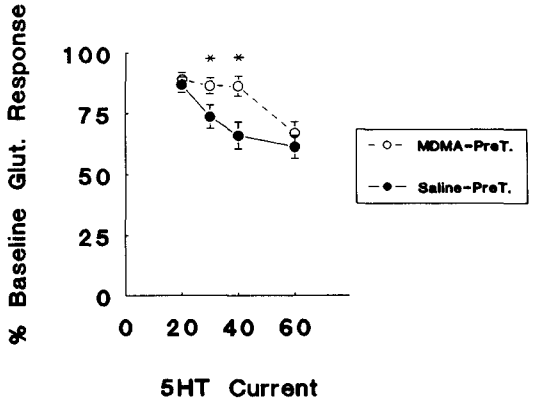


Fig. 17. Dose (current)–response curves for 5HT effects on glutamate-evoked firing of 34 nucleus accumbens core cells from rats in the MDMA-PreT group and 20 cells from rats in the Saline-PreT group. Reponses to 5HT for cells in the two groups were significantly different only for the 30 and 40 nA doses.

tration of an eight-dose sequence of MDMA that is known to be selectively neurotoxic to 5HT axons in rats (20 mg/kg, subcutaneous, twice/day for four successive days). Records of DA and 5HT effects on glutamate evoked firing are compared for cells from saline pretreated (PreT) and MDMA pretreated rats in Fig. 14 and Fig. 15. The inhibitory effects of DA and 5HT on glutamate-evoked firing were virtually abolished in the two cells from MDMA pretreated rats that are shown.

Comparisons of the effects of DA on 33 cells from six MDMA pretreated rats and 28 cells from six saline pretreated rats indicated that the inhibitory effect of DA was significantly ($p < 0.01$) attenuated in the MDMA pretreated rats for all but the lowest dose of MDMA-tested (Fig. 16, two-way repeated measures analysis of variance with *post hoc* comparisons of means). The 5HT was tested on 34 cells from six MDMA pretreated rats and 20 cells from six saline pretreated rats (Fig. 17). The inhibitory effect of the 30 and 40 nA (60 sec) doses of 5HT was attenuated significantly by MDMA-pretreatment, but the highest dose of 5HT (60 nA, 60 sec) was equally inhibitory on cells from both groups of animals. The reduced ability of DA and 5HT to inhibit the nucleus accumbens cells in MDMA pretreated rats was specific in that microiontophoretically applied GABA-produced equivalent dose-dependent inhibitions of glutamate-evoked firing in the nucleus accumbens of both groups of animals. Furthermore, the doses of glutamate that were required to fire cells from both groups of animals at equivalent baseline firing rates did not differ. Thus, pretreatment with neurotoxic doses of MDMA selectively reduced the inhibitory effects of DA and 5HT on nucleus accumbens neurons tested more than a week following the last administration of MDMA.

The finding that the inhibitory effects of DA were reduced following pretreatment with multiple doses of MDMA was opposite to the effect of repeated doses of cocaine. Multiple doses of cocaine, an abused drug that also increases extracellular levels of DA and 5HT in the nucleus accumbens (Kalivas and Duffy, 1990, Kalivas and Duffy, 1993; Parsons and Justice, 1993b), increase the inhibitory effect of DA on glutamate-evoked firing of nucleus accumbens cells (Henry and White, 1991; White *et al.*, 1995a). Cocaine, however, is not neurotoxic to serotonergic axons (Kleven *et al.*, 1988) and this might account for the opposite effects of the two abused drugs. Repeated injections of cocaine might be expected to produced repeated episodes of increased extracellular levels of both DA and 5HT, whereas repeated MDMA injections may produce only an initial episode of increased extracellular 5HT and DA followed by repeated episodes of increased extracellular DA only. Whether this is indeed the means by which repeated exposure to the two abused drugs produces opposite effects on nucleus accumbens neuronal responses to DA and 5HT remains to be determined. Reports of psychopathologies including depression and psychosis following cessation of use are common for frequent users of cocaine and appear to be increasing for frequent users of MDMA, but, interestingly, frequent users of MDMA do not report cravings for MDMA during abstinence from the drug (Schifano and Magni, 1994; Steele *et al.*, 1994). These differential long-term changes in humans may reflect the fact that MDMA is neurotoxic to 5HT-containing axons in the forebrain and cocaine is not.

## 7. CONCLUDING REMARKS

A major mechanism by which MDMA produces euphoric effects is undoubtedly by increasing extracellular levels of DA and 5HT in the nucleus accumbens, a property that it has in common with many other abused drugs such as cocaine, alcohol and opiates. However, the potential ways in which this increased extracellular DA and 5HT might interact with presynaptic and postsynaptic receptors to alter neurotransmission in the nucleus accumbens and in brain regions that project to the nucleus accumbens are exceedingly complex. As a further complexity, the projection neurons from the nucleus accumbens do not seem to act as a functional unit, but rather constitute numerous input/output "neuronal ensembles" that are functionally distinct (Pennartz *et al.*, 1994). Almost nothing is known to date about whether MDMA may affect differentially distinct ensembles within the nucleus accumbens. However, the fact that MDMA is significantly less inhibitory to glutamate-evoked firing in the shell region than in the core suggests that particular locations of the postsynaptic neurons within the accumbens, and perhaps, the neurotransmitter content of the postsynaptic neuron may be determinants of the response to MDMA.

The ability of MDMA to alter neurotransmission in the brain is not restricted to brain regions that are implicated in the rewarding effects of abused drugs. MDMA produces the facilitation of somatic motoneuron excitability that would be expected of a drug that increased extracellular levels of 5HT, DA and NE. It is probable that the muscle hypertonus and increased deep tendon reflexes that occur in some MDMA users are attributable to this action of the drug. It is very likely that MDMA increases extracellular levels of monoamines in every brain region that contains substantial numbers of monoaminergic terminals, and so can mimic most of the neurophysiological effects of monoamines.

It is not yet certain that repeated MDMA ingestion is neurotoxic to forebrain 5HT terminals in humans as it is in laboratory rodents and non-human primates. However, it is now clear that the neurotoxic effects of MDMA in laboratory rats are accompanied by changes in 5HT and DA neurotransmission in the nucleus accumbens that last for at least 2 weeks following the final exposure to MDMA (the longest post-drug time period that has been tested to date). The effects of changes in brain levels of neuropeptides such as CCK and neurotensin that occur following MDMA exposure are just beginning to be studied. Whether changes in the neuropeptides may be long-lasting and what the effects of those changes may be on neurotransmission in the brain are not yet known. However, the numerous and complex effects of MDMA on neurotransmission in the brain combined with increasing reports of psychopathologies from frequent users of MDMA suggest that

repeated exposure to this drug may be much more harmful than seems to be believed by many young recreational users.

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