Perspectives in Pharmacology

3,4-Methylenedioxymethamphetamine (MDMA) as a Unique Model of Serotonin Receptor Function and Serotonin-Dopamine Interactions

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ABSTRACT

(+)-3,4-Methylenedioxymethamphetamine (MDMA; "ecstasy"; "X"; "E") is a popular recreational amphetamine analog that produces a unique set of effects in humans and animals. MDMA use is often associated with dance parties called "raves", but its use has increased in all segments of society and around the world. Like amphetamine, MDMA elicits hyperactivity when administered to rodents. Unlike amphetamine, which has effects mediated by the release of dopamine (DA) from nerve terminals, MDMA-induced hyperactivity is thought to be dependent upon the release of 5-hydroxtryptamine (5-HT). However, MDMA elicits large increases in synaptic concentrations of both DA and 5-HT, and the interaction between these neurotransmitters may account for the unique characteristics of the drug. Comparisons between MDMA, the selective DA releaser amphetamine, and the selective 5-HT releaser fenfluramine are used in the present discussion to highlight the ability of MDMA to model the locomotor activation induced by the interaction of DA and 5-HT. Furthermore, this review summarizes evidence to suggest that the influence of 5-HT receptors on behavioral function is dependent upon the specific neurochemical environment evoked by a given drug, specifically discussed here with regard to the interaction between 5-HT and DA systems.

3,4-Methylenedioxymethamphetamine (MDMA; "ecstasy"; "X"; "E") is an increasingly popular recreational drug in the U.S.A. and abroad. Use of MDMA in teens and young adults occurs commonly in the context of "rave" parties, and the frequency of acute MDMA poisonings (malignant hyperthermia, organ failure, coma, and death) (Fineschi et al., 1999) has been linked to drug use under conditions of dehydration, high temperature, and the extensive strenuous dancing typically experienced at raves (Fineschi et al., 1999). In addition

to this systemic toxicity, exposure to MDMA damages the terminals of serotonin (5-hydroxytryptamine; 5-HT) neurons resulting in neurotoxicity in animals (Schmidt and Kehne, 1987) and possibly humans with repeated recreational abuse (McCann et al., 2000).

The positive subjective effects of MDMA that presumably account for its popularity include feelings of mental stimulation, emotional warmth, closeness and empathy for others, a general sense of well being, and decreased anxiety (Vollenweider et al., 1998). Enhanced sensory perception is an additional hallmark of the "high" associated with MDMA use (Vollenweider et al., 1998); this profile is dissimilar to that evoked by the chemically similar psychostimulant amphetamine and the hallucinogen mescaline. The mode of action for MDMA is based upon its ability to bind to the transporters for 5-HT, dopamine (DA), and norepinephrine (Slikker et

ABBREVIATIONS: (+)-MDMA, (+)-3,4-methylenedioxymethamphetamine; 5-HT, 5-hydroxytryptamine; DA, dopamine; DOI, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; GR 127935, [2′-methyl-4′-(5-methyl-(1,2,4)oxadiazol-3-yl)-biphenyl-4-carboxylic acid (4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl-amide); M100907, [R-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol]; NAc, nucleus accumbens; RU 24969, [5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole]; SB 206553, [N-3-pyridinyl-3,5-dihydro-5-methylbenzo(1,2-b: 4,5-b′)dipyrrole-1(2H) carboxamide]; VTA, ventral tegmental area; SERT, 5-HT transporter; DAT, DA transporter; R, receptor(s); SN, substantia nigra; GABA, γ-aminobutyric acid; MCPP, m-chlorophenylpiperazine

al., 1989), resulting in the release of monoamine neurotransmitters via reversal of the transporter (Rudnick and Wall, 1992). However, while enhanced DA neurotransmission is thought to predominantly mediate the behavioral effects of amphetamine, a unique contribution of 5-HT has been proposed to underlie the neuropsychopharmacology of MDMA (Callaway et al., 1991; McCreary et al., 1999). Thus, the goal of this review is to summarize data supporting the role of specific 5-HT receptors in mediating the in vivo effects of MDMA and to critically analyze the role for 5-HT-DA interactions in the behavioral effects of MDMA. Furthermore, this review will highlight evidence to suggest that the neurochemical environment produced by MDMA provides a unique model of the link between neurotransmitter function and behavior, specifically targeting the interaction between 5-HT and DA. Finally, this review will represent the authors' perspective that the 5-HT system is dynamic and may function in starkly different ways, depending upon the neurochemical environment in the brain.

The literature discussed in this review covers doses of MDMA ranging from "low" [3 mg/kg (+)-MDMA, the more potent isomer] to "high" [20 mg/kg ($\pm$)-MDMA]. The contrast between low and high doses provides evidence that differing doses of MDMA elicit unique effects. Because neuropharmacological studies of the reinforcing and discriminative stimulus effects of MDMA are limited, we focus here on the better-described effects of MDMA on locomotor activity. To appreciate the distinctive aspects of MDMA, we compare MDMA with its congeners, the DA releaser amphetamine and the 5-HT releaser fenfluramine. These studies indicate that 5-HT plays an intricate role in the behavioral effects of MDMA dependent on the tone of DA neurotransmission. Furthermore, 5-HT receptors appear to function in a manner that is unique to the neurophysiological environment elicited by MDMA, setting the stage for its distinctive set of emotional, psychological, and perceptual sequelae and unique pattern of abuse.

MDMA as a Psychostimulant

The drug-induced behavioral syndrome associated with MDMA differs from that evoked by either amphetamine or fenfluramine. Both MDMA and amphetamine robustly increase locomotor activity in rodents (Gold et al., 1989; Gold and Koob, 1989), but the pattern of activity evoked by the two drugs is qualitatively different. Amphetamine increases locomotion throughout the activity monitor, while the activity evoked by MDMA is confined predominantly to the periphery of the chamber (Rempel et al., 1993). On the other hand, hypomotility is evoked by fenfluramine in animals naïve to the test environment (Aulakh et al., 1988), and no change in activity levels is seen following fenfluramine administration in animals habituated to the test environment (M. G. Bankson and K. A. Cunningham, submitted). At higher doses, MDMA (7.5 mg/kg ($\pm$)-MDMA) (Spanos and Yamamoto, 1989) and amphetamine (Ellinwood and Balster, 1974) can evoke repetitive, stereotypical movements, such as head weaving and sniffing, although the stereotypies evoked by high doses of MDMA more closely resemble components of the "5-HT syndrome", including flat body posture, lateral head weaving, forepaw treading, and piloerection (Spanos and Yamamoto, 1989). Fenfluramine, as a more selective

5-HT releaser, can evoke most components of the full 5-HT syndrome (e.g., hyperactivity, hyperreactivity, hindlimb abduction, lateral head weaving, reciprocal forepaw treading, rigidity, Straub tail, and tremor) (Trulson and Jacobs, 1976).

These distinct effects of MDMA, amphetamine, and fenfluramine are apparently based upon the differential interactions of these drugs with the monoamine substrates underlying these behaviors (see Fig. 1). Upon binding to the monoamine transporters, MDMA binds with highest affinity to the 5-HT transporter (SERT) and inhibits 5-HT reuptake into hippocampal synaptosomes ($EC_{50} = 0.35 \pm 0.03$ μM) more potently than DA uptake into striatal synaptosomes ($EC_{50} = 1.14 \pm 0.03$ μM) (Crespi et al., 1997). On the other hand, amphetamine binds with highest affinity to the DA transporter (DAT) and inhibits DA reuptake into striatal synaptosomes ($EC_{50} = 0.13 \pm 0.04$ μM) more potently than 5-HT reuptake into hippocampal synaptosomes ($EC_{50} = 4.51 \pm 0.64$ μM). Lastly, fenfluramine binds with highest affinity to SERT and is a much more potent inhibitor of 5-HT reuptake ($EC_{50} = 0.90 \pm 0.40$ μM) over DA reuptake ($EC_{50} = 11.2 \pm 0.1.3$) (Crespi et al., 1997). It is important to note, however, that although MDMA has a higher affinity for the 5-HT transporter, there is a greater total efflux of extracellular DA over that seen for 5-HT at behaviorally active doses (White et al., 1996). This may be related to higher basal DA levels in a given brain region or to the potentially higher maximal response of the DA system to MDMA over the 5-HT system (for review, see White et al., 1996).

A vast body of literature suggests a significant role for DA in the mediation of the psychomotor stimulation evoked by amphetamine, and neuropharmacological analyses indicate that DA also plays a role in the behavioral effects of MDMA (Gold et al., 1989). However, some unique characteristics of the behavioral effects of MDMA appear to be related to preferential release of 5-HT from nerve terminals (Callaway et al., 1990). Thus, the focus of this review will be to use a comparison of MDMA, fenfluramine, and amphetamine to illustrate that the combination of 5-HT and DA release elicited by MDMA produces a unique behavioral response. More specifically, we will focus on the changing nature of the role of 5-HT$_1$ receptors (5-HT$_1$R) and 5-HT$_2$ receptors (5-HR$_2$R) in mediating the behaviors associated with these drugs.

Serotonin released from terminals by MDMA will expose seven classes of 5-HT receptors and 14 distinct 5-HT receptor subtypes (Barnes and Sharp, 1999) to the endogenous ligand. The 5-HT$_1$ receptor (5-HT$_{1A}$R, 5-HT$_{1B}$R, 5-HT$_{1D}$R, 5-HT$_{1E}$R, and 5-HT$_{1F}$R) exhibits high affinity for 5-HT, is generally negatively linked to adenylyl cyclase activity, and causes induction of membrane hyperpolarization (Barnes and Sharp, 1999). The 5-HT$_2$R (5-HT$_{2A}$R, 5-HT$_{2B}$R, and 5-HT$_{2C}$R) exhibits slightly lower affinity for 5-HT. Stimulation of 5-HT$_2$R evokes a depolarization of the cell membrane via a phospholipase C-mediated activation of the inositol 1,4,5-trisphosphate/diacylglycerol pathway; a 5-HT$_2$R-mediated stimulation of the arachidonic acid cascade via phospholipase A$_2$ has also been identified (Barnes and Sharp, 1999). Although the other 5-HT receptors (i.e., 5-HT$_3$R, 5-HT$_4$R, 5-HT$_{5A}$R, 5-HT$_6$R, and 5-HT$_7$R) may be important in the effects of MDMA and other psychostimulants, the present review focuses on the role of 5-HT$_{1B}$R, 5-HT$_{2A}$R, and 5-HT$_{2C}$R in mediating the behavioral effects of MDMA.

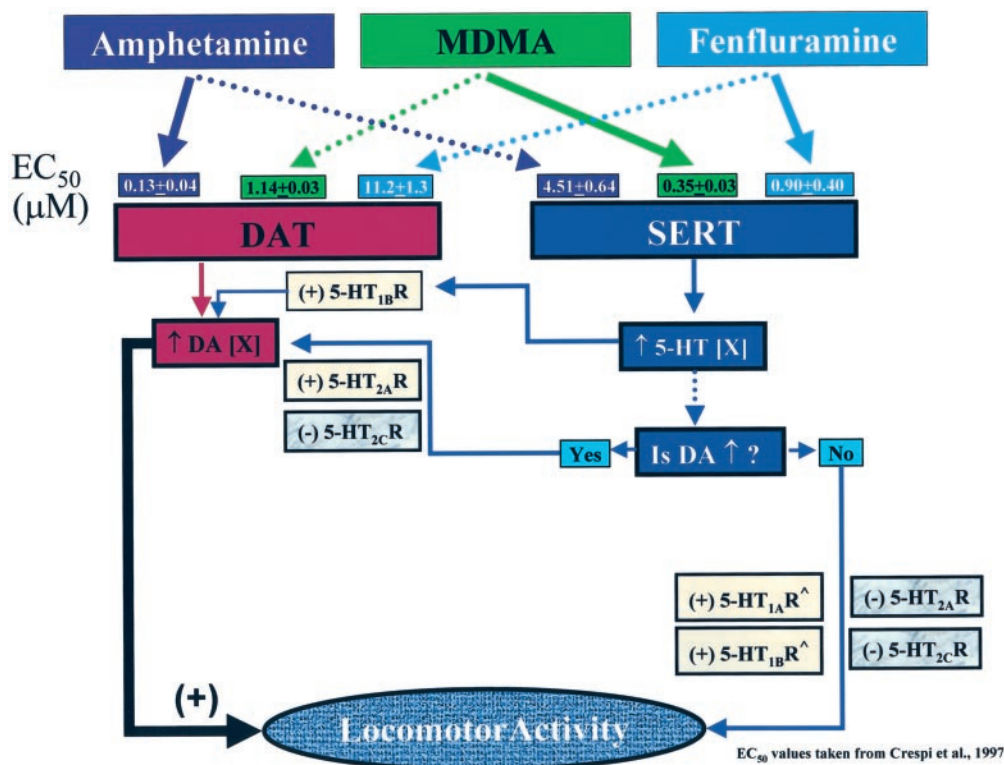Serotonin neurons innervate DA nigrostriatal and meso-

Fig. 1. Schematic representation of the effects of 5-HT$_1$R and 5-HT$_2$R on hyperactivity mediated by amphetamine, MDMA, and fenfluramine. The relative influence of amphetamine (purple), MDMA (green), and fenfluramine (aqua) on the DAT and SERT is illustrated with the primary action of each drug represented by the solid line, while secondary actions are represented by the dotted lines. The EC$_{50}$ (μM) values for each drug are given for inhibition of radiolabeled DA and 5-HT into synaptosomes (Crespi et al., 1997). The consequent increase in predominantly DA efflux associated with amphetamine inhibition of the DAT (pink) results in an enhancement in locomotor activity. The actions of MDMA at the SERT (dark blue) and the DAT (pink) result in simultaneous 5-HT and DA efflux, both of which appear to be necessary for MDMA to evoke hyperactivity (see text). Fenfluramine preferentially acts at the SERT, with little affinity for the DAT, and increases 5-HT efflux but does not evoke hyperactivity. The differential regulatory impact of 5-HT$_{1A}$R, 5-HT$_{1B}$R, 5-HT$_{2A}$R, and 5-HT$_{2C}$R on locomotor activity appears to be dependent upon the neurochemical environment, in particular the degree of elevation of DA efflux. The role of individual receptors is described by (+), increases in DA efflux or locomotor activity, or (−), decreases in DA efflux or locomotor activity preceding each receptor type or adjacent to arrows. The caret (ˆ) represents possible, but unproven, non-DA mediated effects of 5-HT receptors on locomotor activity.

corticolimbic circuits, including the projection from DA cell bodies in the substantia nigra (SN) and ventral tegmental area (VTA) to the dorsal striatum and nucleus accumbens (NAc), respectively, pathways critical in mediating the behavioral effects of psychostimulants. The 5-HT$_{1B}$R, 5-HT$_{2A}$R, and 5-HT$_{2C}$R are among the 5-HT receptors that have been suggested to control brain DA function and also play a role in the behavioral effects of MDMA. The 5-HT$_{1B}$R (and its homolog, 5-HT$_{1D}$R) functions presynaptically as an inhibitory autoreceptor located on terminals of 5-HT neurons and postsynaptically as an inhibitory heteroreceptor to control release of neurotransmitters (Barnes and Sharp, 1999). Localization and lesion studies (Boschert et al., 1994) support the hypothesis that 5-HT$_{1B}$R are localized to the axon terminals of γ-aminobutyric acid (GABA) efferents emanating from the striatum and NAc that provide inhibitory feedback to the origins of nigrostriatal and mesoaccumbens DA pathways. Stimulation of 5-HT$_{1B}$R by direct (5-HT) or indirect agonists (e.g., cocaine) has been shown to inhibit GABA release from terminals that innervate DA neurons in the substantia nigra (Johnson et al., 1992) and VTA (Cameron and Williams, 1994) suggesting an important role for the 5-HT$_{1B}$R in the control of DA function. In support of this hypothesis, microdialysis studies have shown that 5-HT$_{1B}$R agonists facilitate release of dopamine in the NAc (Parsons et al., 1999) and striatum (Ng et al., 1999).

The best characterized 5-HT$_2$R in brain are the 5-HT$_{2A}$R and 5-HT$_{2C}$R (formerly known as 5-HT$_{1C}$R), which have a high degree of homology in their amino acid sequences (Barnes and Sharp, 1999). Modest levels of 5-HT$_{2B}$R are found in brain (Duxon et al., 1997); however, empirical evidence to support or refute a role for central or peripheral 5-HT$_{2B}$R in behavior is limited (see McCreary and Cunningham, 1999, for discussion). The 5-HT$_{2A}$R is synonymous with the classical 5-HT$_2$R and has been implicated in hallucinosis, psychosis, and affective disorders (Barnes and Sharp, 1999). While a tonic role for 5-HT$_2$R to control DA release is debatable (Parsons and Justice, 1993), there is evidence to support the possibility that 5-HT$_{2A}$R may play a "permissive" role in the activation of the DA system consequent to elevated 5-HT activity (Sorensen et al., 1993). In contrast, 5-HT$_{2C}$R appear to limit basal and stimulated DA release in mesoaccumbens and nigrostriatal DA pathways (Di Matteo et al., 2000; Lucas and Spampinato, 2000). For the mesoaccumbens pathway, this control appears to occur at the level of both the VTA and NAc (Benloucif and Galloway, 1991; Prisco et al., 1994). To complicate matters further, DA has also been shown to increase 5-HT release (Matsumoto et al., 1996), and these effects appear to be mediated, at least in part, by stimulation of specific DA receptors. Thus, 5-HT and DA interact via a number of mechanisms, some of which are controlled by 5-HT$_{1B}$R, 5-HT$_{2A}$R, and 5-HT$_{2C}$R. The manner in which

these receptors contribute to the behavioral effects of MDMA is considered below.

Role of 5-HT$_1$ Receptors

An indirect activation of 5-HT$_{1B}$R has been proposed as important to the hyperactivity evoked by MDMA based upon the observation that 5-HT agonists with affinity for 5-HT$_{1B}$R (see Table 1) elicit a behavioral profile similar to that for low doses of MDMA (Rempel et al., 1993). For example, hyperactivity induced by the direct 5-HT$_{1A/1B}$R agonist RU 24969 is blocked by the 5-HT$_{1B/1D}$R antagonist GR 127935, which exhibits no affinity for 5-HT$_{1A}$R (O'Neill et al., 1996). The observation that nonselective 5-HT$_{1A/1B}$R antagonists (e.g., methiothepin and propranolol) blocked MDMA-induced hyperactivity is also in keeping with this hypothesis (Callaway et al., 1992; Kehne et al., 1996). More recent results have shown that GR 127935 potently and completely reversed the hyperactivity caused by a low dose (3 mg/kg) of (+)-MDMA (McCreary et al., 1999) and that transgenic mice lacking the 5-HT$_{1B}$R do not express MDMA-induced hyperactivity (Scearce-Levie et al., 1999). These data solidly demonstrate a critical role for 5-HT$_{1B}$R in MDMA-evoked hyperactivity. However, it is important to note that the 5-HT$_{1A}$R agonist 8-hydroxy-2-(di-n-propylamino)tetralin can also induce a prominent forward locomotion (De La Garza and Cunningham, 2000) that is blocked by 5-HT$_{1A}$R antagonists (Suwabe et al., 2000), and at least one report suggests that a 5-HT$_{1A}$R antagonist can attenuate RU 24969-evoked hyperactivity in mice (Kalkman, 1995). Studies from our laboratory have shown, however, that the selective 5-HT$_{1A}$R antagonist WAY 100635 did not block hyperactivity evoked by a low dose of MDMA (McCreary et al., 1999). Therefore, even though 5-HT$_{1A}$R activation can lead to hypermotility, 5-HT$_{1A}$R stimulation does not seem to be necessary or sufficient for MDMA to evoke hyperactivity.

These data seem to suggest that MDMA-induced 5-HT release leads to the activation of 5-HT$_{1B}$R and thus to increases in locomotor activity; however, an important prediction based upon this hypothesis is that fenfluramine should evoke hyperactivity, which is not the case (Aulakh et al., 1988). MDMA-induced hypermotility can also be inhibited by 6-hydroxydopamine lesion of the NAc (Gold et al., 1989), suggesting that selective 5-HT release (fenfluramine) does

not produce locomotor activation because the neurochemical profile of fenfluramine lacks a dopaminergic component. In other words, 5-HT$_{1B}$R activation is necessary for locomotor hyperactivity induced by the direct 5-HT$_{1A/1B}$ agonist RU 24969 and MDMA but is not sufficient to elicit hyperactivity subsequent to the administration of the selective 5-HT releaser fenfluramine. This could possibly be due to the distinct absence of activation of DA systems via reversal of the transporter following administration of this drug (see Fig. 1).

The Role of the 5-HT$_2$ Receptors

Neuropharmacological analyses of the 5-HT$_2$R involvement in the behavioral effects of MDMA have been historically hampered, as is the case for all studies of this nature, by a lack of ligands that differentiate among its subtypes, especially between the 5-HT$_{2A}$R and 5-HT$_{2C}$R. Although the last decade has seen the development of selective 5-HT$_{2A}$R [e.g., M100907 (MDL 100907); Sorensen et al., 1993] and 5-HT$_{2B/2C}$R antagonists (e.g., SB 206553; Kennett et al., 1996) (see Table 1), the behavioral consequences associated with stimulation of 5-HT$_{2A}$R and 5-HT$_{2C}$R were initially deduced from the study of 5-HT$_{2C/1B}$R agonists, such as MK 212 and m-chlorophenylpiperazine (MCPP) (for review, see Lucki, 1992) and the 5-HT$_{2A/2B/2C}$R agonist (±)-1-(2,5-dimethoxy-4-iodo)-2-aminopropane (DOI), which has equal affinity for all 5-HT$_2$R subtypes (Barnes and Sharp, 1999). With regard to activity levels in naïve, unhabituated rats, administration of MK 212, MCPP (Lucki et al., 1989), and DOI (Krebs-Thomson et al., 1998) all produce hypomotility; DOI-induced hypomotility is reportedly blocked by the 5-HT$_{2A}$R antagonist M100907 (Krebs-Thomson et al., 1998). Nullification of the 5-HT$_{2C}$R by either pharmacological antagonism (e.g., SB 206553; Gleason and Shannon, 1998) or knockout mutation in mice (Heisler and Tecott, 2000) resulted in a loss of MCPP-induced *hypomotility* and unmasked an MCPP-induced *hypermotility*, presumably related to the affinity of MCPP for 5-HT$_{1B}$R because this unmasked hyperactivity was blocked by the 5-HT$_{1B}$R antagonist GR 127935 (Gleason and Shannon, 1998; Heisler and Tecott, 2000). These data suggest that 5-HT$_2$R stimulation may account for hypoactivity induced by such nonselective agonists as MK 212, MCPP or DOI. More importantly, these data illustrate the possibility that activation of 5-HT$_{2C}$R by direct agonists or subsequent to 5-HT release can limit or mask the hyperactivity induced by direct or indirect agonists (e.g., MDMA) that can effectively act at both 5-HT$_{2C}$R and 5-HT$_{1B}$R.

Antagonism of 5-HT$_{2A}$R with M100907 resulted in a blockade of the hypermotility induced by a high dose (20 mg/kg) of (±)-MDMA (Kehne et al., 1996), as well as that evoked by amphetamine (Moser et al., 1996), cocaine (McMahon and Cunningham, 2001), and DA reuptake inhibitors (McMahon and Cunningham, 2001). In contrast, hyperactivity elicited by a low dose (3 mg/kg) of (+)-MDMA was unaffected by M100907 (M. G. Bankson and K. A. Cunningham, submitted) but blocked by GR 129735 (McCreary et al., 1999). The dose-dependent nature of its sensitivity to M100907 suggests a differential role of the 5-HT$_{2A}$R in the control of MDMA-evoked hyperactivity. Although the mechanisms, triggers, and sites of action for 5-HT$_{2A}$R to control stimulated DA function have not yet been thoroughly clarified, M100907 has been proposed to block 5-HT$_{2A}$R which putatively control DA

TABLE 1
Drug selectivity for 5-HT transporters and receptors

Releasers	Transporter Selectivity
MDMA	5-HT > DA
Amphetamine	DA ≫ 5-HT
Fenfluramine	5-HT ≫ DA

Agonists	Receptor Selectivity
DPAT	5-HT$_{1A}$
RU 24969	5-HT$_{1A}$ = 5-HT$_{1B}$
MCPP	5-HT$_{2C}$ > 5-HT$_{1B}$ > 5-HT$_{2A}$
DOI	5-HT$_{2A}$ = 5-HT$_{2B}$ = 5-HT$_{2C}$

Antagonists	Receptor Selectivity
WAY 100635	5-HT$_{1A}$
GR 127935	5-HT$_{1B/1D}$
M100907	5-HT$_{2A}$
SB 206553	5-HT$_{2B}$ = 5-HT$_{2C}$

DPAT, 8-hydroxy-2-(di-n-propylamino) tetralin.

synthesis under conditions of stimulated DA neurotransmission (Sorensen et al., 1993; Lucas and Spampinato, 2000). Assuming this to be the case, the dose-dependent nature of the sensitivity of MDMA-evoked hyperactivity to M100907 suggests a differential role of the 5-HT$_{2A}$R in the control of hyperactivity, possibly dependent upon prevalent levels of 5-HT, DA, and/or MDMA (M. G. Bankson and K. A. Cunningham, submitted). One other possibility is that, at high doses of MDMA, the probability of 5-HT$_{2A}$R stimulation increases as either released 5-HT or MDMA itself could bind to 5-HT$_{2A}$R (Battaglia et al., 1988). At lower doses, a preferential stimulation of 5-HT$_{1B/1D}$R by released 5-HT itself may occur since the affinity of 5-HT for 5-HT$_{1B/1D}$R is higher than for 5-HT$_2$R (Barnes and Sharp, 1999).

Taken together, these data suggest that activation of 5-HT$_{2A}$R, in the absence of reversal of DAT, results in hypoactivity (as in the case of DOI), but in the case of amphetamine or high dose MDMA, activation of 5-HT$_{2A}$R has a potentiative, or at least permissive, role in hyperactivity. Furthermore, the differential effects of M100907 on hyperactivity evoked by a low versus high dose of MDMA may be attributable to a changing relative importance for DA and 5-HT at different doses of MDMA. In other words, by releasing more DA, a higher dose of MDMA may increase the role of DA as compared with the role of this effector at a lower dose of MDMA.

Pretreatment with the nonselective 5-HT$_2$R antagonist methysergide (Gold and Koob, 1988) or the selective 5-HT$_{2B/2C}$R antagonist SB 206553 (M. G. Bankson and K. A. Cunningham, submitted) more than doubled the level of locomotor activation observed after a low dose of MDMA; this potentiation was partially attenuated by GR 127935 (M. G. Bankson and K. A. Cunningham, submitted). In keeping with this observation, a 5-HT$_{1B}$R-dependent MCPP-induced hyperactivity was also seen in 5-HT$_{2C}$R knockout mice (Heisler and Tecott, 2000) and after pretreatment with a 5-HT$_{2C}$R antagonist in mice (Gleason and Shannon, 1998). These findings suggest divergent roles for 5-HT$_{2A}$R and 5-HT$_{2C}$R in modulating the effects of MDMA, as well as direct 5-HT$_2$R agonists, and support the hypothesis that 5-HT$_{2C}$R activation (in the case of MDMA, subsequent to 5-HT release) can inhibit or mask the hyperactivity evoked by 5-HT$_{1B}$R stimulation. However, this simple explanation is complicated by the fact that a robust hyperactivity was *not* evoked by the selective 5-HT releaser fenfluramine after blockade of 5-HT$_{2C}$R with SB 206553 (M. G. Bankson and K. A. Cunningham, submitted). Therefore, the unmasking of a 5-HT$_{1B}$R-mediated hyperactivity upon blockade of 5-HT$_{2C}$R must be dependent on factors in addition to synaptic overflow of 5-HT, such as above baseline 5-HT$_{2C}$R activation in the presence of either direct 5-HT$_{1B}$R activation (MCPP) or elevated DA release (MDMA). Thus, the contrast between the neuropharmacological profiles of MDMA and fenfluramine serves to reinforce the hypothesis that at least some 5-HT receptors (e.g., 5-HT$_{2C}$R) exhibit diversified roles in the control of behavior that may be dependent upon the extant neurochemical milieu.

The Nature of 5-HT and DA Interaction

Countless studies have implicated a critical role for DA release in the striatum and NAc in mediating the hypermo-tive, stimulus, rewarding, and other behavioral effects elicited by psychostimulants such as cocaine, amphetamine, and MDMA. The ability of 5-HT to affect the manner and magnitude of DA release is also an important factor in analysis of the actions of psychostimulants, particularly drugs such as MDMA, which elevates both synaptic 5-HT and DA. The comparison between MDMA, amphetamine, and fenfluramine has shown that MDMA produces a set of behaviors that is qualitatively unlike that evoked by either amphetamine or fenfluramine. This observation is supported by the animal studies described above and self-report studies with humans that indicate that these drugs produce very different subjective effects (Chait et al., 1986; Cohen, 1995). The MDMA literature suggests that this is related to the ability of MDMA to release both DA and 5-HT; however, as noted above, the ability of MDMA to release DA does not depend completely on the action of MDMA at the DA transporter. Microdialysis studies have shown that blocking MDMA-induced 5-HT release by neurotoxic lesion or pharmacological blockade of the 5-HT transporter or 5-HT$_{2A}$R causes a substantial decrease in the amount of subsequent DA release (Yamamoto et al., 1995; Gudelsky and Nash, 1996). In fact, MDMA-evoked increases in DA efflux in the SN and striatum were shown to be partly impulse-dependent and to occur in parallel with a decrease in GABA release in SN; local perfusion of the 5-HT$_2$R antagonist ritanserin blocked these neurochemical effects of MDMA suggesting that 5-HT$_2$R, perhaps 5-HT$_{2A}$R, control DA efflux in SN and striatum in part via GABAergic innervation of the SN. Thus, the DA release evoked by MDMA occurs via reversal of the DAT (Rudnick and Wall, 1992) and secondary to released 5-HT acting at 5-HT receptors to increase normal, vesicular release of DA (Yamamoto et al., 1995; Gudelsky and Nash, 1996).

Studies with amphetamine indicate that reversal of the DAT is sufficient to cause robust hyperactivity (Kelly and Iversen, 1976). Studies with fenfluramine indicate that reversal of the SERT, along with any subsequent 5-HT-mediated DA release, is not sufficient to cause hyperactivity (Aulakh et al., 1988; M. G. Bankson and K. A. Cunningham, submitted). Finally, the ability of MDMA to cause a 5-HT$_{1B}$R-dependent hyperactivity leads to the question of why activation of 5-HT$_{1B}$R mediates hyperactivity subsequent to MDMA (and direct agonists like RU 24969) but not fenfluramine. The answer may lie in the fact that MDMA-induced locomotor activation is dependent on reversal of both the SERT and the DAT. One possibility is that an additive or synergistic effect on DA release overcomes the hypoactivity that selective release of 5-HT (as with fenfluramine) might produce. A second, more complex, model of 5-HT-DA interaction incorporates the possible changing hierarchy of relevance of individual 5-HT receptor subtypes and subpopulations in response to the activation of other 5-HT and DA receptors. In other words, during periods of elevated DA, 5-HT receptors that mediate or potentiate hyperactivity become more dominant or 5-HT receptors that mediate hypoactivity become less dominant (see Fig. 1).

In the case of amphetamine-induced DA release, antagonist studies have shown that 5-HT$_{2A}$R activation is necessary for maximal amphetamine-induced hyperactivity to occur (Moser et al., 1996). Because amphetamine does not cause as large an increase in the concentration of synaptic 5-HT (Kuczenski and Segal, 1989) when compared with MDMA, less

activation of 5-HT$_{2A}$R would be expected. The question then remains: is the efficacy of 5-HT$_{2A}$R antagonists to block amphetamine-induced activity due to antagonism of basal 5-HT$_{2A}$R activation or to antagonism of elevated 5-HT$_{2A}$R activation secondary to amphetamine? More simply, does amphetamine-induced hyperactivity require above-basal activation of 5-HT$_{2A}$R? In the case of MDMA administration, the requirement for elevated 5-HT levels, as noted above, is not in question. Dopamine is released 1) by reversal of the DAT and 2) secondarily to 5-HT release via stimulation of 5-HT$_{1B}$R, 5-HT$_{2A}$R, and/or other 5-HT receptors. These 5-HT receptors, under conditions of elevated 5-HT, have greater receptor occupancy than after amphetamine administration and thus may play a greater role in mediating the unique effects of MDMA. This is supported by the prominent role of the 5-HT$_{1B}$R in mediating MDMA-induced activity. The combination of elevated 5-HT and DA subsequent to MDMA administration may also lead to a more dominant role for the 5-HT$_{2A}$R in the effects of MDMA. Although not effective against a low dose of (+)-MDMA (M. G. Bankson and K. A. Cunningham, submitted), the ability of the 5-HT$_{2A}$R antagonist M100907 to block hyperactivity evoked by a high dose of (±)-MDMA (Kehne et al., 1996) indicates, as with amphetamine, a potential role for the 5-HT$_{2A}$R during periods of elevated DA efflux. It remains to be seen if the elevated 5-HT levels associated with MDMA and the enhanced 5-HT$_{2A}$R occupancy lead to a more important role for 5-HT$_{2A}$R in the effects of MDMA versus amphetamine.

The ability of the 5-HT$_{2B/2C}$R antagonist SB 206553 to robustly potentiate activity induced by a low dose of (+)-MDMA (M. G. Bankson and K. A. Cunningham, submitted) indicates that the combination of elevated 5-HT and DA produces a neurochemical environment that manifests a significant inhibitory role for the 5-HT$_{2C}$R. Again, the question remains: does the greater elevation of 5-HT efflux make the role of the 5-HT$_{2C}$R more significant for MDMA versus amphetamine? While logic predicts this to be the case, empirical evidence in support of this hypothesis has not been established. On the other hand, the lack of hyperactivity induced by SB 206553 administered in combination with fenfluramine (M. G. Bankson and K. A. Cunningham, submitted) implies that 5-HT release, and any DA release secondary to activation of 5-HT receptors (Benloucif and Galloway, 1991), cannot evoke a neurochemical environment that leads to a significant inhibitory role for the 5-HT$_{2C}$R.

The unpredictable aspects of 5-HT pharmacology suggested by the above studies may be related to any one of a number of characteristics of the 5-HT system. In the simplest case, different populations of the same receptor subtype might become dominant under different environments in the brain, such as activation of other receptors and neurotransmitter systems. In the examples discussed here, the presence and possibly magnitude of elevated DA efflux contributes significantly to the behavioral outcomes associated with activation of specific 5-HT receptors. Other aspects of the 5-HT system that add to its complexity include different affinities of the various 5-HT receptors for 5-HT (Barnes and Sharp, 1999), differential effects of 5-HT agonists on second-messenger systems (agonist-directed trafficking), and aspects of receptor desensitization. The fact that 5-HT has higher affinity for 5-HT$_1$R as compared with 5-HT$_2$R (Barnes and Sharp, 1999), coupled with the ability of 5-HT receptors to desensi-

tize, may be of particular importance in the analysis of dose-related differences in the effects of neurotransmitter releasers such as MDMA. For example, a low dose of MDMA may cause little receptor desensitization, while the effects of a larger dose may depend on receptor desensitization. Thus, a very complex set of parameters is important in the manner by which a drug such as MDMA affects behavior via the 5-HT system.

In conclusion, the apparent plasticity of the 5-HT system makes for an infinitely complex system. When studying the effects of 5-HT receptor activation associated with a given pharmacological compound (such as MDMA), the results cannot be reliably extrapolated to other drugs or to other neurochemical environments in the brain. The study of MDMA will help to empirically define the net behavioral outcome associated with activation of multiple 5-HT receptors and the interaction of DA and 5-HT under the specific profile of elevated 5-HT and DA release, a circumstance unique to MDMA. The comparison between MDMA, fenfluramine, and amphetamine demonstrates the dependence of 5-HT receptor function on the neural environment. Furthermore, these comparisons implicate DA as a factor in determining the outcome of 5-HT receptor activation in the brain and the accompanying effect on behavior. Although we have focused our discussion on elicitation of hyperactivity, it will be of particular interest to determine the applicability of these hypotheses to other behaviors, including the reinforcing and discriminative stimulus effects of MDMA that provide models of the abuse liability and subjective effects of MDMA.

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