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Brian John Cummings

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THE ELECTROPHYSIOLOGICAL EFFECTS OF MDMA "ECSTASY" ON DOPAMINE NEURONS OF THE VENTRAL TEGMENTAL AREA IN THE RAT

by

Brian John Cummings

THESIS

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Abstract

Drugs of abuse are thought to exert their "rewarding" effects through common sites of action within the central nervous system (CNS). Through behavioral, pharmacological and electrophysiological studies, such an "endogenous reward system" has been mapped out. It is known that the ventral tegmental area (VTA) and the nucleus accumbens (NAc), which is innervated by the A10 dopamine (DA) neurons of the VTA, play crucial roles in this reward system. Cocaine, ampthetamine and many other drugs of abuse have been tested for their electrophysiological effects on DA neurons in the VTA. Though these drugs vary from direct DA receptor agonists to DA reuptake inhibitors, they all affect the rate of DA neuronal firing. 3,4-methylenedioxy-methamphetamine (MDMA), also known by its street name, "Ecstasy," was tested on VTA DA neurons to determine whether it has similar effects. MDMA is unique, however, in that it is reputed to be both a euphoriant (DA mediated) and an hallucinogen (serotonin mediated). Receptor binding studies indicate that in addition to acting as a DA release enhancer, MDMA is even more potent at releasing serotonin (5-HT), thus supporting the serotonin-hallucinogen hypothesis [Schmidt 1986 and Gehlert et al 1985]. Because of MDMA's dual effects in the CNS, two experiments were conducted to determine MDMA's effects on DA neurons in the VTA. In the first experiment, (±) MDMA and (+) MDMA were shown to have little effect at suppressing the firing rate of DA cells, with the (+) enantiomer being slightly more potent then (±). It was hypothesized that this lack of effect might be due to an excitatory serotonergic influence from serotonin-containing neurons projecting from the dorsal raphè nucleus to the VTA. In the second experiment, animals were pretreated with p-chlorophenylalanine (PCPA), an inhibitor of tryptophan hydroxylase, to decrease CNS levels of serotonin. Animals were then given (+) MDMA, the more potent enantiomer, and a significant suppression of DA firing rates resulted. It is suggested that MDMA's mode of action is similar to that of other drugs of abuse, but that

MDMA's inhibitory effects on DA neurons are "masked" by competing excitatory actions caused by enhanced 5-HT release within the VTA.

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Introduction

Man has used drugs for as long as recorded history. From their use in religious rituals to medicinal purposes, drugs have been used by all known societies. While a specific, generally accepted definition of drug abuse has not yet been agreed upon, it is safe to say that every society has been faced with the proble n. Since drug abuse involves not only physical dependence, overt intoxification, or the use of medically unacceptable substances, but also human drug taking behavior, any definition must consider the behavior of the user. Thus, an acceptable and useful definition must take into account social norms as well as the effect of a drug on the individual. The definition given by the National Institute on Drug Abuse (NIDA) defines drug abuse as "the use of a drug for other than medicinal purposes that results in impaired physical, mental, emotional or social well-being of the user." "After all, it is the person that abuses himself rather than the drug which is abused!" [White, 1985].

Alcohol abuse is probably the single largest drug problem facing our society today. Estimates indicate that five to ten percent of the United States population can be considered alcoholics. Leading the list of illegal drugs, and rapidly increasing in use, is cocaine. In fact, NIDA considers cocaine abuse to have reached epidemic proportions. The number of people who report having tried cocaine rose from 5.4 million in 1974 to 21.6 million in 1982 [NIDA 1983]. Estimates are that of the four million people currently using cocaine more than twice a month, over ten percent of them will go on to heavy, uncontrollable drug abuse.

However, cocaine is not the only drug of abuse experiencing increased usage prevalence. 3,4-methylenedioxy-methamphetamine (MDMA), also know by its street name, "Ecstacy," is quickly becoming more popular amoung drug users. Shafer, in Psychology Today [May, 1985], calls the drug "the Yupple psychedelic because of its increasing popularity with the Big Chill generation... {It} sells for about $10 a dose. It is becoming one

of the most sought after psychedelics on the black market." In the same article, Dr. Ronald Siegel of the University of California at Los Angeles School of Medicine estimates that 30,000 doses of MDMA are taken each month. Schmidt et al concurr, labling MDMA as "one of the most popular members of the class of abused substances know as designer drugs [Schmidt et al, 1986]." Adler et al, in Newsweek [April 15, 1985], goes so far as to state:

> "(MDMA) is the drug LSD was supposed to be, coming 20 years to late to change the world... Users say it has the incredible power to make people trust one another, to banish jealousy and to break down the barries that separate lover from lover, parent from child, therapist from patient. Yet unlike LSD, it does not also break down one's ability to distinguish between reality and fantasy, so that it appears free from many of that drugs unfortunate side effects." (p. 96)

In response to MDMA's quick rise in popularity, the Drug Enforcement Administration (DEA), on July 1, 1985, placed MDMA under an emergency Schedule I Controlled Substance Classification. This is the highest restriction possible and denotes compounds with no accepted value and the highest abuse potential. This is the same rating as heroin and LSD, and a full rating higher than cocaine. What is supprising about MDMA's listing is the speed with which it was restricted. Its appointment to Schedule I was the fastest for any drug in the DEA's history.

While the abuse of some drugs is on the rise, the abuse of others has been on the decline in the last few years. Most noteably declining is the abuse of amphetamine (speed, uppers), methaqualone (Quaaludes®) and phencyclidine (PCP). Drug abuse of specific compounds seems to move in cycles. Thus, it is not really a question of a specific drug being abused and reaching epidemic proportions, but rather what appears to be a never ending

cycle of drug abuse where humans abuse one drug after another, as they become popular or available or newly manufactured, as is the case with MDMA.

The Endogenous Reward System

How is it that man can pass from abusing one substance to abusing another? Are there special, untapped receptors in the brain (like the opiate receptors) that await man's discovery of new compounds to abuse? In examining drug abuse, one must keep in mind that drugs of any kind cannot cause magical effects in man; their effects are mediated through alterations of existing receptors within a given neuronal system. These might include actions such as neurotransmitter release enhancement, attenuation, modulation, or by acting as a direct agonist or antagonist. In addition, drugs might alter metabolism, synthesis or catabolism of neurotransmitters. It is highly unlikely that there are special drug receptors in the brain waiting to be discovered like the endorphins. But rather, as with endorphins, receptors already exist and the effects which they mediate are really nothing new to the human body. It was not a magical morphine receptor waiting patiently for our discovery, but rather a natural brain process of pain supression that was discovered. This is also the case with pleasure, euphoria, hallucinations and excitement, i.e. each of these drugs, in their own way, produces an endogenous-like effect.

Why would such pathways exist in the brain? What possible use could euphoria have? One of these very necessary pathways is that which motivates us to eat. Eating, as well as the pleasure of sex or the satiation of thirst are all, in some way, rewarding. As the studies to be discussed later indicate, it is this hypothesized endogenous reward system which is being activated when we receive pleasure from using a drug. It may be necessary to have this system intact in order to enjoy the normal rewarding effects experienced in daily life.

After the discovery that animals would work for the "reward" of electrical stimulation to certain parts of the brain, [Olds and Milner, 1954], scientists began to investigate the characteristics of this "endogenous reward system". After mapping out the anatomical sites where animals would "self-administer" electrical stimulation by pressing a bar, these sites were compared with the known anantomy of different neurotransmitter pathways [Axlerod 1970; Carlsson 1970].

After initial confusion due to overlapping pathways of norepinephrine (NE) and dopamine (DA), pharmcological studies were undertaken to block receptors for either NE or DA and determine whether animals would still self-administer for electrical stimulation. In this manner, an involvement of NE was ruled out because NE specific antagonists failed to affect self-administration behavior, whereas DA receptor blockers (antagonists such as halperidol or pimozide) completely eliminate self-administration for electrical stimulation [Davis and Smith 1975; deWit and Wise 1978; Risner and Jones 1976; and Yorkel and Wise 1975, 1976]. Futher evidence that DA is the primary neurotransmitter involved in endogenous reward is through studies of self-administration of drugs. Animals self-administer these drugs by bar pressing to receive injections through intravenous catheters or through cannulae implanted in the brain. Animals will self-administer cocaine, amphetamine and many other drugs of human abuse, but only if DA neurotransmission is not blocked [Roberts et al. 1977, 1980; Lyness et al. 1979; Monaco et al. 1980] (see below).

When 6-hydroxydopamine (6-OHDA, a neurotoxin specific to catecholamine neurons) was injected into the VTA or NAc to destroy DA neurons, responding for cocaine or amphetamine injections was rapidly extinguished [Lyness et al 1979 and Roberts et al 1977, 1980]. Local injections of DA antagonists into these areas will also block self-administration of cocaine or amphetamine [Phillips and Broekkamp 1980]. It has also been shown that direct injection of amphetamine into the NAc is rewarding [Monaco et al., 1980]

as well as injections of amphetamine into the medial prefrontal cortex (MPC) of monkeys [Phillips et al ., 1981].

This is all further evidence that there are several projections of the VTA DA system that are involved in endogenous reward. One way or another though, it is the increase of DA concentrations at the post-synaptic receptors (in the NAc and/or MPC) that may result in positive reinforcement. This can occur through several different mechanisms: 1) the direct stimulation of VTA DA neurons and subsequent increase in the release of dopamine; 2) uptake blockade, which results in increased synaptic concentrations of dopamine; 3) direct stimulation of DA receptors by DA agonists, which mimic the actions of dopamine at post and presynaptic receptors; or 4) blocking the inhibitory effects of other neurons (possibly of the GABA feedback loop) upon VTA cells.

While dopamine (DA) is certainly a crucial link in the endogenous reward system, several lines of evidence indicate that it is only one part of the system. The first is that DA neurons are insensitive to differences in the frequency of stimulation which are critical in self-stimulation studies [Wise 1978]. Several studies show that animals prefer high frequencies of electrical stimulation (100 to 400 Hz) and do not respond as well for frequencies below 40 Hz. This indicates that there must be some other non-dopaminergic link in the reward system since catacholamine (CA) systems are already maximally activated at stimulation frequencies of 20 to 30 Hz. [Wise 1980]. The second is that the refractory periods for the directly activated fibers in self-stimulation studies are short, whereas the refractory periods of CA fibers are long [Yeomans 1979; Shizgal, Bielajew and Yeomans 1979]. Next is the problem that the conduction velocities of the self-stimulation target fibers are considerably faster than those for CA fibers [Shizgal et al., 1980; Wang 1981].

Taken together, these findings indicate that the neurons involved in self-stimulation reward are myelinated. Even though DA fibers are found in the medial forebrain bundle

(MFB, one of the most active self-stimulation sites), these DA neurons are not myelinated [Wang 1981]. Thus self-administration cannot be due to the direct activation of these fibers [Wise 1980], but more likely to the stimulation of myelinated fibers within the MFB. Wise [1980] also suggests that the myelinated MFB fibers synapse directly upon the self-stimulation sites in the lateral hypothalmus and upon self-stimulation and self-administration sites in the ventral tegmental area (VTA).

Shizgal et al. [1980] report that when stimulation pulses are alternated between the lateral hypothalamus and the VTA, evidence of axonal collision is seen. That is:

> "If the pulses to the lateral hypothalmus are given too closely in time to the ventral tegmental pulses, the effects of one of the sets of pulses are blocked. It is assumed that this is due to collision of orthodromic action potentials generated at one of the sites with antidromic potentials generated at the other, and in fact it is an analysis of the critical interval at which pulses must be spaced which provides estimates of the conduction velocity of the fiber assumed to connect these regions [Wise 1980]." (p. 214)

This evidence, coupled with the aforementioned evidence, suggests that the myelinated MFB fibers which are stimulated in self-stimulation studies descend upon (and innervate) the VTA. From reports that DA antagonists are able to attenuate self-stimulation regardless of the proximity of DA fibers to the site of stimulation and also that the MFB does not project caudally beyond the DA cells of the VTA, but does take the exact dorsal-ventral and medial-lateral distribution of these DA cells [Corbett and Wise 1980], we can infer that the VTA is efferent to many MFB fibers.

From the VTA, there are three major anatomical projections: the limbic, the striatal and the cortical. Of these, the limbic system, which is made up in part by the NAc and the MFB connecting the NAc to the VTA, is the most clearly implicated in the actions of

psychomotor stimulants. In the experiments that follow, the serotonergic (5-HT) projections from the dorsal raphè nucleus (B7) to both the NAc and VTA will play an important role. These pathways are thought to play an important role in hallucinations. The dorsal raphè nucleus, implicated in hallucinations, sends its single largest projection of axons directly to the VTA [Phillipson 1979].

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Insert Figure I

"DA Pathways"

About Here

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While it is evident that the VTA is crucial to the reward system, it is equally evident that it is not the only link in the reward chain. The NAc also plays an important role and is linked to the VTA via its own DA fibers and possibly a feedback loop of gamma-aminobutyric acid (GABA) fibers projecting back to the cell bodies interwoven amid the VTA.

Further complicating the theory of endogenous reward is the possibility that rewarding compounds need not directly affect DA neurons. As was briefly mentioned above, there are several possible ways to alter DA firing rates other than direct stimulation. Thus, not only can DA firing rates be changed by reuptake inhibition or other mechnisims, but additionally, feedback pathways or efferent pathways comprised of neurons containing neurotransmitters other than DA can synapse upon VTA neurons and thus indirectly affect DA firing. NE neurons, as well as GABA neurons, which are always inhibitory, synapse directly upon DA neurons of the VTA. Drugs which stimulate these neurons would increase their firing rates resulting in greater inhibition of DA cells. Conversely, drugs which inhibit inhibitory neurons to the VTA would have a net result of disinhibiting DA neurons (ie. they would speed up). Wise [1984], speculates that this disinhibition may be the route of action

of ethanol, barbiturates and benzodiazepines. Since DA itself is an inhibitory neurotransmitter, it is speculated that the rewarding effects of typical DA agonists is not through their DA-like inhibitory effects of post-synaptic DA cells, but rather by their inhibitory effects on DA neurotransmission itself via DA autoreceptors. Thus, if DA as a whole is an inhibitory neurotransmitter, a decrease in DA transmission would disinhibit, or excite some other neurotransmitter elsewhere, resulting in reward.

Anatomy of a Dopamine Neuron

One would assume, then, that there would be one prototypical response to be seen when recording from VTA DA neurons. That is, if the endogenous reward system hypothesis is correct, all drugs of abuse would produce the same electrophysiological effects. Things are not so simple, mainly because neurons themselves are not so simple. Neurons can not only be innervated by one or more different neurotransmitter containing axons, but they can also have ways of monitoring their own activity through different subclasses of receptors.

VTA DA neurons possess D2 type receptors whereas the synaptic targets of DA neurons possess both D1 and D2 receptors [White and Wang, 1986]. The classification, in part, has to do with varying sensitivity to various dopaminergic agonists, as well as to what second messenger they are coupled. More importantly, however, is the sensitivity of these receptor subtypes. Evidence indicates that pre-synaptic DA receptors (D2 autoreceptors) are significantly more sensitive (three to ten times more sensitive) to DA then are post-synaptic receptors of either the D1 or D2 type [White and Wang, 1986]. Thus, depending upon efficacy, or D1-D2 selectivity of a drug, one could either find an increase or a decrease in basal firing rate with identically classified drugs (i.e. two antagonists or two agonists).

Insert Figure II

"Anatomy of a Dopamine Neuron"

About Here

In addition, due to possible feedback loops, release and synthesis modulating receptors (also called autoreceptors), or very short, local interneurons employing a different inhibitory neurotransmitter, there is reason to expect more than one single response. In order to confirm the endogenous reward system hypothesis, first, a drug must be found to change, whether through an increase or a decrease, the basal firing rate of VTA cells. Increases would result in enhanced release of DA in the NAc whereas decreases would result from enhanced release of DA in the NAc, ie. via negative fedback loops. The VTA criteria is used because it is considered central to the system. The most compelling argument for this criteria is that regardless of where self-stimulation or self-administration occur, these behaviors can always be blocked by selective DA antagonists. Thus the DA - VTA link must play a crucial, central role in the reward system. Further studies of other areas involved will be needed to determine the drug's effects on the system as a whole.

Insert Figure III

"The Proposed Endogenous Reward System"

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Drugs of Abuse

Since it was hypothesized that the VTA was central to the endogenous reward system, many major drugs of abuse have been tested for their effects on the activity of DA neurons within the VTA following intravenous administration. The basal rate of the recorded cell can be compared with rates after various concentrations of a drug have been administered. By recording from different types of cells in different regions, a given drug's effects can be mapped throughout the system. Although this is the basic technique, others, such as microiontophoresis have also been employed with equal success. Amphetamine, cocaine, morphine, ethanol, many benzodiazepines, nicotine, phencyclidine (PCP) and lysergic acid diethylamide (LSD) have all been tested with such methods. There are only a few major drugs of abuse that have yet to be tested. Testing these remaining drugs is one of the future goals of our lab, and the intent of this study.

Methylenedioxyamphetamine (MDA) and MDMA are two drugs currently enjoying a major increase in abuse prevalence. Neither of these drugs have been tested in the VTA. In fact, MDMA is so new, very little is known about it. MDA, unlike MDMA, has been around since the early seventies. However, relatively few behavioral and pharmacological studies have been conducted with MDA. In addition, because of the current street interest in MDMA and accompaning media attention, as well as the complete lack of electrophysiological data related to this compound, our lab opted to conduct this more detailed study of MDMA. Additionaly, because of the dual euphoriant/hallucinogenic effects of MDMA, a greater understanding of MDMA could help bridge the gap between euphoriants, which animals will readily self-administer, and hallucinogens, which animals will not self-administer.

Current MDMA Research

Behavioral Studies on MDMA

Very few studies have been conducted with MDMA (3,4 methylenedioxy-methamphetamine). Although most of its pharmacological properties had yet to be investigated, it was listed as a Schedule I narcotic by the Federal Comprehensive Drug Abuse and Control Act [Shulgin 1978] (This is the highest and most controlled rating possible, comparable with that given to heroin and LSD). And although MDA has been found to produce both amphetamine-like and LSD-like effects in humans [Shulgin 1978 and Anderson et al 1978] as well as in animals [Nozaki et al 1977; and Marquardt et al 1978] only one similar discrimination study with MDMA investigating these possiblities has been conducted. Rats that had been trained to discriminate between the stimulus properties of DA or serotonin (5-HT) active drugs generalized MDMA's properties to both drug class catagories (Schechter et al 1985). This indicates that MDMA, too, has both amphetamine-like and hallucinogenic-like effects. Schechter concludes (Parentheses mine):

> "results would suggest that MDMA is acting both as an indirect dopaminergic agonist and upon a serotonergic subtype of receptors, viz., 5-TH2. This amphetamine-like (dopaminergic) and hallucinogenic-like (serotonergic) duality for the effect of a drug has previously been suggested to occur with the MDMA analogue MDA (Glennon et al 1984, and Nozaki et al 1977) and with MDMA (Nichols et al 1982). It is these stimulant and hallucinogenic properties that may account for the present abuse potential of MDMA [Schechter 1985]." (p. 1536)

Behavioral studies on MDMA in primates indicate that, contrary to earlier reports that MDMA may be an adjunct to psychotherapy because it may promote interactions between individuals, Schlemmer et al [1986] report that MDMA actually disrupts social behavior

and interactions between primates. While users report that, like MDA, MDMA has both hallucinogenic as well as amphetamine-like effects, no controlled human studies (contrasted to MDA human studies) to confirm or disconfirm this have been reported to date.

Pharmacological Studies of MDMA and Dopamine

Pharmacological studies of MDMA are not as lacking as behavioral studies. MDMA's effects on DA have been noted in three recent studies. The first reported that MDMA was ten times more potent than p-chloroamphetamine (a serotonergic release enhancer) at enhancing DA release in superfused rat striatal slices [Levin et al 1986]. Amphetamine itself is considered a DA release enhancer as well as reuptake inhibitor. Cocaine's route of action is similar. Chronic MDMA does not significantly alter tyrosine hydroxylase levels (the rate limiting enzyme for DA synthesis), nor does it reduce striatal DA concentrations. However, acute doses of MDMA significantly increase striatal concentrations of DA [Stone et al 1986]. Stone concludes:

> "alterations of DA and DA metabolite levels after single or multiple drug injections indicate that these agents do affect the dopaminergic system...and that...such an initial, transient elevation of striatal DA has been observed following acute administration of other amphetamine analogs, including amphetamine [Stone et al 1986]." (p. 46)

Stone also reports that, since striatal levels of homovanillic acid were elevated following drug administration, DA turnover (utilization) is also being affected. Schmidt et al [1986] reach identical conclusions that MDMA is a DA release enhancer and that homovanillic acid elevations indicate an increase in DA turnover. These effects are similar to those seen by amphetamine. They also add, as later studies have also reported, that (+) MDMA is the more active of the stereoisomers (stereoisomers being composed of two enantiomers with

identical chemical formulas but the bonds between particular atom groups in one enantiomer, called the (+) enantiomer, are a mirror image of the atomic connections in the other, (-), enantiomer. As a result, these two identical/different stereoisomers rotate polarized light to a different degree. A recemic mixture, (±), is one that contains both (+) and (-) enantiomers.)

The third study on MDMA and DA reported that MDMA produced a biphasic effect on DA efflux in the neo-striatum, with the slight increase in DA efflux lasting aproximately forty minutes (a probable result of increased DA release), followed by a significant decrease in DA efflux [Takeda et al 1986] (possibly either due to greater DA metabolism because of an increased time in the synaptic cleft or due to action at the autoreceptor signaling the cell to decrease DA synthesis). The same study also reported that MDMA significantly increased serotonin efflux, which returned to normal within three hours. These data, and the 5-HT data that follow, are consistent with the claim that MDMA is both and amphetamine–like and hallucinogen-like compound.

Pharmacological Studies of MDMA and Serotonin

In addition to MDMA's biphasic DA effects and DA release enhancing, further research of MDMA's effects also confirm its biphasic effects on 5-HT neurons as well as enhancing 5-HT neurotransmitter release. Studies on MDMA in the last year indicate that MDMA is even more potent than amphetamine in inhibiting the reuptake of 5-HT [Steele et al 1986], the neurotransmitter suspected of being involved in the hallucinatory effects of LSD and DOM. As with DA, MDMA has a biphasic effect on 5-HT levels in cortical neurons, where levels were reduced to 16% of control within three to six hours. Yet this dramatic reduction had returned to normal by twenty-four hours, only to decline again to 74% of control by seven days (at 10 mg/kg s.c.). Higher doses of MDMA (20 mg/kg s.c.) had similar biphasic effects

with final whole brain concentrations of 5-HT reduced to 65% of controls after seven days and 5-HT synaptosomes reduced to 50% of control in the same amount of time [Schmidt 1986]. These were properties of the (+) enantiomer only.

In high performance liquid chromotography studies, it has been reported that MDMA can reduce the striatal concentration of serotonin by as much as 75% [Schmidt and Lovenberg 1986]. In vitro, MDMA was reported to be a potent 5-HT releasing agent (release enhancer). In fact, this study indicates that MDMA is even more effective at causing 5-HT release than it is in causing DA release [Schmidt 1986 and Gehlert et al 1985].

Hypothesis of Experiment One

We predicted that 1) MDMA would have effects on DA neuronal firing similar to those of cocaine and amphetamine because of the apparent similarity in the site of action (i.e. release enhancer) shared by these compounds. Thus, MDMA should slow down the spontaneous firing rate of these cells due to increased stimulation of DA autoreceptors. In addition, we predicted that 2) the (+) stereoisomer would be more potent than either the (±) or (-) stereoisomers in suppressing DA firing rates.

General Methods

Animals

In two similar experiments, MDMA's effects on DA cells within the VTA were examined. All experiments used male, Sprague-Dawley rats weighing 225-325 grams. The rats were housed in groups of two to four with free access to food and water. The colony room was kept at a constant temperature of 22^{o}, ± 1^{o} C. The animals were kept on a 12 hour

light-dark cycle (07:00-19:00), and all experiments were performed during the light phase of the cycle.

Experimental Groups

Three groups of animals were tested. Each of the three groups of eight was housed, cared for, and prepared for single-unit recordings in the same way. The two groups of experiment one were non-pretreated animals. Group one received seven injections of (±) MDMA ranging from 1mg/kg and doubling thereafter till 64mg/kg (i.e. the noncumulative doses were 1, 2, 4, 8, 16, 32, and 64 mg/kg) Group two received (+) MDMA on an identical dose schedule.

In addition to the normal rats tested with, (±) MDMA, and (+) MDMA, resepectively, in a second e periment, a third group was pretreated with 400 mg/kg i.p. of p-chlorophenylalanine (PCPA) 24 hours before recording to deplete brain stores of 5-HT (See experiment two for further explanation).

Preparations

Single-unit recordings were taken using the techniques described by Bunney et al [1973] and O'Brien and White [In press]. The rats were anesthetized with an intraperitoneal injection (i.p.) of chloral hydrate (400 mg/kg) and placed in a stereotaxic device (David Kopf Instruments). A lateral tail vein was cannulated with a 25 gauge needle and used to give additional anesthetic injections as needed. Body temperature was maintained at 37^{0} C with a heating pad (Fintronics). After placement in the stereotaxic apparatus and a final check to insure that the animal was fully anesthetized, the skin of the scalp was shaved, incised and retracted. Burr holes were then drilled over the VTA, which is 3.0-3.4 mm anterior to the lambdoid suture, 0.5-1.0 mm lateral to the midline and 6.5-9.5 mm ventral

to the dura [Paxinos and Watson, 1982]. The dura mater was retracted and the electrode lowered.

Single-unit Recordings

Extracellular, single-unit recordings were obtained through a single barrel glass micropipette. Micropipettes were prepared from 2.0mm glass capillary tubing (Corning) preloaded with two to three fiberglass strands to insure proper filling of the NaCl solution. The tubing was pulled with a verticle pipette puller (Narishige) and broken back under a microscope to produce a pipette with a tip diameter of approximately 1-2μm. They were then filled with 2M NaCl solution saturated with 1% fast green dye to mark to site of recording. The in vitro impedance was 2-5 MΩ measured at 135 Hz (Winston Electronics).

The electrodes were lowered near the VTA and slowly advanced with a hydraulic microdrive (David Kopf Instruments). The electrode signal was passed through a high impedance amplifier/filter (band settings: 100 Hz and 3kHz) and a window discriminator (Fintronics WDR 420). The signal was displayed on an oscilloscope (Tekronix 5110) and monitored with an audioamplifier (Grass AM8). A polygraph recorder (Gould 220) plotted integrated rate histograms generated by the analog output of the window discriminator. The digital output of the window discriminator was fed into a microprocessor-based data acquisition system (Medical System Corp.) which calculated mean firing rates and generated interspike interval histograms.

DA neurons were identified by their location in the VTA, (0.5-1.0 mm lateral to the midline, 3.0-3.2 mm anterior to Lamda and 6.5-9.5 mm ventral to the dura mater), as well as by well established physiological criteria [Bunney et al 1973; Grace and Bunney 1983, Wang 1981b; White and Wang; 1983a; and Yim and Mogenson 1980]. These criteria include a spontaneous firing rate between 0.5 and 10.0 Hz, either a slow variable firing pattern or a

slow bursting pattern with decreasing spike amplitude, and a biphasic wave form of long duration (>2.5 msec), with a prominent notch in the first ascending portion of the positive wave.

Baseline firing rates were observed for at least three minutes prior to any drug administration other than anesthetic. Various drugs were then administered via the lateral tail vein. Animals were injected on a regimen in which the total dose was doubled in comparison to the previous administration. Following each dose, the firing rates were recorded for one minute. Only one cell per rat was observed. Depending upon the observed results, reversal of inhibition via haloperidol (a selective D2 antagonist) was attempted.

Histology

After administration of all drug doses, or upon loss of the cell being recorded, the postion of the cell being recorded was marked by passing a -25µA current through the unmoved recording electrode for 15 minutes. This current ejects a small amount of the fast green dye which was used to fill the pipette electrode into the area of the brain surrounding the electrode tip. The animals were then sacrificed and immediately perfused with saline, followed by 5% phosphate buffered formalin solution. After the perfused brain had fixed in the formalin solution far at least a week, frozen serial sections (50µm thick) surrounding the VTA were cut, stained with cresyl violet and then counterstained with neutral red. Recording sites were then verified to have been in the VTA by microscopic examination for the dye spot.

Drugs

The drugs used in this study were (±) MDMA, and (+) MDMA (supplied by NIDA); PCPA (supplied by Sigma Chemical Company, St. Louis, Mo.); and Chloral hydrate (supplied

by Sigma Chemical Company, St. Louis, Mo.). All drugs in these experiments were disolved in deionized water.

Results: Experiment One

(±) MDMA had no significant effect in suppressing DA VTA neurons. Of the eight cells tested, only two were inhibited by greater then 25% of spontaneous baseline firing rates. In addition, in half of the subjects, firing rates increased by an insignificant amount. Thus, from the animals tested, it is not possible to quantify a specific effect of (±) MDMA due to the variable responses. For a list of the means and standard errors at each dose for both (±) and (+) MDMA, see Table 1.

(+) MDMA was more effective than (±) MDMA at inhibiting firing rates, compared to a saline control from earlier studies with cocaine, but was unable to inhibit DA firing to the same extent as cocaine or amphetamine.. Thus, (+) MDMA failed to inhibit DA neurons to the extent hypothesized. In no cases did (+) MDMA inhibit the firing rate of DA neurons by greater than 50% of spontaneous baseline firing rates. Non-pretreated animals receiving doses as high as 3.2 mg/kg (±) MDMA or 2.6 mg/kg (+) MDMA still did not exhibit inhibition greater than 50%. Table 2 summarizes the number of animals in each group inhibited by the amount shown in the colums. However, a repeated measures analysis of variance (ANOVA) indicates that there is a significant difference $\{F(8,96)=18.732, p<0.01\}$ between successive doses of (+) MDMA, indicating that (+) MDMA has at least some effect on DA firing rates as doses increase.

Insert Figure IV

"Dose Response Curve for (±) MDMA"

Insert Figure V

"Dose Response Curve for (+) MDMA"

About Here

Figures 4 and 5 are dose response curves of (±) MDMA and (+) MDMA in non-pretreated animals. Note that the dose regimens across groups one and two are not the same. (+) MDMA, being the more potent of the enantiomers, had to be administered at lower doses than (±) MDMA. Initial doses of (+) MDMA that were comparable to the initial doses of (±) MDMA (i.e. 0.1 mg/kg and 0.2 mg/kg etc...) were detrimental to the animals in that they severly suppressed respiration. The initial dose for (+) MDMA was thus reduced by tenfold (ie .01 mg/kg and .02 mg/kg etc...).

Discussion: Experiment One

The results indicate that MDMA does not have the efficacy to inhibit DA firing that was predicted. Such an effect was expected because MDMA is an amphetamine analogue, because there is evidence indicating that MDMA enhances release of endogenous DA, and because users report that MDMA's euphoriant properties are similar to those of amphetamine. Thus, the absence of supporting data from this experiment is suprising. But it is important to note that due to the different doses given each group, they cannot be statistically compared. (+) MDMA's effects, when compared with saline controls from other experiments on cocaine, were significant. Thus, it is just the (±) MDMA group that lacked efficacy. This difference between the two groups would be expected, based on evidence from other researchers that the

(+) enantiomer was more potent at releaseing both DA and 5-HT and from users reports that the (+) enantiomer was more euphoriant.

As discussed earlier, however, MDMA is even more potent at releasing endogenous 5-HT than it is at releasing DA. This release of 5-HT is consistant with reports that MDMA is also an hallucinogen. It was noted above that the dorsal raphè nucleus's greatest concentration of 5-HT afferents project directly to the VTA. These excitory afferents could either synapse directly upon VTA DA neurons or upon interneurons influencing DA firing rates. Thus, although MDMA's DAergic effects may well be similar to those of cocaine or amphetamine, its other properties, (ie. enhancing 5-HT release) may "mask" some of its euphoriant effects on DA neurons. It should therefore be possible to more exactly identify MDMA's specific effects on DA neurons in the VTA by eliminating or blocking MDMA's effect on 5-HT transmission. This was the aim of experiment two.

Hypothesis: Experiment Two

We predicted that 3) when CNS levels of 5-HT were depleated by PCPA, the resulting loss in competing excititory afferents to the VTA would allow MDMA's effect on DA neurons in the VTA to be visible as an inhibition of DA neuronal firing rates.

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Insert Figure VI

"5-HT Depletion via PCPA"

About Here

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Specific Methods

All methods in experiment two were identical to those in experiment one, with the exception that all animals were pre-treated with 400 mg/kg i.p. of p-chlorophenylalanine

(PCPA) 24 hours prior to electrophysiological recording. Several investigators report that PCPA (which inhibits tryptophan hydroxylase, the rate limiting enzyme in 5-HT synthesis) reduces whole brain 5-HT concentrations by as much as 85% of controls [Wang et al 1978 and Baraban et al 1978] This will be verified by high preformance liquid chromotography (HPLC) analysis of whole brain concentrations of 5-HT in control rats verses similarly PCPA pretreated time permitting.

PCPA was supplied by Sigma Chemical Company, St. Louis, Mo., and was disolved in deionized water in a concentration of 100 mg/ml. All animals treated in this group received the same doses of (+) MDMA as the animals in group two, experiment one.

Results: Experiment Two

There was a significant difference ($p < .05$) in the inhibition of DA firing rates between group two of experiment one, (+) MDMA, and the PCPA pre-treated (+) MDMA group three of experiment two. Table 3 lists the means and standard deviations for group two (repeated for comparison) and group three.

A repeated measures analysis of variance (ANOVA) between the dose response curves of groups two and three revealed a statistically significant difference ($F(1,12)=5.038$, $p<0.05$) between the firing rate means at identical doses of pre-treated with PCPA or non-pre-treated groups.

Insert Figure VII

"Dose Response Curve of PCPA/(+) MDMA Versus (+) MDMA"

About Here

Figure 7 demonstrates the increase in inhibition caused by PCPA with (+) MDMA as compared to (+) MDMA alone. Groups two and three are shown together because they received identical dose regimes. Group one has been omitted because its doses, as discussed earlier, cannot be compared with either group two or three. Table 4, conversely, provides a comparsion between both groups of experiment one, where no dose caused inhibition greater than 50% of baseline and the PCPA pre-treated group, where nearly all of the animals showed an inhibition of greater then 75%. Group one was included in this comparison because only the overall cumulative dose is being considered, and in all cases, animals in group one received a greater cumulative dose of (±) MDMA than any animal in either group two or three did of (+) MDMA. Final results (within two minutes after the last dose of (+) MDMA was given) of the PCPA pre-treatment were: 7 of the 8 cells recorded from had been completely inhibited (ie. to 0% of baseline). No other group had cells which were inhibited below 50% at any time.

Insert Figure VIII

"Comparison of Inhibition Across All Groups"

About Here

Figure 8 graphically depicts the number of cells in each group that were inhibited by either 0%-25%, 25%-75% or >75% of baseline. It is clearly evident PCPA pre-

treatment to deplete 5-HT allowed MDMA's DA effects to be "unmasked". Lastly, Figure 9 contains a representative rate-histogram for each group.

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Insert Figure IX

"Rate-Histograms for All Groups"

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Discussion: Experiment Two

The aim of experiment two was to elicit more clearly MDMA's DAergic effects by blocking the ability of MDMA to enhance 5-HT release. Even though data from experiment one suggested that MDMA indeed had an inhibitory effect on DA neuronal firing rates, this inhibition was not as great as was predicted. We had originally hypothesized that MDMA should be able to completely inhibit DA neuronal firing, as is the case with cocaine or amphetamine. We reasoned that the weakness of MDMA's effects on DA neurons in the VTA revealed in experiment one was due not to a flaw in the endogenous reward model and DA's role in this system, but rather to the dual properties of MDMA. The results of experiment two indicate clearly that 5-HT pathways have an influence on VTA DA firing rates.

When CNS stores of 5-HT were depleted via PCPA, the efficacy of (+) MDMA was doubled. Without 5-HT depletion, the average inhibition by 2.56 mg/kg of (+) MDMA was 61.9% (ie. 39.1% lower than baseline of 100%). Comparatively, the average inhibition by the same dose of (+) MDMA in a PCPA pretreated animal was 15.7% of baseline. Though there was a large variation in the effects of (+) MDMA in PCPA animals across doses, after all injections had been administered (a cumulative dose of 5.1 mg/kg (+) MDMA), fully seven out of eight cells were completely inhibited. In no case did (+) MDMA inhibit cells to this extent in "normal" rats.

Though it might be argued that the complete inhibition witnessed with PCPA was a result of losing the cell, and not that of the cell shutting off, this is likely not the case. Of the seven cells that shut off in group three, two recovered spontaneously after approximately ten minutes to 40% of baseline. Another completely inhibited cell returned to baseline after administration of halperidol, a selective D2 antagonist. Halperidol is routinely used to verify that the cell one is recording from is a DA cell because, even at very low doses, halperidol blocks the D2 impulse regulating autoreceptor, causing the cell to increase its own firing. In group two, four of the seven cells were recovered, either by administration of halperidol or being allowed to recover spontaneously, ie. in these instances, though the last dose of (+) MDMA was given and the firing rate for the following minute was recorded as usual, the animal was allowed ten minutes to spontaneously recover.

Conclusions

The two experiments reported here help confirm reports by other researchers concerning the mode of action of MDMA in the CNS. As indicated by the inhibition of VTA DA neurons by (+) MDMA alone, MDMA is working via DAergic pathways. Though the evidence outlined here does not determine whether MDMA is acting as a DA agonist or as a release enhancer (such as amphetamine), it is reasonable to assume that MDMA's DAergic effects are a result of the endogenous release of DA acting at it own terminals, given the evidence reported by other investigators that MDMA causes an increase in DA release. Supporting this assumption is the indirct evidence from our experiments that the (-) enantiomer is indeed less potent than the (+) enamtomer. This is based on (±) MDMA's composition being 50%

(-) and 50% (+). Yet even at the higher doses group one received of (±) MDMA, no significant inhibition occured. This difference is also compatable with earlier reports.

The second experiment points to the importance of 5-HT in the mechanism of action of MDMA. It is interesting to note that enhanced 5-HT neurotransmission is suspected to play a major role in hallucinogenesis. MDMA is reputed to be a weak hallucinogen with euphoric properties. Unlike other hallucinogens such as LSD, MDMA is self-administered by laboratory animals, probably because of its DAergic effects. MDMA may play an important role in understanding the mechanism of hallucination.

Though the experiments reported here answer some basic questions about MDMA, they also indicate that there is much more to learn. Most importantly, and in the interest of completeness, experiments should be conducted to determine the effects of the (-) enantiomer alone. This was originally our goal, however, the limited efficacy revealed in the first two groups of experiment one partly answered this question, and more importantly, pointed to other underlying actions of MDMA that were more relevant to a better understanding of the compound. Because of time constraints, (-) MDMA studies were abandoned to pursue experiment two. However, (-) MDMA studies alone, and both (-) MDMA and (±) MDMA in PCPA pretreated animals should also be conducted.

Usefulness of the Endogenous Reward System Model

Aside from the guidance the endogenous reward system model gives us in determining prospective research, the model is useful in many other ways. From a practical standpoint, the model, had it been adhered to by pharmaceutical companies, could have prevented nomifensine (a new antidepressant) from reaching the market. Recently, after years of research, nomifensine was put on the market as an antidepressant without being tested with the endogenous reward model. Only months after its release, nomifensine was withdrawn

from the market because, amoung other problems, patients were abusing the drug. Subsequent tests revealed that nomifensine, in fact, affects DAergic transmission in the VTA [Einhorn et al 1986]. Had the drug been tested in the VTA before release, this adverse effect would most likely have been predicted.

In addition, a greater understanding of the reward system could lead to prevention of drug abuse or intervention in drug abuse. Once the feedback mechanisms and autoregulatory controls of DA neurons are better understood, drugs may be developed which either block the rewarding effects of psychomotor stimulants without blocking the normal rewarding properties of food, sex and the like, or help the system of a drug addict return to normal without the difficult and often psychologicaly trying effects of drug withdrawl. Some progress in this area has been made already with the use of tricyclic antidepressant treatment on cocaine abusers [Kleber and Gawin 1984; Gawin and Kleber 1984].

Since depression has been linked to an underactivity of DAergic neurons (and possibly an underactivity in the entire endogenous reward system), and the depressed symptoms of patients withdrawn from cocaine was similar to that of profound depression, it was hypothesized that tricyclic antidepressants, which have been reported to increase DAergic receptor binding [Borison et al 1979; Taylor et al 1979], could reverse the depression caused by cocaine withdrawl. DA receptor changes that follow antidepressant treatment are also in the opposite direction of those that occur after chronic cocaine abuse [Koide and Matshushita 1981; Naber et al 1980]. Kleber and Gawin report some success in their efforts.

This example is given to illustrate the potential benefits a better understanding of this system can offer. Even more could be done if new drugs were discovered that could affect just one part of the system and not the others, or a drug that could somehow bypass the autoregulatory mechanisms that are so often active. Contrary to the hypothesis that

depression is due to an underactivity in this system, there is the hypothesis that schizophrenia is a result of an overactivity of this system. The indistingushable difference between amphetamine psychosis and paranoid schizophrenia is just one piece of evidence. No doubt, once new drugs are developed and the system as a whole is better understood, this will also lead to a better understanding of schizophrenia and possibly new treatments.

Uncovering the endogenous reward system is important not only for our understanding of how reinforcers such as food, water, sex and sleep effect our behaviour, (In fact, all reinforcing stimuli are thought to exert their effects, at least in part, through this same system, [White 1986; Wise et al., 1978a, 1978b; Spiraki et al., 1982; Xenakis and Sclafani 1982].) but also how drugs of abuse exert their effects. A greater understanding of this reward system could lead to better treatment or prevention of drug abuse and also pre-screening techniques to detect potential drugs of abuse before they are allowed onto the market.

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Table 1

Group One	Dose	Number	Mean	Standard Deviation
	0.1 mg/kg	8	100.0	±7.1
	0.2 mg/kg	8	97.0	±20.3
(±) MDMA	0.4 mg/kg	8	91.3	±20.4
	0.8 mg/kg	8	106.4	±25.6
	1.6 mg/kg	7	106.2	±27.0
	3.2 mg/kg	3	78.1	23.1

Group Two	Dose	Number	Mean	Standard Deviation
	.01 mg/kg	7	94.7	±4.8
	.02 mg/kg	7	92.6	±4.6
	.04 mg/kg	7	90.7	±6.9
	.08 mg/kg	7	92.3	±8.0
(+) MDMA	.16 mg/kg	7	89.9	±6.8
	.32 mg/kg	7	82.5	±4.6
	.64 mg/kg	6	74.6	±8.0
	1.28 mg/kg	6	67.9	±7.9
	2.56 mg/kg	6	61.9	±11.4

Table 2.

	Greatest % of Inhibition from Baseline			
Group	Number	0%-25%	25%-75%	>75%
#1 ±MDMA	8	6	2	0
#2 +MDMA	7	2	5	0

Table 3.

Group Two	Dose	Number	Mean	Standard Deviation
	.01 mg/kg	7	94.7	±4.8
	.02 mg/kg	7	92.6	±4.6
	.04 mg/kg	7	90.7	±6.9
	.08 mg/kg	7	92.3	±8.0
(+) MDMA	.16 mg/kg	7	89.9	±6.8
	.32 mg/kg	7	82.5	±4.6
	.64 mg/kg	6	74.6	±8.0
	1.28 mg/kg	6	67.9	±7.9
	2.56 mg/kg	6	61.9	±11.4

Group Three	Dose	Number	Mean	Standard Deviation
	.01 mg/kg	8	96.9	±7 2
	.02 mg/kg	8	94.5	±7.2
	.04 mg/kg	8	93.2	±12.6
	.08 mg/kg	8	73.3	±33.5
PCPA with	.16 mg/kg	8	60.9	±37.1
(+) MDMA	.32 mg/kg	8	50.4	±45.4
	.64 mg/kg	8	40.5	±42.1
	1.28 mg/kg	8	30.7	±38.7
	2.56 mg/kg	8	15.7	±24.2

Table 4.

% Inhibition from Baseline at Most Potent Dose

	Group	Number	0%-25%	25%-75%	>75%
#1+2	Normal	15	8	7	0
#3	PCPA/+MDMA	8	0	1	7

Figure Legend

Figure One: Coronal and sagittal views of the dopamine pathways of the rat brain. From Ungerstedt, [1971].

Figure Two: Schematic drawing of a dopamine neuron's anatomy, receptor subtypes and locations.

Figure Three: Diagram of the "Endogenous Reward System" as outlined by Wise, [1980].

Figure Four: Dose response curve of (±) MDMA's effects on A10 DA neurons. Each point represents the mean inhibition produced at each dose of (±) MDMA. The vertical bars represent the standard error of the means.

Figure Five: Dose response curve of (+) MDMA's inhibition of A10 DA neurons. Each point represents the mean inhibition produced at each dose of (+) MDMA. The vertical bars represent the standard error of the means.

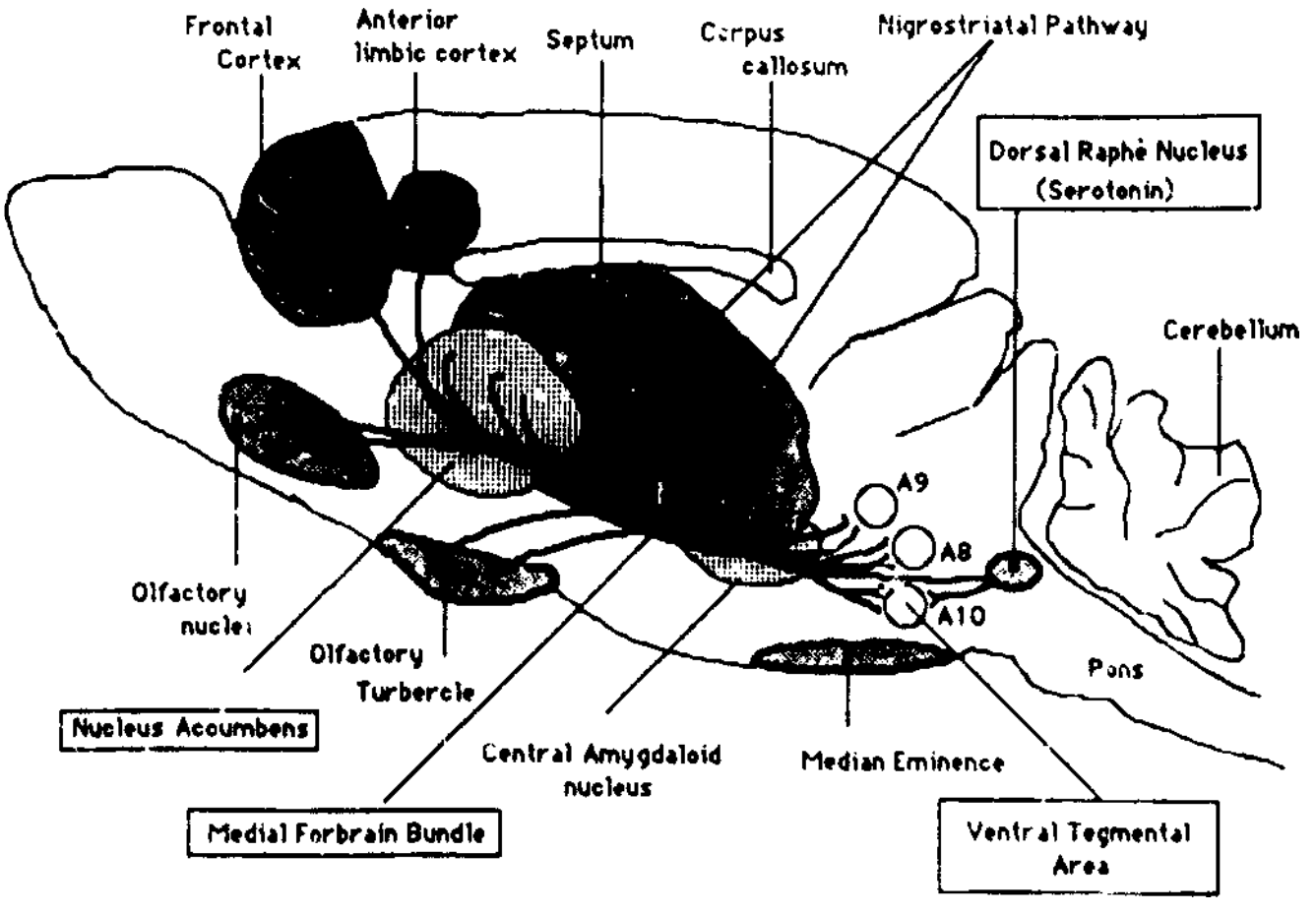
Figure Six: Dorsal raphè innervation of the VTA. PCPA and its role in inhibiting 5-HT influence on the VTA.

Figure Seven: Dose response curves of (+) MDMA/PCPA versus (+) MDMA. A comparison of their effects on A10 DA neurons. Each point represents the mean inhibition produced at each dose of + MDMA. The vertical bars represent the standard error of the means.

Figure Eight: Comparison of the greatest inhibition caused by MDMA across all groups. 0%-25% indicates that cells in this group were inhibited by no more than 25% of baseline (ie. a cell inhibited by 20% would be firing at 80% of baseline, and would thus be placed in this grouping. 25%-75% indicates that the cells were inhibited by at least 25% of baseline but not more then 75% of baseline. >75% is for all cells that were inhibited by at least 75%, and includes those cells that were completely inhibited (ie. they shut off).

Figure Nine: Rate histograms for all groups. These are photographs of the actual polygraph recordings produced by each subject.

Figure One:


Frontal Cortex
Anterior limbic cortex
Septum
Corpus callosum
Nigrostriatal Pathway
Dorsal Raphè Nucleus (Serotonin)
Cerebellum
A9
A8
A10
Olfactory nucle
Olfactory Turbercle
Pons
Nucleus Accumbens
Central Amygdaloid nucleus
Median Eminence
Medial Forbrain Bundle
Ventral Tegmental Area

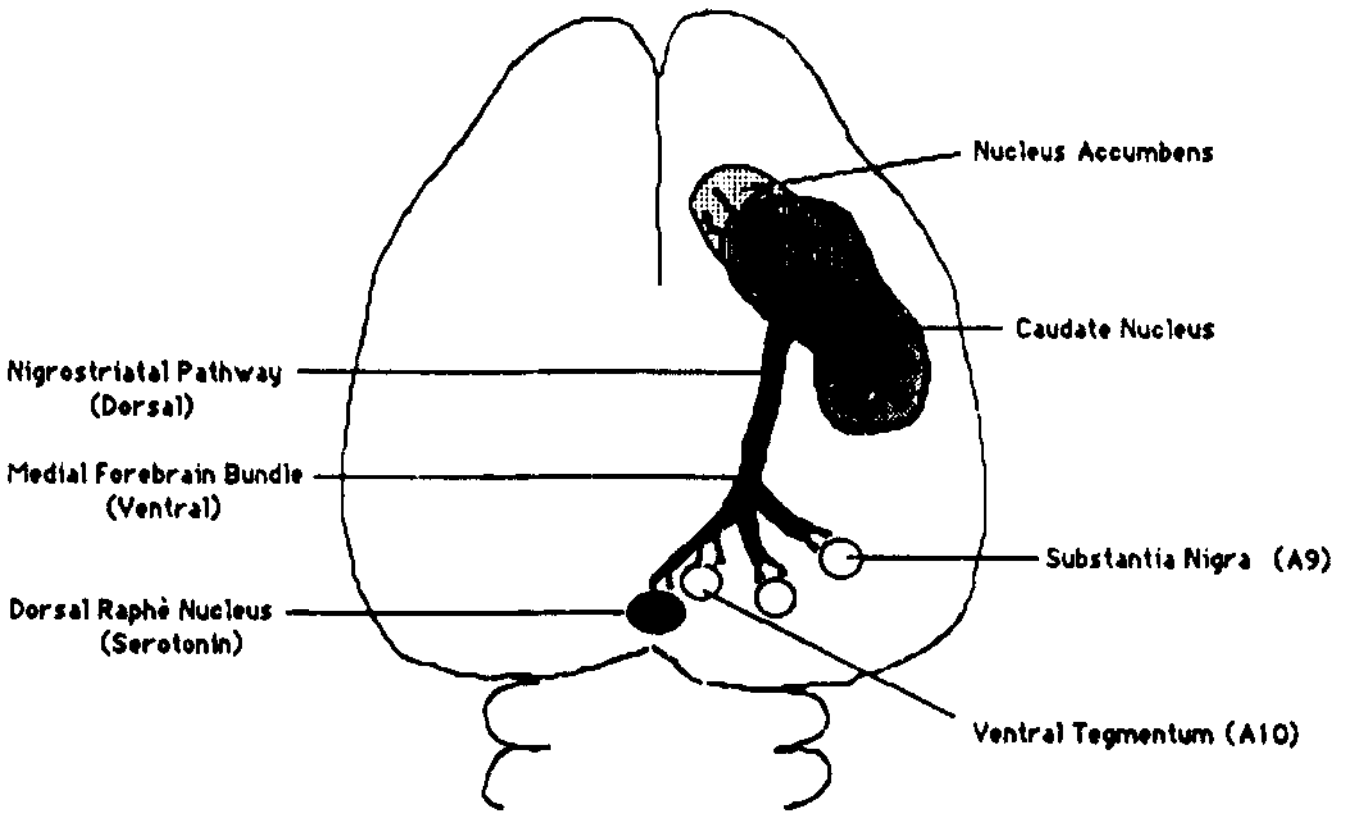

Nucleus Accumbens
Caudate Nucleus
Nigrostriatal Pathway (Dorsal)
Medial Forebrain Bundle (Ventral)
Substantia Nigra (A9)
Dorsal Raphè Nucleus (Serotonin)
Ventral Tegmentum (A10)

Figure Two:

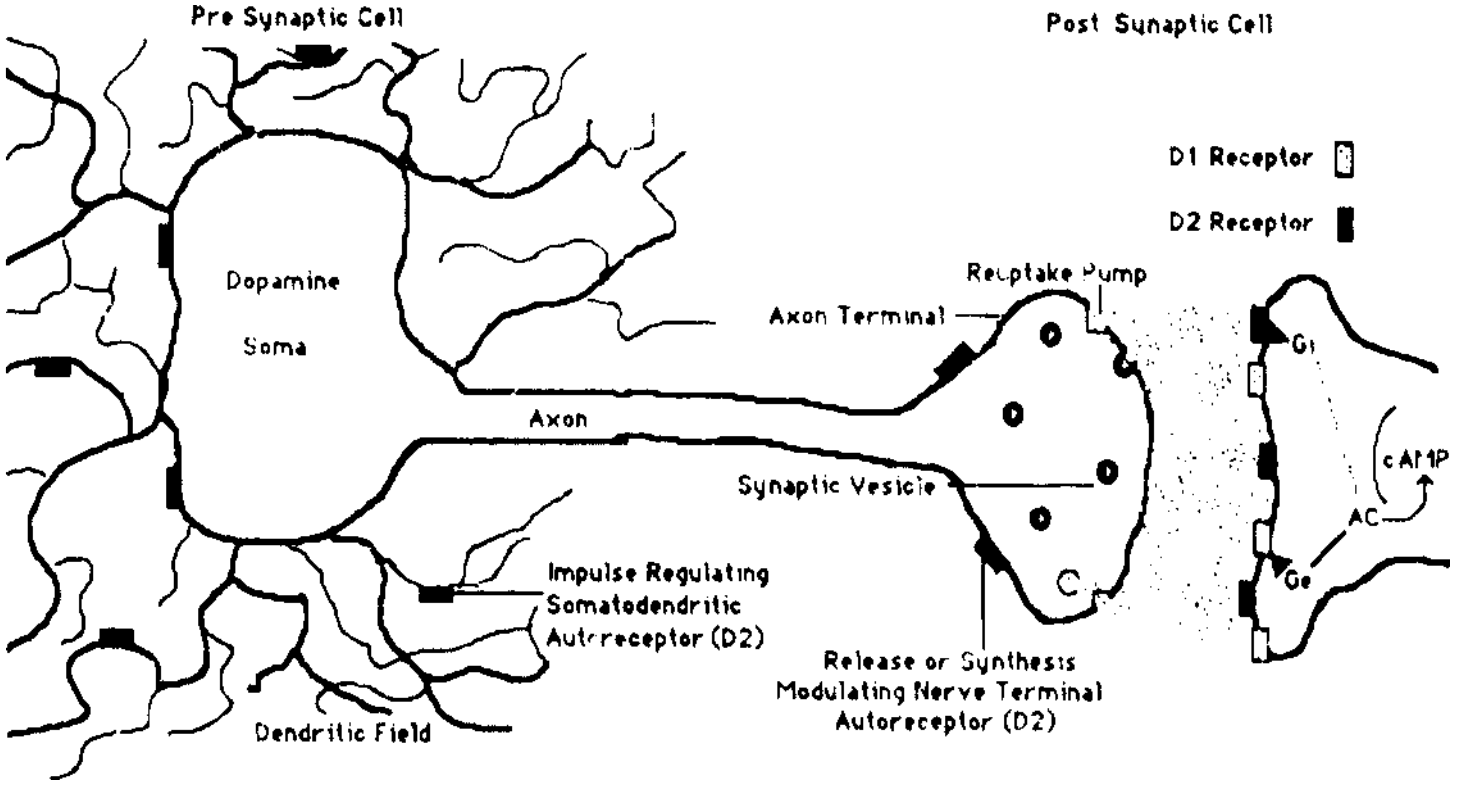

Pre Synaptic Cell
Post Synaptic Cell
D1 Receptor
D2 Receptor
Dopamine
Soma
Reuptake Pump
Axon Terminal
Axon
Synaptic Vesicle
cAMP
AC
Impulse Regulating
Somatodendritic
Autoreceptor (D2)
Release or Synthesis
Modulating Nerve Terminal
Autoreceptor (D2)
Dendritic Field

Figure Three:

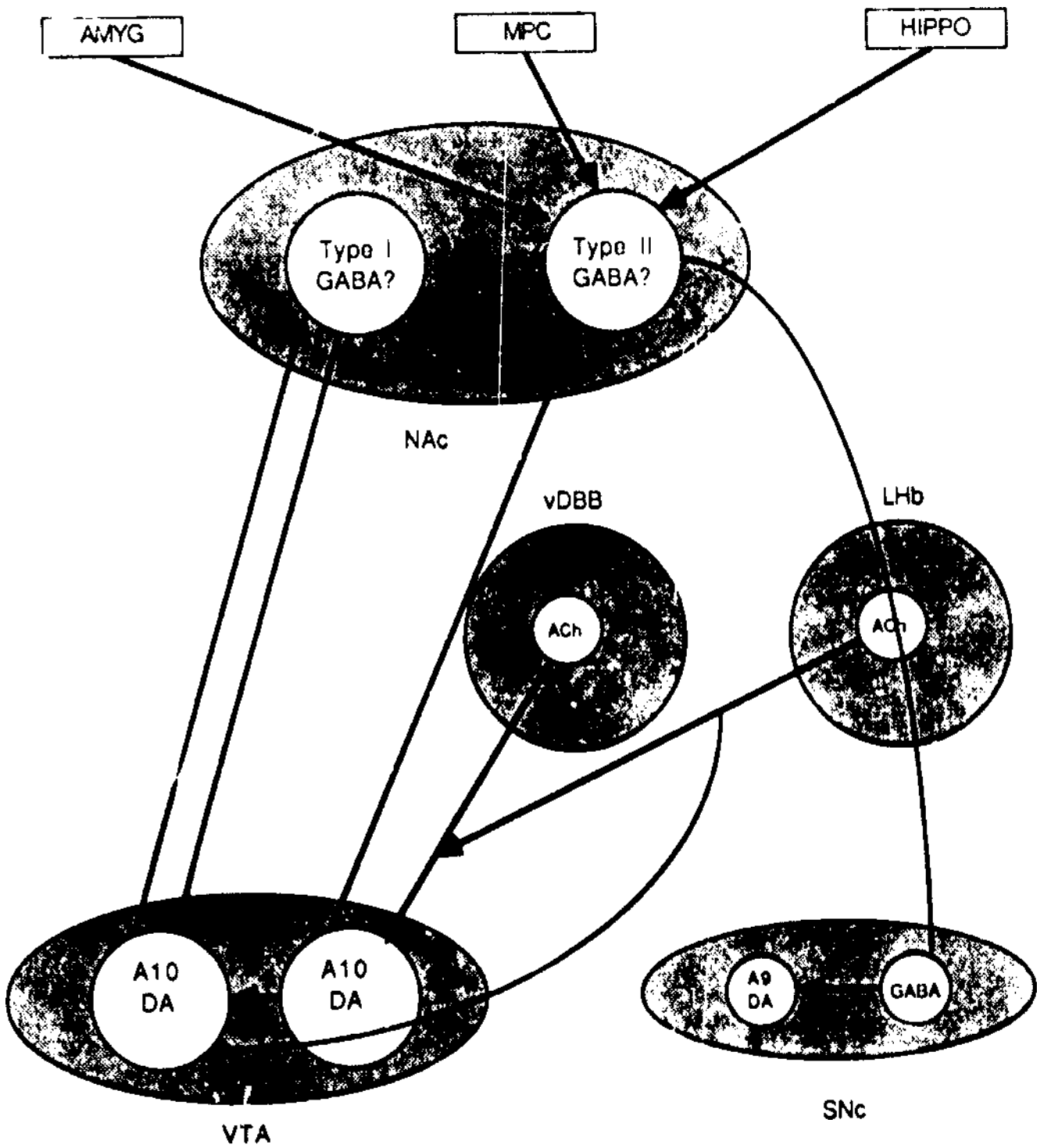


Figure Four:

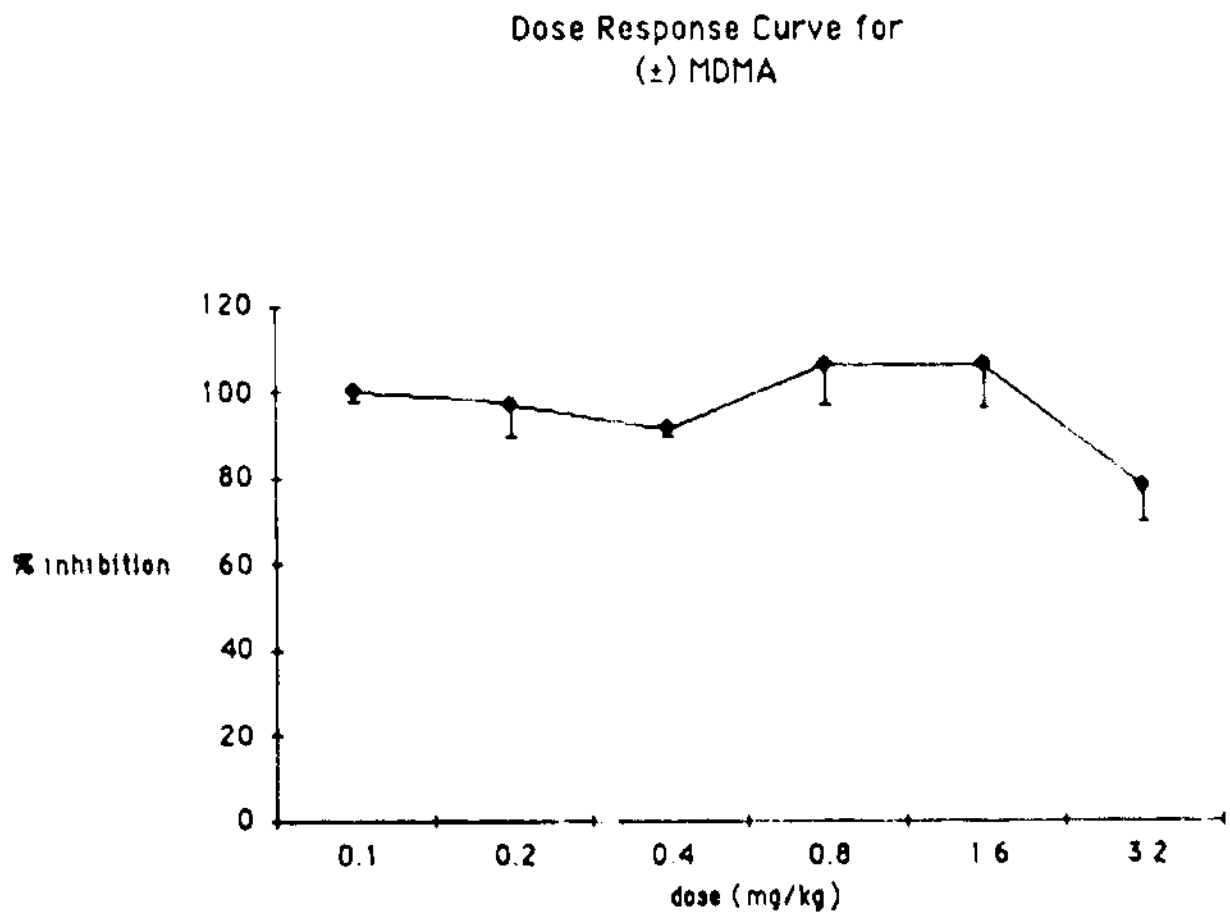

Dose Response Curve for
(±) MDMA
% inhibition
120
100
80
60
40
20
0
0.1
0.2
0.4
0.8
1.6
3.2
dose (mg/kg)

Figure Five:

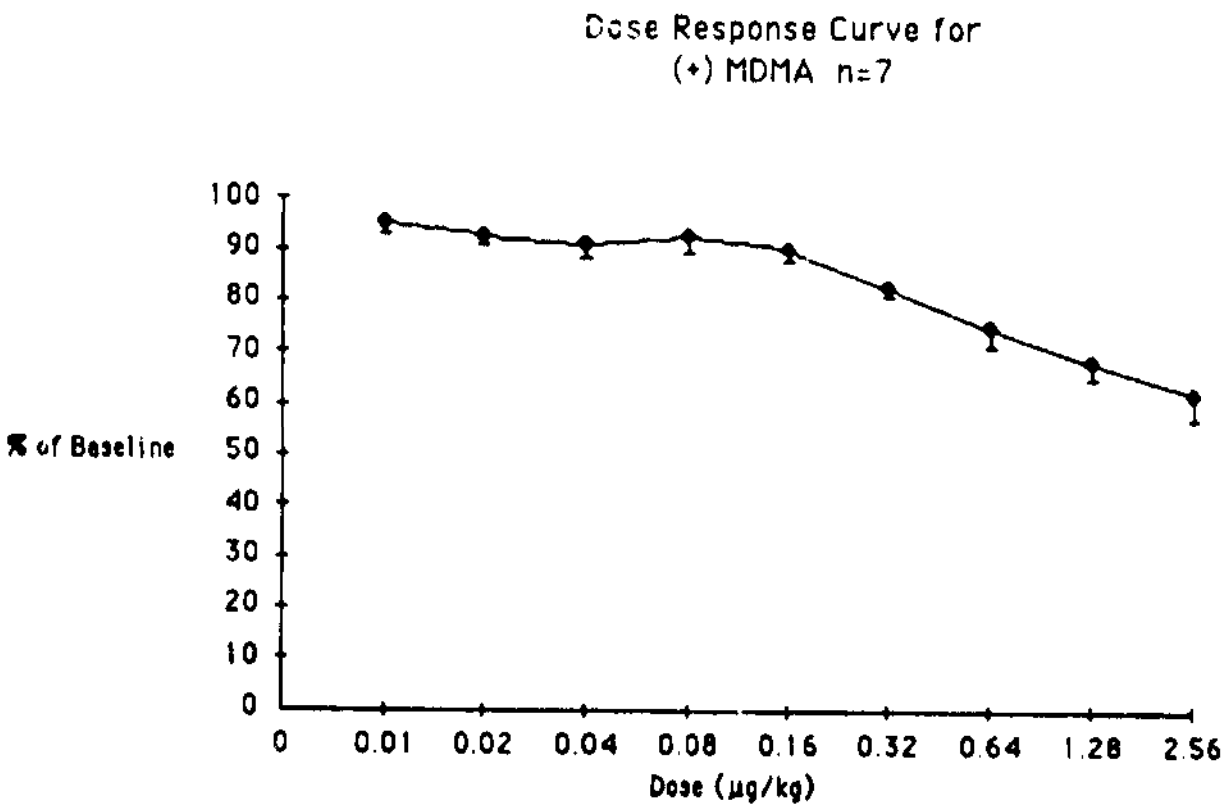

Dose Response Curve for
(+) MDMA n=7
% of Baseline
100
90
80
70
60
50
40
30
20
10
0
0.01
0.02
0.04
0.08
0.16
0.32
0.64
1.28
2.56
Dose (µg/kg)

Figure Six:

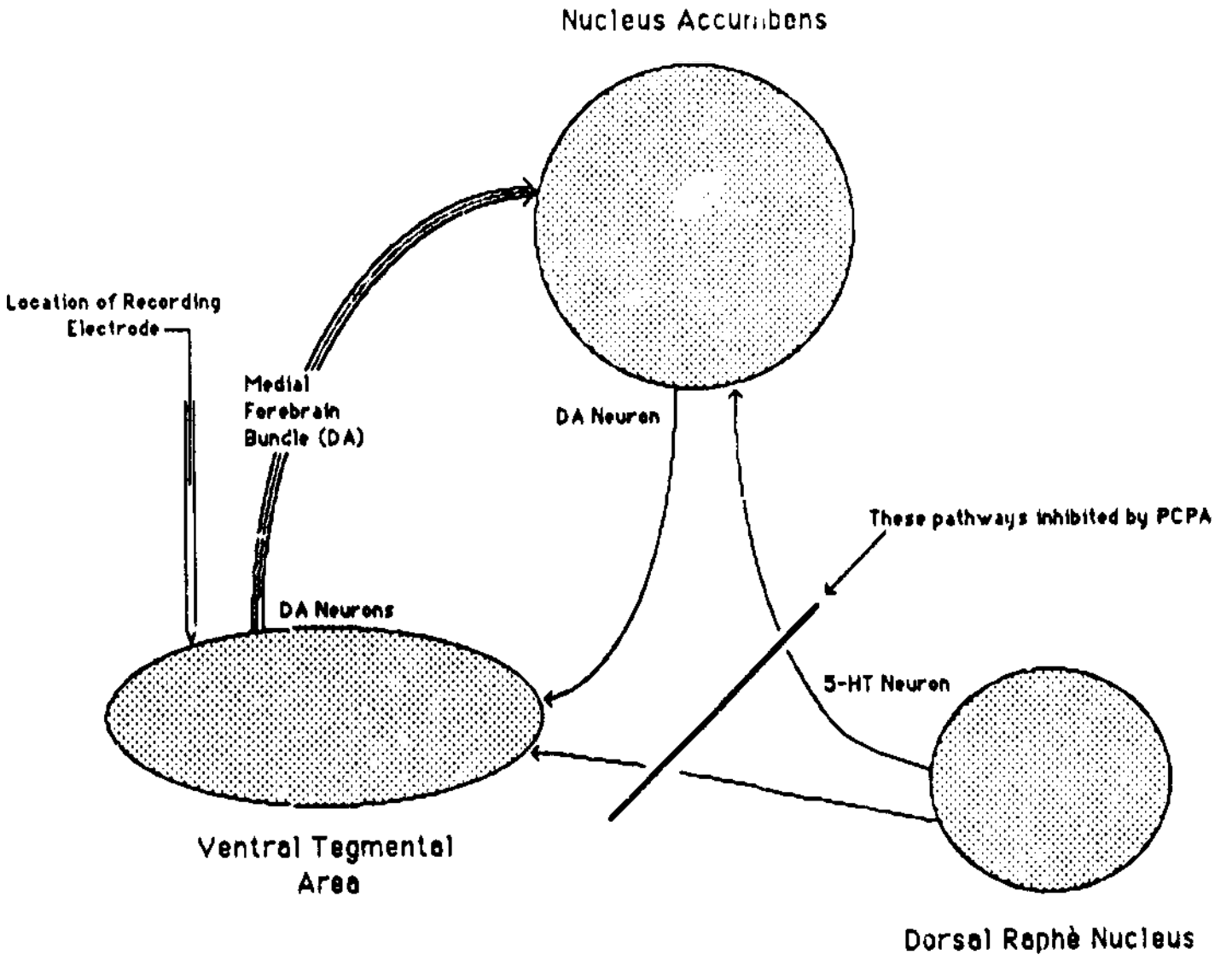

Nucleus Accumbens
Location of Recording Electrode
Medial Forebrain Bundle (DA)
DA Neuron
These pathways inhibited by PCPA
DA Neurons
5-HT Neuron
Ventral Tegmental Area
Dorsal Raphè Nucleus

Figure Seven:

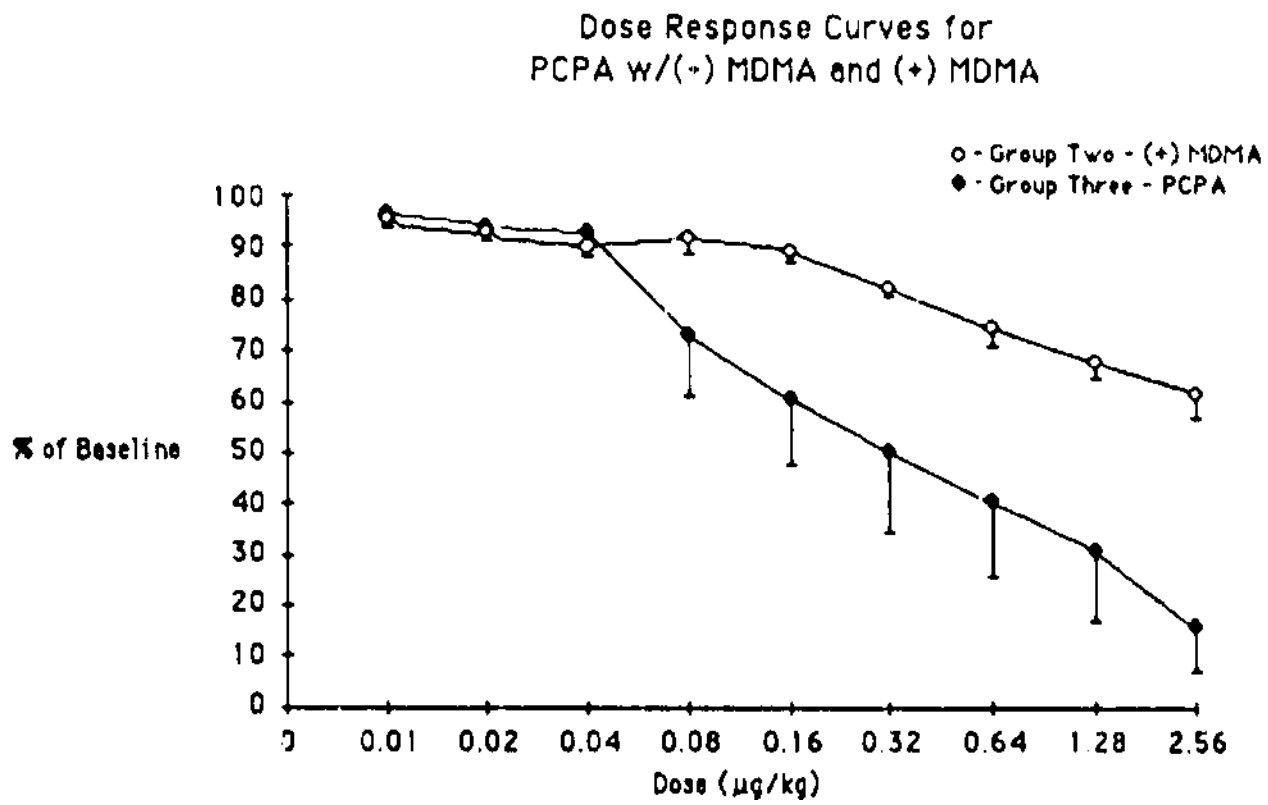
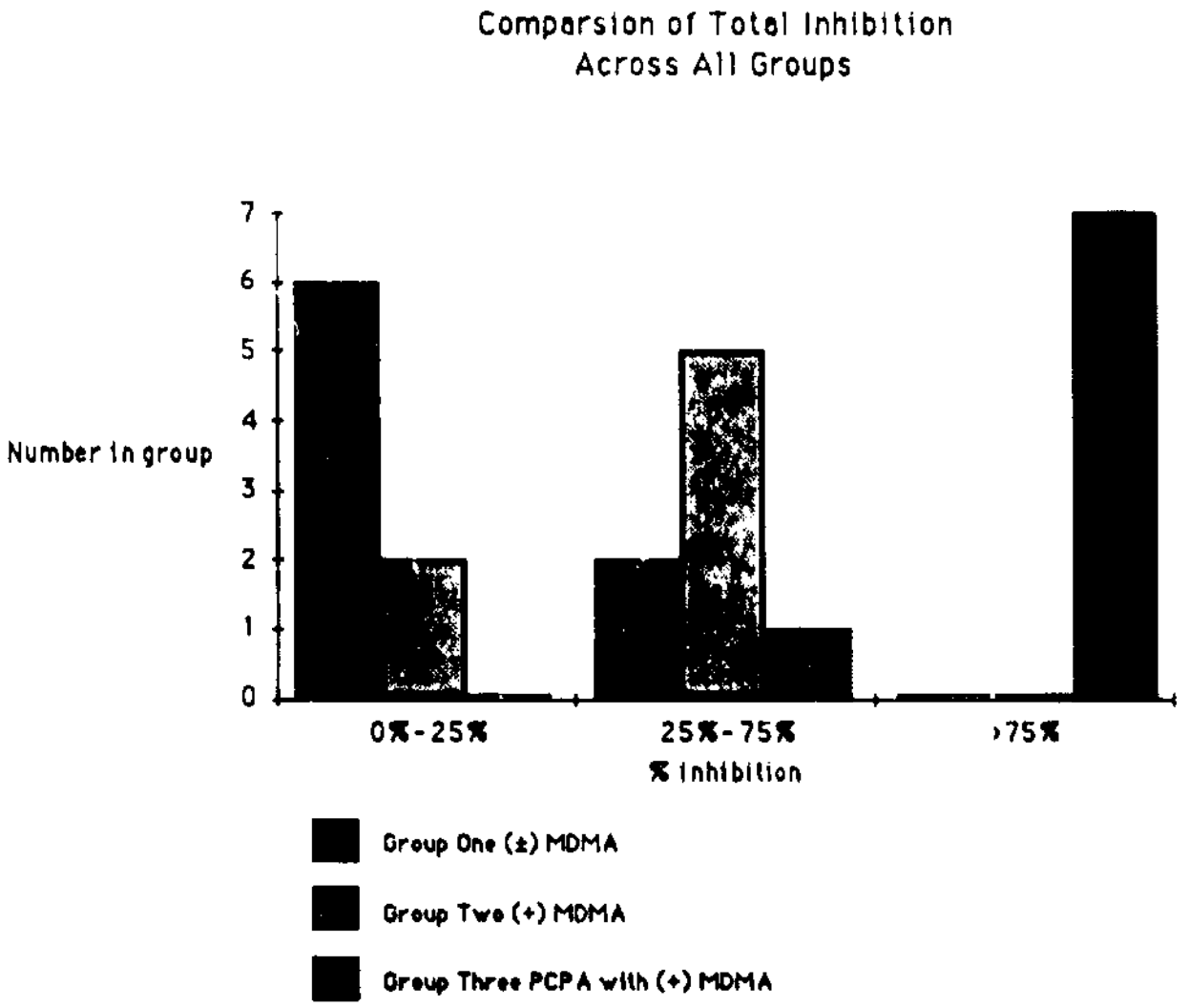
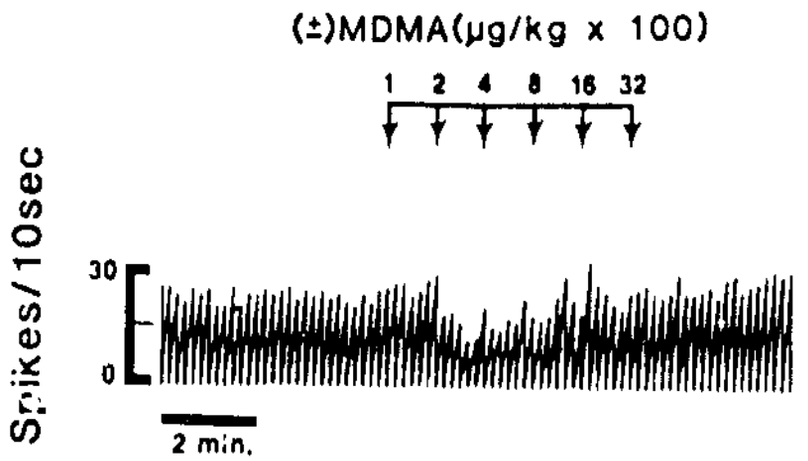


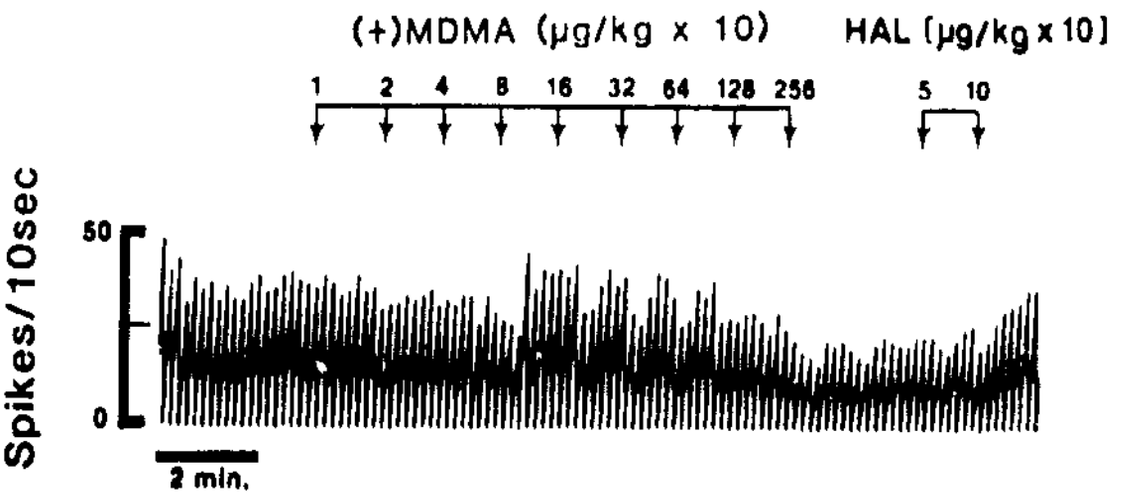
Figure Eight:



A: (±)-MDMA



B: (+)-MDMA



C: PCPA Treated-(+)-MDMA