The Effects of MPP^+ on the Dynamics of Dopamine Release in the Corpus Striatum of the Rat Brain

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ABSTRACT

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Parkinson's disease is a progressive disorder affecting mainly persons of middle age or older. The main disturbance of this disease is a loss of motor function caused by a pattern of cell loss in the zona compacta of the substantia nigra. As a result of substantia nigra degeneration, a depletion of a neurotransmitter, dopamine, occurs in the corpus striatum. By the depletion of the neurotransmitter, the symptoms associated with Parkinson's disease, such as tremor or rigidity often appear.

Certain endogenous or exogenous hypotheses have emerged as to the cause of Parkinson's disease. MPP^+, a potent neurotoxin, was found to cause symptoms equivalent to Parkinson's disease plus the degenerative effects in the nigrostriatal dopaminergic system of humans and rodents. MPP^+ has given researchers an animal model for the specific investigation of Parkinson's disease. Researchers have proposed that the dopamine cell loss in the corpus striatum might lead to a "retrograde system" causing the loss in the substantia

nigra. Presently, this study examined the effects MPP^+ had on dopamine release in the rat brain. The technique of *in vivo* voltammetry was used to monitor the releases of dopamine directly in the major area of dopamine cell loss, the corpus striatum.

Results of this study demonstrate that dopamine release is substantially decreased in the corpus striatum when induced by MPP^+ compared to the potassium-stimulated (control) responses. Decay times, clearance rates, and overall secretion rates of the MPP^+- induced releases suggest that this neurotoxin not only effects the release of dopamine but the reuptake mechanisms as well.

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CHAPTER I

INTRODUCTION

James Parkinson, in 1817, described the disease and its symptoms that corrulate to the syndrome that is given his namesake - Parkinson's Disease (PD). At this point in time, he had no autopsy studies to substantiate the pathological site until Brissaud, in 1895, had three reports of tuberculomas in the substantia nigra (SN) of individuals with Parkinson's (Forno, 1995). It was not until 1960, when Ehringer and Honykiewicz would establish the dopamine deficiency in the striatum, as well as, the nigrostriatal dopaminergic pathway, that nerve cell loss in the SN was accepted as a crucial part of the pathology of PD (Forno, 1995). Other contributors such as, Cotzias et al. (1967) strengthened this view of dopamine deficiency. Cotzias was able to reverse the effects of Parkinson's by giving large oral doses of the precursor to dopamine, D,L-dihydroxyphenylalanin (dopa) to Parkinson's patients. Contrary to this finding, giving patients B-melanocyte-stimulating hormone intramuscularly made the Parkinson's patients worse (Cotzias et al., 1967). Confirmation was still needed though in the relationship of the decrease in dopamine to the nerve cell loss in the SN. In 1973, Bernheimer et al. confirmed this corrulative relationship. In their large study, 69 patients with various forms and degrees of Parkinson's disease were investigated. Biochemical examinations were performed on the 28 brains from the Parkinson's group, as well as, the 28 controls and 14 Huntington's disease brains. They found that the dopamine decrease in the striatum was

congruent to the degree of nerve cell loss in the SN (Bernheimer et al., 1973). Therefore, all of these former studies have given present day researchers a good working knowledge of the pathology of PD. Now other disciplines can use this knowledge to investigate possible hypotheses of Parkinson's disease.

DEFINITION AND SYMPTOMS

Parkinson's disease is defined clinically as a progressive disorder which affects persons of middle age or older. The major manifestations of this disorder occur on the disturbance of motor function. The degenerating loss of motor function is caused by a characteristic pattern of neuronal cell loss in the zona compacta (dense area) of the SN with the formation of Lewy bodies. As a result of substantia nigra degeneration, a depletion of the neurotransmitter, dopamine, in the striatum also occurs. It is this depletion of dopamine that causes the signs and symptoms of PD (Yahr, 1993). Tremor, rigidity, and bradykinesia are the major features of this disease. The tremor associated with PD occurs at rest and can involve the hands, arms, legs, head, and even the jaw. The rigidity of the Parkinson's patient appears in both the flexors and extensors of a joint. The axial and appendicular musculature are effected and can lead to the displacement of one's center of gravity which may eventually lead to falling (Pahwa and Kollar, 1995). Bradykinesia, which can be defined as the "inability to iniate or perform common motor movements with normal speed", is probably the most debilatating factor (Pahwa and Kollar, 1995). Normal functions such as eye blinking, facial movements, and movements of the arms when walking are impaired and cannot be done without conscious effort.

In addition, there are other nonmotor manifestations of this disease. A loss of smell, dementia, and depression represent a few of the changes that can occur with patients, but patients may not display each of these symptoms (Pahwa and Kollar, 1995). The loss of smell that occurs in approximately 75-90% of PD patients may occur many years prior to the onset of the motor symptoms (Pahwa and Kollar, 1995). This specific observation may have interesting implications for the cause of PD. First, there is the hypothesis that since the olfactory receptors are exposed directly to the environment, a toxic agent can enter the nasal cavity and make its way to the brain. Secondly, the entire disease process may decrease the resistance of the olfactory epithelium. By decreasing this resistance an exogenous agent can easily damage the olfactory epithelium (Pahwa and Kollar, 1995). Dementia, as a subsequent nonmotor manifestation, is also a matter of debate and scutiny. It is known that approximately 10-40% of cases show the prevalance of dementia in PD (Fearnley and Lees, 1990). The variability that is seen could be due to the assessment criteria for a particular study. Research conducted by Marder et al. (1990) found that " first-degree relatives of demented patients with Parkinson's disease had at least six times the risk of dementia compared with nondemented patients with Parkinson's disease". This investigation by Marder et al. (1990) also substantiated that Parkinson's patients had a high prevalance of dementia, as well as, a high prevalance of PD in first degree relatives. This statement by these researchers can raise the question of shared genetic risk factors in Parkinson's. Finally, depression in PD is also quite common. In a clinicopathological study of striatonigral degeneration by Fearnley and Lees (1990) all 5 patients with PD in this study were diagnosed as having depression or even suicidal thoughts. Depression

in Parkinson's patients may be reactive or endogenous to this disease, however, researchers are still uncertain of its origin.

Congruent to these motor and nonmotor manifestations, there are several symptoms that involve the autonomic nervous system. These symptoms include the following: gastrointestinal problems (swallowing, delayed gastric emptying, excessive salivation and constipation), sexual dysfunction, excessive sweating, and urinary problems (Pahwa and Kollar, 1995). These manifestations collectively can cause emotional, mental, and substantial physical trauma.

INTRODUCTION TO NEUROTOXIN

As the advancement in technology has occurred over the years, so has the realm of possibilities opened to test varied hypotheses concerning PD. The probable causes of PD can be grouped into either exogenous or endogenous exposures to potentially harmful agents. These hypotheses suggest that exposure to toxic agents, such as air or water pollutants, cause PD. It is known that not everyone who is exposed to a pollutant becomes a PD patient. This has lead reserachers to believe that there might be a genetic predisposition required for this causation. The most probable cause could be one of a "multifactorial pathogenesis" (Forno, 1995). This multifaceted causation suggests that it is not just one neurotoxin or one genetic defect that causes PD but many agents combined. The main hypotheses for the causes of PD are as follows: (1) aging (2) environmental toxins (heavy metal accumulation or pesticides) (3) metabolic and genetic areas (free radicals, mitochondrial toxins, neuromelanin) and (4) Andre Barbeau's "Global Hypothesis" - includes all of these mentioned (Forno, 1995). Of these

hypotheses, the one of our interest and research is in the use of a neurotoxin, 1-methyl-4-phenyl pyridinium (MPP^+).

MPP+

MPP^+ is the pyridinium derivative of the contaminant called MPTP or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. MPTP was first discovered in 1977 by a 23 year old college student. This student was referred to the National Institute of Health because of an onset of a parkinsonian syndrome. The college student had used a variety of illicit agents, but during the past summer he had begun to use a mixture of cocaine and a demerol-like compound, 1-methyl-4-phenyl-4-propionoxy-piperidine (MPPP). He perpared the mixtures successfully many times. The last batch, though, was sloppily prepared, and he used the drug without purification. Researchers were able to identify a side product, MPTP, as the contaminate responsible for the Parkinson's-like symptom. This patient eventually died of a drug overdose, and an autopsy revealed striking nerve cell loss in the substantia nigra (SN). This nerve cell loss now confirmed their suspicions. This was a toxin that leads to symptoms of PD and produces the degenerative effects in the nigrostriatal dopaminergic neurons of humans (Kopin et al., 1986).

Now researchers needed an animal model to work with that could be used to further test the effects of MPTP on neuronal systems. Several animal species were tested to observe their responsiveness to this chemical. First, chemical lesions were introduced in the rodent brain to try and initiate parkinsonian syndrome, but this failed (Kopin et al., 1986). Next, monkeys were given MPTP, intravenously for several days. The monkeys did exhibit an extrapyramidal syndrome, accompanied by decreases of dopamine, metabolites of dopamine, and norepinephrine. Increases of serotonin were

evident as well. In addition, the changes seen in monkey behavior with MPTP were the most comparable to human behavior with MPTP induced PD (Siegal et al., 1989). Further research conducted by Mortatalla et al. (1992) confirmed that MPTP treatment in monkeys can produce a pattern of nigrostriatal degeneration characteristic of that seen in Parkinson's patients. Even though the use of the monkey model was successful, mice proved just as sensitive to MPTP plus are quite economical for research purposes (Langston, 1995).

These differences of selective dopamine neurotoxicity of MPTP are still not fully understood. Could there be specific receptor sites for MPTP analogous to high affinity receptors? MPTP does bind with high affinity to brain membranes and can easily cross the blood brain barrier (Itano et al., 1995). MPTP also has small variations in its structure that can abolish affinity for dopamine and serotonin binding sites. Cotzias (1986) studied the binding of MPTP to several brain areas in humans. By autoradiographic localization, MPTP binding sites were most dense in the caudate nucleus of the basal ganglia. The caudate nucleus is the area involved in motor function, and it is the primary area of dopamine depletion causing the symptoms of PD. Accordingly, there was considerable grain density in the substantia nigra by autoradiographic localization. This is confirming MPTP's binding and potential degenerative properties. The locus ceruleus displayed high density as well. The locus ceruleus is an area of norepinephrine cell bodies. Therefore, it can be concluded that MPTP is binding in catecholamine containing areas.

One particular finding of Cotzias concerns the sensitivity of MPTP in the rat brain. There are much fewer binding sites for MPTP in the rat substantia nigra and caudate than in the human. This equates to a much lower

sensitivity of the rat brain to the neurotoxic actions of MPTP (Cotzias, 1986). Therefore, the use of MPTP in our rat brain experiments would be futile. This lead us to stimulate dopamine release by MPP^+, the active metabolite, instead.

The nature of the MPTP receptor was still a mystery until Chiba et al. (1984) showed that monoamine oxidase in brain mitochondria converts MPTP to MPP^+. Subsequent research with inhibitors of monoamine oxidase reported that inhibitors of monoamine oxidase B (MAO-B) were effective against MPTP binding (Cotzias, 1986). Since MAO-B is known to be located in astrocytes and serotonergic neurons, MPTP is proposed to be oxidized in these cells. MPP^+, as described by Itano et al. (1995) is found to cause degeneration of cultured mesencephalic dopaminergic neurons. This suggests that MPP^+ is the substantial neurotoxin. MPP^+ can be taken up into dopaminergic neurons via the dopamine reuptake system (Chiba et al., 1985). MPP^+ is then allowed to accumulate by reversibly binding to neuromelanin. Neuromelanin is a waste product of catecholamine metabolism and is derived from the oxidation of dopamine, norepinephrine, and related compounds of quinones (Graham, 1979). Monkeys and humans have a high content of neuromelanin in the SN while little or no neuromelanin exists in the SN of rodents (Kopin et al., 1986). D'Amato et al. (1985) reported in monkeys that there was high affinity binding of MPP^+ to synthetic neuromelanin. Actual neuromelanin is present in abundance in the dopaminergic neurons. By MPP^+ binding to this waste product, this neurotoxin can finally cause neuronal death (D'Amato et al., 1985). So far, the characterization of the selectivity of MPP^+'s toxic effects include both the uptake into the dopaminergic neurons and the binding to neuromelanin. Additionally, there have been reports that MPP^+ interferes with mitochondrial respiration as well. Langston (1995)

reports that once MPP^+ has gained entrance to cells, it can accumulate in the mitochondria. This process is dependent on the mitochondrial membrane gradient and, therefore, may be driven by the mitochondrial membrane potential. By MPP^+ entering and accumulating in the mitochondria, MPP^+ interrupts the process of cellular energy production which causes a rapid depletion of ATP. Although definitive evidence is lacking, researchers suggest that this energy depletion by MPP^+ is the ultimate mediator of cell death in neuronal systems, thereby, potentially leading to symptoms of Parkinson's disease. (Langston, 1995).

CATECHOLAMINES/ DOPAMINE

In order to understand the MPP^+ induced depletion of dopamine, it is necessary to explain the general mechanisms of the chemical transmitters affected by this neurotoxin. Neurotransmitters, in general, are synthesized by neurons to influence the activity of other neurons, muscle fibers, or gland cells (Tortora and Grabowski, 1996). Neurotransmitters are synthesized from the products of normal metabolism and consists of a simple chemical structure. Catecholamines are a specific group of neurotransmitters that are prevalent in peripheral tissue, as well as, the central nervous system. This group consists of such chemical transmitters as dopamine, norepinephrine, and epinephrine. They are termed catecholamines due to their catechol nucleus (benzene ring with two hydroxyl groups) and an amino acid. The pathway of catecholamine synthesis is quite simple and takes place in the cytosol. Tyrosine, a natural amino acid found in protein foods, is converted into L-Dopa, then into dopamine (DA). DA is then taken up into the storage vesicles of nerve terminals. DA can be further converted into norepinephrine

and finally epinephrine. Each conversion requires an enzyme. This enzyme that converts tyrosine into L-Dopa is tryrosine hydroxylase. It is this enzyme that is the rate limiting step for the formation of these substances. A rate limiting step is one critical step either in the synthesis or storage of the transmitter which will limit the amount of the transmitter given. Aromatic amino acid decarboxylase is the enzyme that converts L-Dopa into DA. Thus, in order to synthesize DA specifically both enzymes, tyrosine hydroxylase and aromatic amino acid decarboxylase must be available in the neuron terminal (Thompson, 1985).

Once these catecholamines are synthesized, they can be concentrated within the storage vesicles by an ATP-dependent process which is linked to a proton pump. This intravesicular concentration of catecholamines is approximately 0.5M, and they exist in a complex with adenosine triphosphate (ATP) plus acidic proteins known as chomogranins. This vesicular uptake process can transport a variety of biogenic amines including tyramine, which can compete with endogenous catecholamines for vesicular storage sites. These storage vesicles play a dual role in which they have a ready supply of catecholamines, such as DA, at the terminal ready for release and mediate the process of release. For example, when an action potential reaches a nerve terminal, calcium channels will open, allowing an influx of calcium into the terminal; increased intracellular calcium promotes the fusion of DA vesicles with the presynaptic neuronal membrane receptors (Siegal et al., 1989). The DA vesicles will discharge their contents into the synaptic space. The neurotransmitter can diffuse back and be taken up by the presynaptic terminal (Thompson, 1985). This reuptake process of DA is a Na^+-dependent uptake process which is a characteristic feature of all catecholamines. The uptake process is mediated by a carrier or transporter specific on the outer membrane

of each type of catecholaminergic neuron. The dopamine-containing neurons, for example, have a specific carrier verses other catecholamines such as norepinephrine. The uptake process is energy-dependent, and by coupling with the Na^+ gradient can cross the neuronal membrane. This linkage of uptake to the Na^+ gradient may be of physiological significance, since transport temporarily ceases at the time of depolarization-induced release of catecholamines. Because of this differential specificity of the transport processes between dopamine and other catecholamines, uptake of catecholamines can be inhibited. Therefore, DA reuptake can be inhibited by various drugs including the potent neurotoxin, MPP^+ (Siegal et al., 1989).

After this action of reuptake of DA, there is also the aspect of catabolic inactivation of the catecholamine. First, inactivation can occur by enzymes which breakdown the transmitter at the presynaptic receptor sites or postsynaptic receptor sites. Otherwise, inactivation can occur when the transmitter is released and degraded. Two important enzymes which are involved in DA breakdown are monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT). MAO, a flavin-containing enzyme, is present in both the presynaptic and postsynaptic cells and will breakdown excessive amounts of DA. MAO is also located on the outer membrane of the mitochondria. Because of MAO's intracellular localization, it plays a strategic role in inactivating DA. COMT is only present on the postsynaptic cell, and will methylate any catechol including DA (Siegal et al., 1989). After inactivation the transmitter or parts of the transmitter can be taken back up into the terminal and reused. This recyclable process is quite economical but is not perfect. Some transmitter is lost and will be metabolized. If no new transmitter is synthesized, the stores will be depleted. Again, if the rate

limiting factor is not properly activated, a depletion of the transmitter will follow (Thompson, 1985).

NIGROSTRIATAL DOPAMINERGIC SYSTEM

The nigrostriatal dopaminergic system is just one of three major dopamine circuits of the midbrain. This particular system consists of dopamine-containing cell bodies within the substantia nigra (SN). The SN is an unique structure of the lower midbrain, due to its various dark-colored cell bodies. Indeed, the meaning of substantia nigra is "dark substance". These dark colored cell bodies are found in the region known as the pars compacta or zona compacta. This specific region contains the dopaminergic neurons, but the dopaminergic neurons are light colored bodies within the SN (Kandel et al., 1991). The dopamine-containing SN cells project to the corpus striatum. The corpus striatum, for reference, consists of the caudate nucleus and the putamen. The globus pallidus is a third area involved in this system. A more inclusive term for all three areas is termed the basal ganglia (Thompson, 1985).

The majority of dopamine-containing neurons, which consists of three-quarters of all the dopamine of the brain, are found within this nigrostriatal dopaminergic system (Thompson, 1985). On average, only seven thousand Da cell bodies are located within the intact SN and project to the corpus striatum of the rat (Yurek and Sladek, 1990). This is a relatively small number of cell bodies accomplishing a majority of striatal function.

Experiments have shown that, if the nigral dopamine neurons are depleted or lesioned in animal species, surviving DA neurons do compensate. In these cases, there are also losses of striatal dopamine. Gerald Stern (1966)

first substantiated this ideology. Stern observed the effects of large bilateral lesions of the SN of primates. He concluded that with bilateral lesions of the SN plus basal gangliar regions, severe motor disturbances such as those associated with PD, were evident. Further study by Yurek and Sladek (1990) reported that most of these lesioned animals, either rodents or primates, do not produce symptoms of motor dysfunction untill a threshold of 80-90% reduction of striatal dopamine is exceeded. Clinical studies have provided analogous findings in patients with Parkinson's disease in which major symptoms are not detected until DA levels drop to less than 20% of their normal level (Yurek and Sladek, 1990).

SUBSTANTIA NIGRA

Since Stern's experiments in the 1960's, various means of exploring the SN and its dopaminergic pathway have developed, and more precise areas of the SN have been labeled and identified. The regions of concern for the SN are known as nucleus A8, nucleus A9, and nucleus A10. A8 consists of the retrorubral area, which is located behind the red nucleus. A9 is the area of the substantia nigra (zona compacta) which is often further divided into ventral and dorsal tier in some literature. A10 is known as the ventral tegmental area. A10 is directly adjacent to the SN. Each one of these areas projects to the striatum (German et al., 1989, Cooper et al., 1986). A study conducted by German et al. 1989, used a computer visualization technique in order to see the consequences of DA cell loss in PD patients. They found that in the Parkinsonian brain that there was a consistant pattern of midbrain DA cell loss throughout the three areas. The greatest cell loss was in the A9 or SN region. By all nuclear areas being effected, this indicates that the

striatal regions of each nuclei are effected. These three nuclear areas generally called the mesostriatal DA system or nigrostriatal dopaminergic system are the primary targets for Parkinson's disease (German et al., 1989). Further studies by Fearnley and Lees(1990) labeled the areas of the SN most severely involved in PD. They had divided the SN into 6 zones - ventromedial (VM), ventrointermediate (VI), ventrolateral (VL), dorsomedial (DM), dorsolateral (DL) and the most lateral - pars lateralis (PL). Brains of 10 patients had been donated and sectioned for histological study. Of the 6 zones in these PD patients, the most affected zone was the ventrolateral of the SN which projects to the putamen. The ventrolateral "strip" consists of a compact cell group extending for almost half the width of the SN. This is the most dominant cell group, and contains the most compact dopamine-containing cell group plus the areas of the greatest neuronal cell loss in PD patients (Gibb, Fearnley, and Lees, 1990).

LEWY BODIES

The Lewy body, which is always found in the SN and other brain regions of patients with PD, is a distinctive neuronal inclusion. These inclusions are generally not seen in patients younger than 60 years old (Gibb and Lees, 1988). There are a few characteristics of the Lewy body within the SN of patients with PD which can be stated. First, Lewy bodies can be spherical or oblong in structure and contain cytoskeleton proteins in the form of filaments. Secondly, these filaments in the SN radiate out from the core of the body. This filamentous material is randomly oriented, and other cell constituents may be included or trapped within the Lewy body. Lastly, the

Lewy bodies present in the SN are seen in the perikaryon, the part which surrounds the nucleus of the nerve cell (Forno, 1986).

Lewy bodies are also reported in other neurological disorders. Lewy bodies have been identifed in cases of Alzheimer's disease, dementia (Lewy-body form), neuroaxonal dystrophy, and ataxiatelangiectasia. Common with these disorders listed is that they all have an association with nerve cell degeneration in the SN as does PD. Therefore, the Lewy body is found in diseases where the SN is involved and where the patients have symptoms of Parkinsonism (Forno, 1986). Characteristically, though, these disorders do contain neurofibrillary tangles especially in the case of Alzheimer's disease. In Alzheimer's disease, the tangles constitute a greater brain area and are found throughout the cerebral cortex. The Lewy bodies, if present in an Alzheimer's patient are few and found in the cortex. It is the neurofibrillary tangles that are generally associated with Alzheimer's, and equivocally it is the Lewy bodies that are associated with PD (Forno, 1986).

Research that has been done pertaining to Lewy body formation has centered on the occurance of Lewy bodies in aging individuals. In an extensive study by Forno (1969), further investigated this aspect of aging, lewy body formation, and PD. The results of this study found that no patient under the age of 62 years old had Lewy bodies. Also, the sites of Lewy body formation were in the SN, locus ceruleus, and dorsal motor vagus nucleus. Although a clinical diagnosis of PD was not made, the hypothesis of Lewy body formation presymptomatically occuring before PD was being formed (Forno, 1969). By 1988, further research by Gibb and Lees (1988) suggested that the Lewy body formation does have some progressive pathology as seen in PD. As is known about PD, the rate of cell destruction

is constant and occurs at a modest rate. Nevertheless, some cases of Lewy body formation could represent predisposition to PD.

In addition, animal models have been used to investigate the relationship between exposure to MPTP and Lewy body formation. Forno et al. (1993) infused squirrel monkeys of advanced age with MPTP. Inclusions were noticed, but these inclusions lacked a central core and had only one inclusion per nerve cell present. This is in contrast to a very frequent array of Lewy bodies present in actual PD patients. One conclusion made from this study was that the Lewy bodies were stress-related and could possibly be reversible (Forno et al., 1993). It is still uncertain, though, if the presence of Lewy bodies is of direct consequence of MPTP infusion is the aging primates.

CORPUS STRIATUM

The final brain area of concern is the corpus striatum or CPU. As stated previously in concern to the nigrostriatal system, the dopamine-containing cells of the SN project directly to the CPU. The CPU consists of two areas, the caudate nucleus and the putamen. Besides being called the CPU, this aggregation of brain nuclei is also commonly called the basal ganglia. The basal ganglia also includes one other area, the globus pallidus, which does have an impact in the brain of a PD patient. The major input to the CPU comes directly from the cerebral cortex. Virtually all regions of the cerebral cortex convey input to the CPU. The major output of the CPU is back to the motor areas of the cerebral cortex but this time by way of the thalamus (Thompson, 1985). For example, if a person were to have a voluntary movement, the "decision" would originate in the association cortex. This area can act directly upon the CPU. The CPU would increase its

activity, and activate the motor area of the cerebral cortex via the thalamus. The motor cortex then projects to the motor neurons of the spinal cord to produce movement (Thompson, 1985). It is this motor circuit that is damaged in patients with PD due to the depletion of DA-containing cell bodies by the original source, the SN.

Each brain nuclei region of the CPU has a known specific function, and it is these functions that are degenerated in PD patients. First, the caudate nucleus is known to be involved with cognitive functions. The caudate is also the major projection site of the SN. If this brain nucleus is damaged, activity is decreased and a major relay station back to the motor area of the cerebral cortex is lost (Kandel et al.,1991). Indeed, researchers have discovered that maximal striatal dopamine loss occurs in the caudate (Fearnley and Lees, 1990). Secondly, the putamen is involved in motor programming. The putamen is organized in a somatopical way. Each area of the motor cortex is connected to a zone of the putamen. For instance, the leg area of the motor cortex projects to the dorsal zone of the putamen in non-human primates, the arm area to the intermediate zone, and head area to the ventral zone. For most PD patients, it is most common for the symptoms of the disease to begin in the arm. This would then suggest an association with neuronal deficiency in the intermediate zone of the putamen (Fearnley and Lees, 1991). Congruent to this research, Fearnley and Lees (1990) suggested that the midputamen or intermediate zone of the putamen was most effected by neuronal cell loss and pigmentation. Finally, the globus pallidus, consistant with the basal ganglia, is also intervated by the nigrostriatal dopaminergic system. The globus pallidus lies medial to the putamen and is divided into an internal and external region. The globus pallidus has a striking similarity to the pars reticulata (ventral pale zone of SN). These two nuclei sometimes are

considered a single structure much like the CPU. Functionally, the globus pallidus with the pars reticulata of the SN constitute the major output nuclei of the basal ganglia (Kandel et al., 1991). Therefore, in PD patients it can be hypothesized that the globus pallidus may have some degenerative aspects because of its SN involvement. Again, Fearnley and Lees (1990) investigated morphological aspects and conducted neuronal cell counts in the globus pallidus of PD patients. This study suggested considerable atrophy of the cells and decreased neuronal cell counts in the globus pallidus.

Most research to date emphasizes the loss of dopamine-containing cell bodies in the SN as the factor which causes PD. Still, there is a possibility that the disease process responsible for PD attacks the dopamine terminals in the CPU before the cell bodies of the SN. This would entail a retrograde axonal transport of the pathogen that causes PD (German et al., 1989). The specific character of the dopaminergic synaptic terminal in the CPU has been quite controversal. It was thought that dopamine innervation was nonspecific in nature towards the activity of striatal neurons (Forno, 1995). However, Smith and Bolan (1990) presented convincing evidence that medium spiny output neurons in the CPU do receive symmetrical (inhibitory) input on their distal dendritic spines and shafts from dopaminergic terminals. It is known that these same dendritic spines also receive excitatory input from the cerebral cortex. This particular arrangement may permit important interaction between the two afferent systems. This interaction could mean that during PD these dopaminergic terminals are devoid of DA, and the inhibitory actions of these neurons is lost. Therefore, the cerebral cortex and its stimulatory actions would be increased causing the symptoms of PD. Hence, the new acquired evidence is leading researchers in a different direction, which is not

just investigating the SN but the relevance of the dopamine cell loss in the CPU.

MPP^+ is thought to have a variety of effects in the CPU. It is thought that once MPP^+ gains entrance into the extracellular space of the synapse, it is taken up into the dopaminergic neurons. This reuptake has been referred to as the "trojan horse" phenomenon. The cells are recognizing MPP^+ as a friendly substrate but are accumulating a substance that will kill them (Langston, 1995). The reason for MPP^+ selectivity of dopaminergic neurons is still unclear. One might believe that all catecholamine uptake sites would be vulnerable, but that is not the case. MPP^+ selects dopaminergic neurons by being actively taken up into the dopaminergic terminal through the dopamine transporter (Chiba et al., 1985). MPP^+ will first stimulate DA release but will also iniate neurotoxic effects as well (Chang and Ramirez, 1986). Similar findings of dopamine release by MPP^+ *in vivo* by a microdialysis technique have also been reported (Obata et al., 1992). Examples of these neurotoxic effects of MPP^+ would be as follows: 1. decrease in concentration of neostriatal dopamine and its metabolites, 2. decrease in the ability to take up DA, and 3. disapperance of nerve cells in the zona compacta of the substantia nigra (Heikkila et al., 1984). By understanding the selectivity of MPP^+, certain clues pertaining to PD could be unraveled and the degenerative processed halted.

HYPOTHESES

Since Parkinson's disease is thought to be caused from either exogenous or endogenous toxins, a whole realm of hypotheses has emerged.

Besides the exogenous hypotheses of the "pollutant" and "toxin - MPP^{+}" already mentioned, there are other hypotheses of concern. These forms of causation are of the endogenous point of view. The areas of interest are the following: simple aging, hormonal regulation by estrogen, and dopamine regulation.

AGING HYPOTHESIS

It has been well established that PD affects mainly middle-aged to older individuals. The average age of onset is usually 58-62 years old, but onset after the age of 70 is not uncommon (Forno, 1995). Controversy arises when patients over 90 are diagnosed as having PD. Do these older persons have PD or is their nerve cell loss because of their age? In general, as a person ages, nerve cell loss does occur in the SN. This nerve cell loss is not extensive enough to cause PD in all older individuals. As previously stated, PD symptoms do not occur until 50% of nigral neurons and 80% of striatal dopamine is lost (Fearnley and Lees, 1991). Fearnley and Lees, in their 1991 study of aging plus degenerative cell loss in PD, found that these are unrelated events. They are considered unrelated because the nerve cell populations most vulnerable in PD appear to be relatively resistant to the effects of aging. This extensive cell loss known to be present in PD patients is not evident in all individuals over the age of 60 (Fearnley and Lees, 1991). Even before this 1991 study of Fearnley and Lees, Scherman et al.(1989), found interesting data concerning normal aging and Parkinson's disease. They reported that dopamine neurodegenerative processes in the caudate were accelerated about twofold in PD patients when compared to normal aging. Futhermore, aging does not enhance the rate of progression of

dopamine lesions. From these studies, it can be concluded that normal aging can not cause PD. Why then is this a disease of only older persons? One way of approaching this question could be the genetic implications. There could be a genetic factor that contributes to the beginning degenerative process while normal aging compounds these effects. Some researchers have even suggested that an illness early in life could iniate the cell loss in the brain and that aging enhances this as well. This "latency" concept in PD derives from the symptoms of depression, dementia, motor disfunction, and loss of olfaction years before actual diagnosis (Pahwa and Kollar, 1995). Again, a multifactorial hypothesis is plausible due to these several areas of investigation.

GENDER DIFFERENCES: MALE VS. FEMALE

Studies conducted have expressed a male predisposition to PD, and varied death rate statistics have shown that a higher percentage of men than women are dying from Parkinson's disease. A study published in 1990 by Kurtzke and Murphy clearly showed a male:female variance. The ratios of 2.7 (male) to 2.1 (female) for every 100,000 persons in a population were given. This male prevalence is not just a modern phenomenon. Even going back to the 1950's, Kurtzke and Murphy saw the same pattern. The male:female ratios for 1950 were 1.6 (male) to 1.1 (female) for every 100,000 persons in a population (Kurtzke and Murphy, 1990). The 1950 statistical numbers for their study were considerably lower because Parkinson's disease was usually not diagnosed as accurately as it is in this decade. A change in statistics can be seen around 1975 when both male and female ratios rose sharply. This diagnosis can be attributed to technological advances and

health care changes. This increase continued till 1985, when this particular study had terminated and then published (Kurtzke and Murphy, 1990). Concurrent to the Kurtzke and Murphy studies, Diamond et al., in 1990, also found that there was an increased male predisposition to Parkinson's disease. Of course, in this study, a depletion of dopamine was seen within the striatum of the deceased persons.

Contrary to these findings of male predisposition, there have been studies claiming that the opposite is true. In a study done by Schoenberg et al., 1985, they claimed that male:female ratios with PD to be 0.75 (male) to 0.81 (female) for 100,000 persons in population. These statistics of Schoenberg et al. are the exception to most findings presented today.

ESTROGEN - A NEUROPROTECTANT

A plausible explaination for the decreased occurance of Parkinson's disease in females could be the role of the hormone, estrogen. Estrogen or estradiol, a gonadal steroid hormone, is mainly synthesized and secreted by the female ovaries, particularly the ovarian follicle. It is during the follicular phase of the menstrual cycle that estradiol is secreted. Estradiol's function at the follicular phase is one of a negative-feedback mechanism. By the follicle increasing estradiol secretion, this steroid hormone can feed back and inhibit the secretion of the anterior pituitary hormone FSH (follicle stimulating hormone). The inhibition of FSH will ultimately only allow one follicle to become mature (Hadley, 1996). Besides its role in follicular selection and maturation, estradiol has several physiological actions. Estradiol is known to maintain libido and sexual behavior, increases pituitary gonadotropin releasing hormone receptor number, stimulates secondary sex characteristics

(pubic hair growth, adipose tissue), and even decrease plasma cholesterol formation (Hadley, 1996).

The effects of estradiol in relation to striatal dopamine release was first elucidated by Becker and Ramirez in 1980 and 1981. The researchers at this time had concluded that endogenous gonadal hormones did modulate striatal DA (dopamine) release and nigrostriatal DA-mediated behaviors (Becker, 1990). These conclusions were only true for female rats not male rats. Further experimentation by Becker and Ramirez lead them to using an *in vitro* superfusion system to show distribution of DA levels. In this second study, they used amphetamines to stimulate DA release from striatal tissue. In female rats without ovaries, DA release was decreased. In male rats, removal of the testes had no effect on the release of DA from striatal tissue (Becker and Ramirez, 1980, 1981). These particular studies lead Becker to delve further into the possibility of estrogen having a direct effect on the striatum to induce changes in DA release. In 1990, Becker confirmed this direct-effect hypothesis. The results of this study concluded that, "estradiol acts directly on striatal tissue *in vitro* to modulate DA release and that there are sex differences in this effect of estradiol" (Becker, 1990). These effects of estradiol include stimulation of DA release and an increase of striatal DA responsiveness.

Other avenues of study by McDermott, Leiu, and Dluzen (1994) expounded on Becker's findings. Now, it was common knowledge that estrogen treatment increased stimulation and responsiveness of DA release. Of course, estradiol exerts hypothalamic effects as seen by follicle selection and maturation. It can also have effects upon extrahypothalamic sites, such as the nigrostriatal dopaminergic system (McDermott et al., 1994). McDermott et al. (1994) then observed the differences of nigrostriatal

dopaminergic function by measuring dopamine and DOPAC (3,4-dihydroxyphenylacetic acid) release rates in male and female mice. Subsequent treatment with estrogen or oil vehicles was done after measurements were taken (McDermott et al., 1994). First, this experiment proved that male mice show increases in striatal dopamine release with decreases in dopamine content. Secondly, it also appeared that estrogen is acting specifically in female mice as a modifier of neuronal dopamine release. By modifying release, this would entail effects upon striatal dopamine metabolism specifically increasing neuronal dopamine synthesis and release without depleting dopamine content (McDermott et al., 1994). These findings are of particular interest in relation to Parkinson's disease. As statistical figures indicate the increased male predisposition, experimental analysis substantiates this claim.

Indeed, estrogen could be acting as a neuroprotectant in which this hormone modifies DA release. In a study by Disshon and Dluzen (1997) this modification was noticed. By using an *in vitro* superfusion technique on striatal female rat tissue, infusions of MPP^+ alone, MPP^+ estrogen, and a no-treatment control were performed. MPP^+ alone did display a notable increase of DA release while MPP^+ with estrogen displayed a reduced increase in DA release. This analysis suggests that estrogen has a direct effect on striatal dopaminergic neurons and may prevent the initial increase in DA release. Again, this theory of gonadal steroid involvement has been proposed as a substantial mechanism for the gender differences in PD patients.

DOPAMINE HYPOTHESIS

The role of dopamine, itself, as a potential causative agent in PD has also been investigated. DA is found in high concentrations in the nigrostriatal neurons. These particular DA-containing neurons contain DA in greater concentrations than other catecholamine areas of the brain. Therefore, dopamine may be a causative agent because of the three following reasons: (1) the production of hydrogen peroxide in normal dopamine metabolism (2) the tendency of DA to autoxidize, a process that leads to formation of toxic dopamine analogs, and (3) an association of its specific chemical reactivity with neuromelanin (waste product) within the substantia nigra neurons (Irwin and Langston, 1995). The most important mechanism mentioned by which DA might damage neurons, is in its normal metabolism. Dopamine is oxidized into an aldehyde called 3,4-dihydroxyphenylacetic acid (DOPAC) which occurs rapidly in the central nervous system. The actual breakdown of DA uses molecular oxygen, and in doing so, produces a single molecule of hydrogen peroxide for every molecule of DA that is deaminated. Hydrogen peroxide has damaging effects to neurons and could possibly be damaging dopaminergic neurons. This neuronal cell loss could lead to Parkinson's disease (Irwin and Langston, 1995).

Furthermore, a vicious cycle could ensue by this continuous release and metabolism of DA. Likely, the hydrogen peroxide damage causes other surviving neurons to compensate by increasing DA release. However, increased release of DA would lead to enhanced production of hydrogen peroxide. In turn, hydrogen peroxide would produce a further degeneration of dopaminergic neurons. This dopamine degenerative cycle would be set in

motion and could eventually result in sufficient neuronal cell loss, and symptoms of PD would soon follow (Irwin and Langston, 1995). This particular hypothesis does have a logical basis but has not yet been proven by animal models. The MPP^+- stimulated animal model in regards to the dopamine hypothesis might prove how the dopaminergic neuron is degenerated. Since, MPP^+ is known to stimulate an increase in DA release, as well as, decrease the ability to take up DA, perhaps this could contribute to the demise of the dopaminergic neurons in this degenerative cycle.

CONCLUSIONS

Although extensive research has been done pertaining to the degenerative striatal and substantia nigra dopamine cell loss in PD, several integral parts have not been substantiated. The MPP^+-treated animal model, though, has opened new pathways of investigation. Already, this model has given researchers a means to test working hypotheses to envision how the disease process of PD actually happens. Accordingly, the present study with the use of an MPP^+-treated animal model and the electroanalytical technique, *in vivo* voltometry, reports on the dynamics of DA release in the corpus striatum in the female rat brain. DA release is induced by the classical potassium-stimulated release (control) and by MPP^+-induced dopamine release. K^+-stimulated dopamine release via the depolarization of neurotransmitter is by a Ca^{++} dependent exocytosis, while the MPP^+-stimulated dopamine release is by the uptake mechanism through the dopamine transporter or carrier. The relative amounts of DA released, as well as, the time course of DA is evaluated. Several parameters of investigation have also been elucidated in this study which are as follows: (1) amplitude of

dopamine release, (2) dopamine's secretion rate, (3) clearance rate from the probe, (4) rise time of the dopamine release from the initial infusion of MPP^+ or potassium to peak amplitude, and (5) the various time phases from peak amplitude back to baseline. By understanding the entire nature of dopamine release, an important key to unlocking the mystery surrounding dopamine's role in Parkinson's disease could be solved.

CHAPTER II

MATERIALS AND METHODS

EXPERIMENT

This experiment was designed to assess the effects of MPP^+ (neurotoxin) or high potassium (control - K+) on dopamine release in the CPU of the rat brain. *In vivo* voltammetry, an electroanalytical technique was used to monitor dopamine release. Twelve successful experiments were run from a total of twenty-four rats. Of these twelve successful experiments, 43 dopamine responses stimulated by K^+ were recorded in six animals, and 39 dopamine responses were stimulated by MPP^+ in the remaining six animals. Several dopamine responses by both groups were recorded in each rat as the electrode was passed through the CPU at 0.5mm intervals.

ANIMALS

Twenty-four female Long-Evans rats ages 6-12 months were housed in groups of two to four with a reversed 12 hour light : dark cycle, with lights off between 1000 and 2200 hour. Food and water were available ad lib. All twenty-four female rats were bilaterally ovariectomized (OVX) two - four weeks prior to experimentation with the use of the dual anesthetics, ketamin and xylorin. Also, by removing the ovaries, no possible hormonal interaction

by estrogen should be visible with the dopamine releases. The average weight of the female rats used in this experiment was 420.2 grams.

STEREOTAXIC SURGERY

Rats were deeply anesthesized with urethane (1.25-1.50g/kg IP, Sigma). The total dose was divided into three injections of approximately 15-20 minute intervals. Rats were tested for any withdrawl reflexes and was placed evenly into the ear bars of a stereotaxic apparatus and securely fastened. The rat's temperature was monitored by a rectal thermometer, and a heating pad was placed under the rat's body. The urethane caused the rat's body temperature to decrease so constant care was taken to keep its temperature steady at 37 degrees Celsius. Next, the dorsal portion of the rat's head was shaved from nose to ears, and a midline incision was made in an anterior (cephlad) to posterior (caudal) direction. The tissue seen was scraped aside to expose the skull. Bregma was located and highlighted by a marker pen (See Figure 1). Two square windows were drawn anterior to bregma on the right and left sides of the frontal bone. By having two windows, two full passes with the probe apparatus could be made on the right and left sides. A Dremel drill was used to outline the windows and then gently shave the innermost bone sections. The bone chips were removed, and small strips of saline-soaked gauze were placed over the brain exposed windows. Two other small holes (side-by-side) were also drilled vertically in the right side of the parietal bone posterior to bregma. In one of the holes, the two ends of the reference electrodes used for *in vivo* voltammetry were placed. In the second hole, a small machine screw 1/8" long was fashioned into the hole for stability. Lang's jet acrylic powder (Lang Dental M.F.G. Co., Inc.) was spread around the reference electrode and machine screw.

Two drops of Lang's jet liquid acrylic were added to the powder areas for adhesiveness. The final step in this process was to determine the area of the corpus striatum where the probe apparatus would be placed. Three specific coordinates were needed and assessed which were anterior-posterior (A-P), medial-lateral (M-L), and vertical (V) positons. Coordinates were determined according to Pellegrino et al. (1979). The following coordinates in millimeters were used: 1.5 to 2.5mm (A-P), 2.0mm (M-L), 1.5mm (V) to start then .5mm intervals vertically to the corpus striatum. (See Figure 2 for CPU location)

IN VIVO VOLTAMMETRY

PRINCIPLES

The technique of *in vivo* voltammetry was adapted from the well used electrochemical detection (ECD) which had been coupled with HPLC for amine and neurotransmitter assay. Ralph N.Adams and his team of researchers made *in vivo* voltammetry available for experimentation in the early 1970's (Adams, 1990). This *in vivo* voltammetry technique involves implanting into the brain a smaller version of the HPLC electrochemical detection. This specific electrochemical detection relies on a well-known property of catechol and indole-based substances - their susceptibility to undergo oxidation (Maidment et al., 1990). Dopamine, norepinephrine, and serotonin can all be detected because of their oxidizable properties. In voltammetry, the oxidizing agent is a carbon-based working electrode. At the surface of this electrode, a positive potential of a certain strength sufficient to remove electrons from the oxidizable species occurs with respect to a

reference electrode. The flow of electrons from working to reference can be measured in the form of an electrical current. The amplitude of this current is directly proportional to the amount of material oxidized (Maidment et al., 1990).

Some electroactive species will undergo oxidation with greater ease than others. With respect to the present study, dopamine oxidizes quite easily because it has a catechol nucleus. During the oxidation process, hydroxyl groups surrounding the catechol nucleus lose hydrogens, as well as, electrons. The oxidation number also becomes greater due to the electrons moving to the oxidants. Electrons are also more readily removed from hydroxyl groups than from NH-groups. Therefore, DA consisting of two hydroxyl groups surrounding a catechol nucleus allows oxidization at a lower potential. This basic knowledge is important for proper interpretation of the *in vivo* signals (Maidment et al., 1990).

Obviously, there are hundreds of chemical compounds in the brain, but fortunately there are few species that are both present in high enough concentrations to be detected together by this electroanalytical technique (Maidment et al., 1990). So by this technique, dopamine and serotonin should not be detected together but as separate entities. It should be stated that the electrodes work in the extracellular fluid (ECF). Therefore, all voltammetric measurements record the concentration of compounds within the ECF (Marsden et al., 1988).

ELECTRODES

Two working electrodes, 30um tip diameter, (Quanteon, Rocky Mountain Center for Sensor Technology, Denver, CO.) were prepared for

each experiment. The working electrodes are comprised of a single carbon fiber that protrudes 0.3mm from the resin and glass capillaries that surrounds them. Gold pin connectors (Newark Electronics) were soldered to the end of the exposed electrodes.

One area of concern for this technique is within the ECF of the brain. There are other compounds, such as, ascorbic acid, which are present in excess in the brain. Ascorbic acid is present in high concentrations in the brain and is easily oxidized. Therefore, it has the potential to interfere with recording monoamine signals. The working electrodes were then coated by the electrochemical substance Nafion (Aldrich Chemical Company, Inc., Milwaukee, WI) to eliminate any interference by other substances. Nafion is a perfluorosulfonated derivative of Teflon and is highly negatively charged (Maidment et al., 1990). Electrodes were coated 6-8 times with nafion and baked at 160 degrees Celsius after each coat was applied. By coating with Nafion, this increased selectivity of the electrodes for dopamine over ascorbic acid.

In vitro calibration tests, data acquisition, and data analysis was performed with a computer-based neurotransmitter analyzer system, IVEC-10 (Medical Systems Corp., Greenvale, NY). This system allowed high-speed chronoamperometric measurements to be recorded as described by Friedemann and Gerhardt (1992). Measurements, by this type of *in vivo* volammetric method, could be taken at 5 Hz, and then averaged over 1 s to enhance the signal-to-noise of the recordings. A potential of +0.55 V verses a Ag/AgCl reference was applied to the electrode for 100 msec. The resulting oxidation current was digitally integrated during the last 70msec of the pulse. When the electrode was returned to resting potential (0.0V), the

reduction current produced by the oxidized electroactive species was integrated in the same manner (Friedemann and Gerhardt, 1992).

In order to calibrate *in vitro*, dopamine-HCL (Sigma Corp.) 0.038g DA/100 ml 0.1M perchloric acid for a 2mM solution was made fresh weekly. Ascorbic acid (Sigma Corp.) at a concentration of 0.352g AA/100ml distilled water for a 20mM solution was made fresh daily. 40ml of a 0.1M phosphate buffered saline (pH 7.3) was placed in a 50ml Pyrex beaker, and this beaker was positioned on a stage to which the headstage of the IVEC-10 was overhead. The Nafion-coated electrode was placed into the beaker in solution, and the gold pin connector of the electrode was placed into the headstage. A stable reference electrode (silver wire) was also connected to the headstage and placed in buffer solution. The gain was set on the headstatge at 100V/uA. The calibration mode was entered in the computer, and the electrode was allowed to stabilize for about 150-200 seconds. Gain adjustments could be made at this point to ensure correct oxidation and reduction waveforms on the computer screen. These gain numbers were recorded so that when the electrodes were to be used for *in vivo* measurements the correct gain for each electrode was used. After stabilization, the working electrode was challenged with 500$_{\mu}$l of 20mM of ascorbic acid to test for the selection of the electrode. After the addition of ascorbic acid, four subsequent additions of 40$_{\mu}$L of 2mM DA solution, was added to the beaker and mixed after each addition. Each addition of dopamine raised the concentration by 2$_{\mu}$M in order to generate a standard curve. After all additions were made, linear regression of the oxidation and reduction currents were calculated by the computer and printed. (Refer to Figure 3) Oxidation currents were seen on the y-axis of the graphs while the x-axis of the graphs represented the $_{\mu}$M concentration of dopamine. The

oxidation slope, which representes the calibration factor that determines the sensitivity of the probe to the test chemical, should be greater than 100,000. The reduction/oxidation (RED/OX) ratio, the ratio of the two slopes which does help to characterize the identity of the chemical, should be between 0.2-0.8. The selectivity of the electrode for dopamine over ascorbic acid should be greater than 500 to 1. The signal- to -noise value listed is important to this working system to tell how small a signal that will be seen over the level of noise in the system or electrode. This value needed to be under 0.1000 to be accurate. Lastly, the correlation coefficients needed to be greater than 0.997 for the oxidation and reduction slopes (*In Vivo* Electrochemistry Computer System Version 3.0 User's Manual, 1993).

PROBE APPARATUS

Once the working electrodes had been calibrated, the probe apparatus could be constructed. A single barrel micropipette (Quanteon, Rocky Mountain Center for Sensor Technology, Denver, CO.) had to be positioned tip to tip with the working electrode. Melted sticky wax (Kerr Corporation, Romulus, MI) was applied to the area surrounding the outer wall of the electrode and micropipette carefully. The ends of the electrode and pipette were pressed together till the wax hardened. The tip of the micropipette and the tip of the electrode had to be even with each other and between 270-330 um apart. To check each apparatus, a microscope with a micrometer was used. Once the correct measurements were secured the probes could be set aside in buffer solution until implanted.

REFERENCE ELECTRODES

In electrochemical detection, the application of the needed potential is given to the working electrode but with respect to a reference electrode. The currents generated are read from working to reference as a result of chemical oxidation and reduction. Also, the chemical reaction at the reference electrode has to be reversible to maintain a stable reference potential (User's Manual, 1993).

Two good reference electrodes were used for each experiment. The reference electrodes used were silver/silver chloride wires. The silver/silver chloride wires were insulated in Teflon, and a strip of approximately 5mm of this Teflon was removed by razor blade. Also, a gold pin connector was soldered to one end of the wire in order to plug into the headstage of the IVEC-10. Once the Teflon was removed, the silver/silver chloride wire needed to be plated to become a stable reference electrode. A plating bath containing 1M HCl solution saturated with NaCl (40g/100ml of distilled water) was used. The reference electrode was placed in the bath and connected to a positive pole (anode) of a regulated power supply. A second wire (silver or platinum) was immersed in the plating solution and connected to the (cathode) or negative pole. This completed the plating circuit. The silver wire was anodized by applying 2 volts for 15-20 minutes. Afterwards a thin layer of AgCl would have formed on the exposed wire making it a dark grey. The reference electrode is checked against a stable reference in a 3M NaCl solution. An Autorange Digital Multimeter (Tenma Corp.) was used to check the voltage difference between the two electrodes. The difference should be less than 15.0mV inorder to be stable. These two stable reference

electrodes are then placed in the hole of the parietal bone in the rat skull and secured.

FILLING MICROPIPETTES

Before actual data acquisiton, the micropipettes needed to be filled with either high potassium consisting of 70mM KCl, 79mM NaCl, 2.5mM CaCl or MPP^+(Research Biochemicals International, Natick, MA) - 2.97mg/10ml buffered saline for 10^{-3} M solution. Preliminary investigations were done in order to test MPP^+concentrations for verification that the electrode showed no interference. Three concentrations of MPP^+(10^{-3}, 10^{-4}, 10^{-5}) were tested. None of these concentrations interfered with the electrode and the 10^{-3} proved to be the best concentration for these experiments. Otherwise, when filling the micropipettes, the solutions were filtered to eliminate any particular contaminants. MPP^+ was handled with extreme caution because of its toxic nature. Proper attire was worn at all times, and gloves were used during the entire experiment. Test solutions were drawn up in a 1cc syringe, and a micropipette filling needle (30 gauge) was attached to the end of the syringe. The filling needle was inserted into the micropipette, and each micropipette was backfilled with the desired solution.

PROCEDURE / DATA ACQUISITION

PROCEDURE

After all prepatory work had been done, the working and reference electrodes were connected to the headstage of the IVEC-10 and the micropipette was connected to the Picospritzer II (General Valve Corp.) The

Picospritzer II was needed to regulate the duration (1-10 milliseconds) and pressure (15-30 psi) of nitrogen that was used to pressure eject pulses of the potassium or MPP^+ solutions. A test pulse was done to make sure solution came free from the micropipette. The probe assembly was connected to the vertical movement of the stereotaxic apparatus and a slip of white paper was placed behind the probe in order to see the meniscus of the micropipette solution. A dissecting scope with a micrometer was placed in front of the probe assembly and adjusted so the meniscus was visible. The dissecting scope was calibrated using a micrometer of 0.01mm per division. This allowed a magnitude of 1.8 (for the dissecting scope) x 10x ocular for proper determination of the volumes of solution ejected with each pulse. The volumes of solution ejected during the experiments averaged (301.94 +/- 32.0 nl).

DATA ACQUISITION

In order to acquire data, the computer was set to acquisiton mode. A check list of the parameters of the specific electrode used in calibration was verified, and the gain setting checked. The electrode verification screen was displayed, and showed the size and shape of the waveform which should have been equivalent to the waveform of calibration. If the waveform was equivalent, this test was halted and the data acquisition screen was prompted. The data acquisition screen showed several areas of analysis. The electrode waveform was seen in the lower left corner in a box. The oxidation current data was seen in the upper section of the screen. If noise was noted, additional time for stabilization of the electrode and the baseline was allowed before preceding with the experiment. The y-axis of the screen displayed the

micrometer concentrations detected by the electrode. The x-axis displayed time in seconds. Two time clocks were also present in the display. One clock showed the time remaining in the file while the other recorded the time since a previous event mark (TTL).

A typical pulse in the corpus striatum of the rat brain with this electrochemical technique would occur as follows: (1) baseline reading (yellow color visible on acquisition screen), (2) 2.0ms duration and 20psi pressure set on picospritzer II, (3) TTL marker pressed which ejected test solution (line magenta), (4) dopamine release (peak back to baseline, shown as cyan color), (5) amplitude, time course, signal/noise, RED/OX ratio, rise time, percent decay times from peak amplitude, clearance rate were recorded, (6) volume recorded, and (7) probe moved ventrally .5mm to record next release. Subsequent releases would be stimulated untill about 7.0mm down from the surface of the brain. Twelve stimulated releases would be analyzed from each pass. Furthermore, a right and left pass of the brain was accomplished from each experiment totalling twenty-four releases.

HISTOLOGY

PERFUSION

After the end of the experiment, some of the female rats were perfused with a 10% formaldehyde solution, 1:4 dilution - 250ml/1000ml distilled water (Fischer Scientific) plus a 0.9% NaCl solution (Sigma Corp.). A female rat was taken from the stereotaxic apparatus and placed securely to a tray. The thoracic and abdominal cavities were opened and flushed with water. A puncture with a blunt needle was inserted into the left ventricle of

the animals, and a 50cc syringe with a 0.9% NaCl solution was injected. At the same time, the right atrium was cut. Usually, three full 50cc injections of the 0.9%NaCl were necessary to flush all the remaining blood from the animals. Next, 1-2 50cc injections of 10% formaldehyde were injected into the left ventricles as well. These injections allowed the entire animal and its tissue to be fixed. After perfusion was completed, the rats were decapitated by a small animal decapitation device (Harvard), and their brains removed by Rongeurs. The brains were placed in small glass jars with formaldehyde fixative (10%) till cross-sectional slides could be made.

SLIDE PREPARATION

Slides were coated with 20% Bovine serum albumin (BSA), prior to tissue placement. BSA allows the brain tissue to adhere properly, so the tissue is not lost during the staining procedure. The slides were allowed to dry for 5-10 minutes on a slide warmer after BSA was applied. The brain tissue was placed on the stage of a microtome and frozen for tissue slicing. The brain tissue was cross-sectionally sliced in 30 micron increments by the microtome (Cryo-histomat mK 2 model, Hacker Instruments, INC., Fairfield, NJ.). Each slice was carefully placed into small trays with 20% ETOH. The tissue slices were removed from these trays, floated in 20% ETOH, and placed onto the prepared slides. Fuchsin staining was done on all the slides. Staining procedure was as follows: 1) 20% ETOH -3 min., 2) D-water-3min, 3)D-water-3min, 4)D-water-3min, 5) B-Fuchsin-1.5min, 6)D-water-3min, 7)D-water-3min, 8) Acid formalin-5sec, 9)D-water-rinse, 10)D-water-3min, 11)70%ETOH-3min, 12)95%ETOH-3min, 13)100%ETOH-3min, 14)100%ETOH-3min, 15)xylene-5min, 16)xylene-5min, and 17)cover with

permount and cover slip. Slides were then set aside for visualization by light microscopy.

STATISTICS

Potassium and MPP^+- stimulated dopamine releases were analyzed by Jandel Scientific Sigma Statistics program for differences in their amplitudes, rise times, secretion rates, times from peak value to baseline, total time courses, and clearance rates. Unpaired t-tests compared the average response values for each group, high potassium ($n = 43$); MPP^+ ($n = 39$). For each group data, the P value assigned for statistical significance was ($p < 0.05$).

CHAPTER III

RESULTS

Forty-three K^+- stimulated and thirty-nine MPP^+- stimulated dopamine releases were recorded in these experiments. The stereotaxic coordinates where most potassium and MPP^+- stimulated responses took place were A-P (2.0mm), M-L (2.0mm), and V (3.0-7.0mm). An example of a potassium - induced dopamine response is shown in figure 4, while an example of a MPP^+-stimulated dopamine response is shown in figure 5. The oxidation curve (solid line) and reduction curve (dotted line) were recorded for each response. The asterick in the bottom left corner was generated when the event marker was pressed. This intiated the time sequence and also triggered the pressure ejection of the test solution. Several parameters of each release event are shown to the right of the response. Amplitude is known as the maximum change from baseline of the oxidation current peak and was expressed in $_{\mu}M$ concentrations of dopamine. Signal-to-noise (S/N) was reported, and it was defined as the smallest change from baseline that can be considered a response. The IVEC-10 computer-based system analyzed the S/N value as three times the standard deviation of the baseline. Next, the RED/OX ratio was reported and defined as the ratio of the reduction current value to the oxidation current value when the oxidation current was at maximal amplitude. Generally, this parameter was between (0.20-0.80) for potassium and for MPP^+- stimulated responses. Hystersis was also calculated and reported as the time point at which the reduction current intersected the

oxidation current. Next, rise time was recorded as the time from the point of the event marker (TTL) to maximal amplitude of the oxidation current. Also, T1/2 was explained as 50% decay from peak amplitude of the oxidation curve response. Finally, the time course was given for the entire curve from the event marker untill response returned to baseline for each response. In figure 4, the time course was not noted by the computer as it was for figure 5. The time course was estimated for this response at 110 seconds. For ease of comparison, figure 6 depicts an MPP^+- stimulated response (top) verses a K^+-stimulated response (bottom). Visually, it appears that the dopamine signal stimulated by MPP^+ is markedly different from that stimulated by K^+.

Figures 7-13 compare differences between groups with respect to several parameters of the release events. For each group the following parameters were calculated: amplitude, rise time, secretion rate, T-50, T40-80, Tc (clearance), and total time course. These parameters were used to determine differences between the K^+ and MPP^+- stimulated dopamine releases. The amplitude of the dopamine response for K^+ and MPP^+-stimulated groups is shown in figure 7. Amplitude of the dopamine signal was substantially reduced following MPP^+ stimulation (0.968 +/- 0.628$_\mu$M DA) when compared to K^+- stimulated controls (4.709 +/- 0.132$_\mu$M) $p = 0.0001$. The rise time of the dopamine response for K^+ and MPP^+-stimulated groups is shown in figure 8. Secretion rate of the dopamine response for both stimulated groups is shown in figure 9. Secretion rate demonstrates a better overall representation of the amount of DA released per second from the corpus striatum in each group. Secretion rate of the dopamine signal was significantly reduced following MPP^+ stimulation (0.0746 +/- 0.0129$_\mu$M DA/sec) when compared to K^+- stimulated controls (0.2309 +/- 0.0321$_\mu$M DA/sec) $p = 0.0001$. Almost three times more DA

was released from the K^+ group (control) compared to the MPP^+ group. T-50 of the dopamine response for both stimulated groups is shown in figure 10. The T-50 of the dopamine signal was substantially lengthened following MPP^+ stimulation (75.6 +/- 14.03s) verses the K^+- stimulated controls (40.6 +/- 2.52s) $p = 0.0113$. T40-80 of the dopamine response for both groups is shown in figure 11. The T40-80 of the dopamine signal was also lengthened following MPP^+ stimulation (64.2 +/- 9.14s) verses the K^+- stimulated control (18.7 +/- 1.47s) $p = 0.0001$. MPP^+ took nearly three times as long to decay in this 40-80% range. Total time course for both groups when stimulating dopamine responses is shown in figure 12. The total time course overall was lengthened by the MPP^+ stimulation (3.65 +/- 0.389 min) when compared to K^+- stimulated controls (2.37 +/- 0.251 min) $p = 0.0061$. The total time course for MPP^+ took a little more than one and a half times longer than K^+-stimulated dopamine responses. The clearance rate for both groups when stimulating dopamine responses is shown in figure 13. Clearance was markedly reduced following MPP^+ stimulation (0.0138 +/- 0.00195$_\mu$M) verses the K^+ -stimulated controls (0.1665 +/- 0.02369$_\mu$M) $p = 0.0001$. Clearance of dopamine occurred eight times faster with K^+ stimulation than by MPP^+ stimulation.

Figure 1. Illustration of the rat skull displaying bregma (midline suture point) , plus the nasal, frontal, and parietal bones. Bregma was used to determine where the "windows" should be placed in stereotaxic surgery for the specific location of the CPU. (Redrawn from Laboratory Anatomy of the White Rat, 5th ed. by Robert B. Chiasson and William C. Brown)

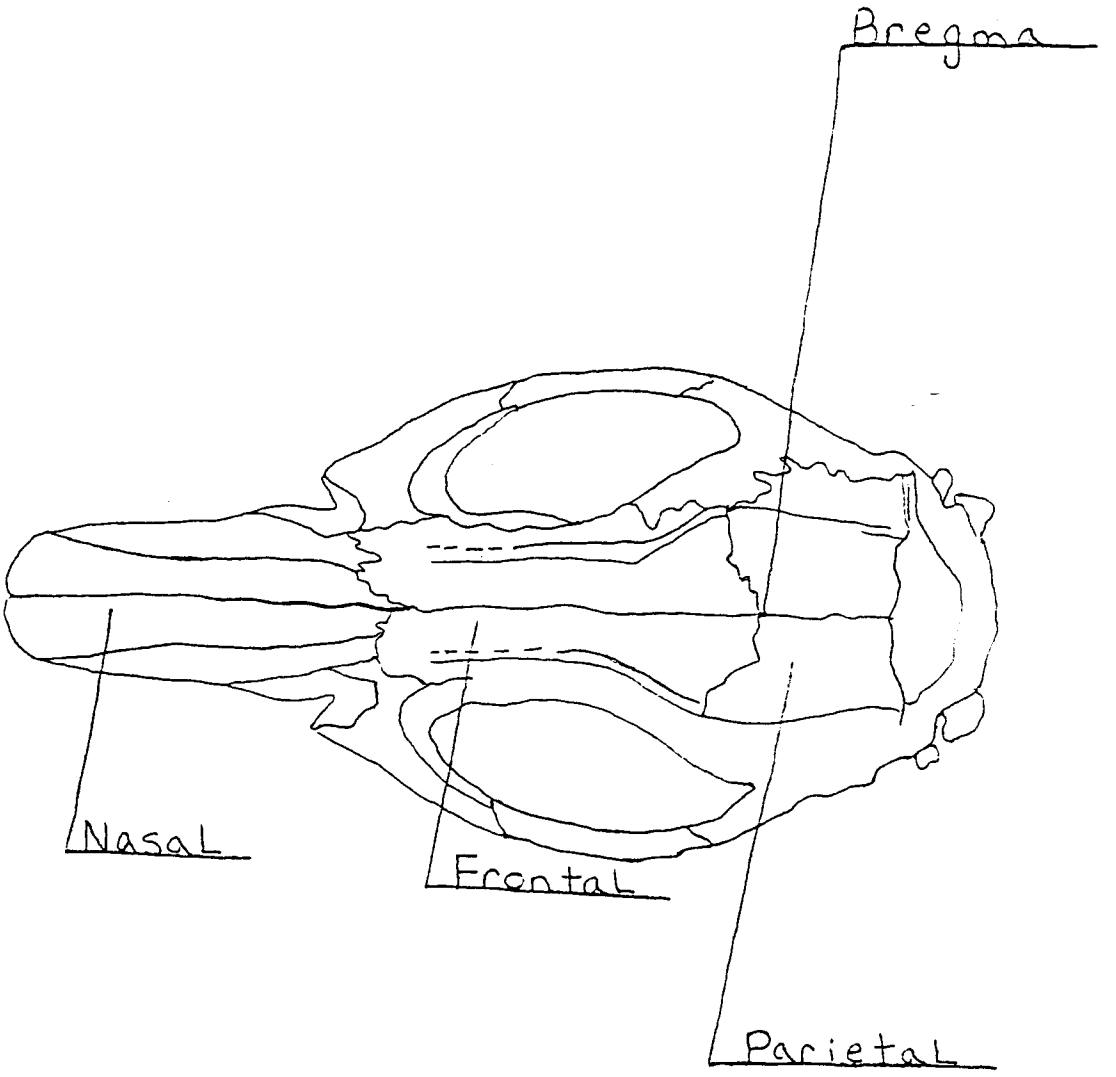

Bregma
Nasal
Frontal
Parietal

Figure 2. Illustration of the cross-section of the rat brain used in determining coordinates for finding the corpus striatum (CPU) and globus pallidus (GP). Coordinates were as follows: A-P(1.5-2.5mm), M-L(2.0mm), and V(1.5mm starting down to 7.0mm) (Redrawn from A Stereotaxic Atlas of the Rat Brain, by Pellegrino et al., 1979).

1.8

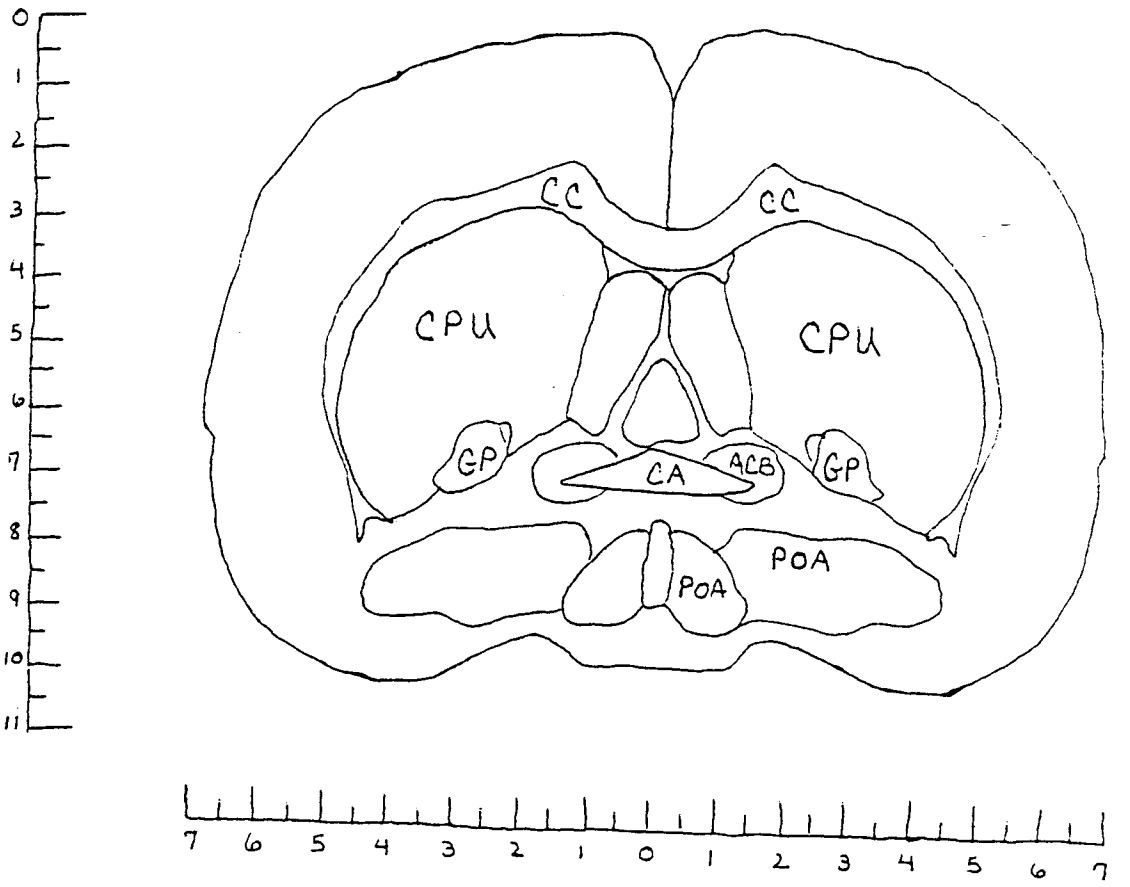
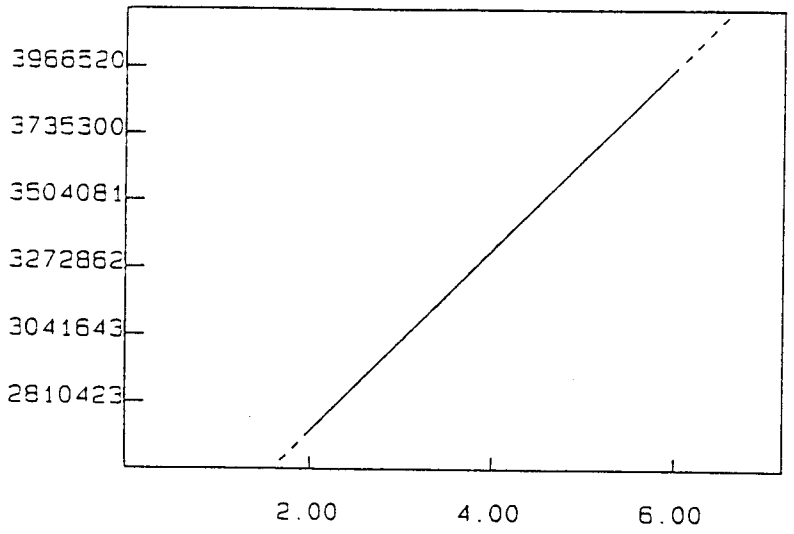
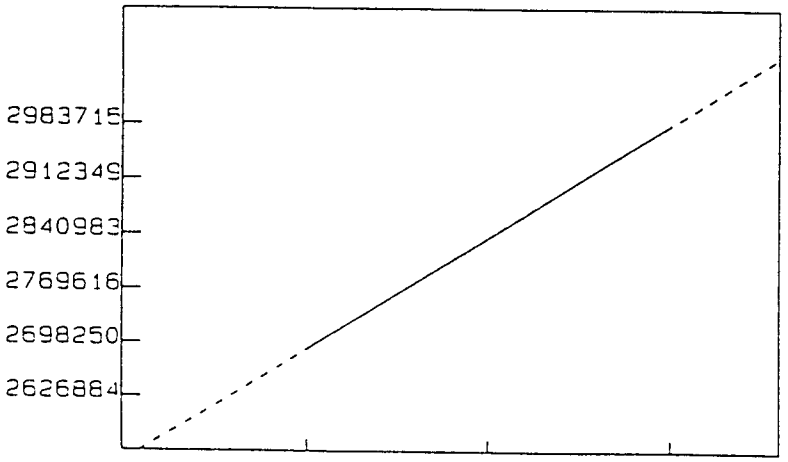

0
1
2
3
4
5
6
7
8
9
10
11
CC
CC
CPU
CPU
GP
CA
ACB
GP
POA
POA
7 6 5 4 3 2 1 0 1 2 3 4 5 6 7

Figure 3. An example of a printout of an *in vitro* calibration curve showing the oxidation currents (y-axis) and dopamine concentrations (x-axis). Specific parameters such as RED/OX ratio, selectivity, signal-to-noise, and oxidation/reduction slopes were analyzed for proper calibration of the working electrode.





09: 39: 39 1997

OXIDATION

SLOPE	312892.00
INTERCEPT	2087473.00
R^2	1.0000

ELECTRODE # 3

TRANSMITTER	Dopamine
RED/OX RATIO	0.23
SELECTIVITY	1079: 1
SIGNAL/NOISE	0.02765
FREQUENCY	5 Hz

REDUCTION

SLOPE	73424.00
INTERCEPT	2542736.75
R^2	1.0000

GAIN SETTING _____

FILTER SETTINGS _____

NAME _____

Figure 4. An example of K^+- induced (control) dopamine release. The oxidation curve is shown as a solid line while the reduction curve is displayed in a dotted fashion. The micromolar concentration of dopamine is given on the y-axis verses time on the x-axis.

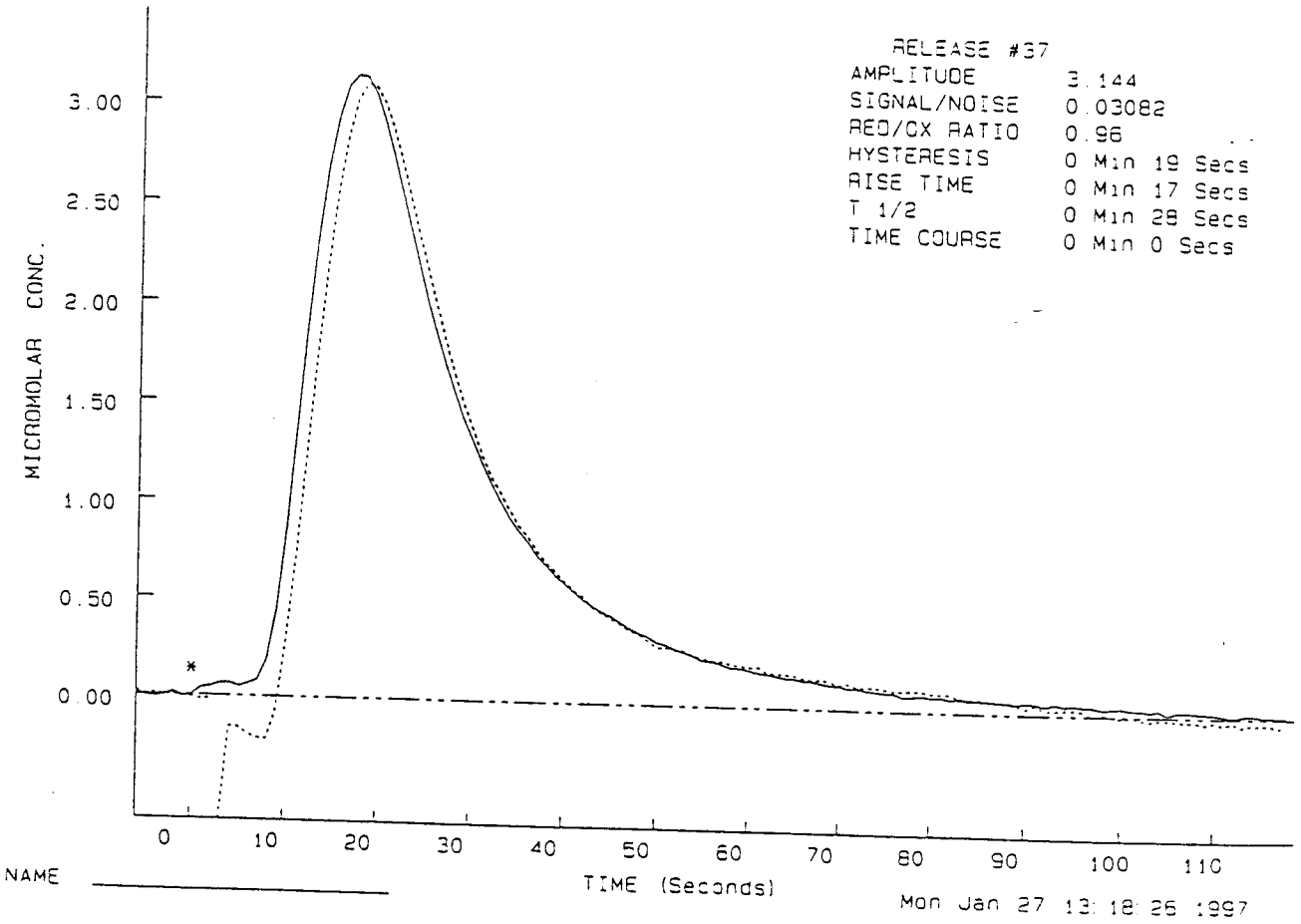

RELEASE #37
AMPLITUDE 3.144
SIGNAL/NOISE 0.03082
RED/OX RATIO 0.96
HYSTERESIS 0 Min 19 Secs
RISE TIME 0 Min 17 Secs
T 1/2 0 Min 28 Secs
TIME COURSE 0 Min 0 Secs
MICROMOLAR CONC.
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0
10
20
30
40
50
60
70
80
90
100
110
TIME (Seconds)
NAME
Mon Jan 27 13:18:26 1997

Figure 5. An example of a MPP^+-induced dopamine release. The oxidation curve is shown as a solid line while the reduction curve is displayed in a dotted fashion. The micromolar concentrations of dopamine is given on the y-axis verses time on the x-axis.

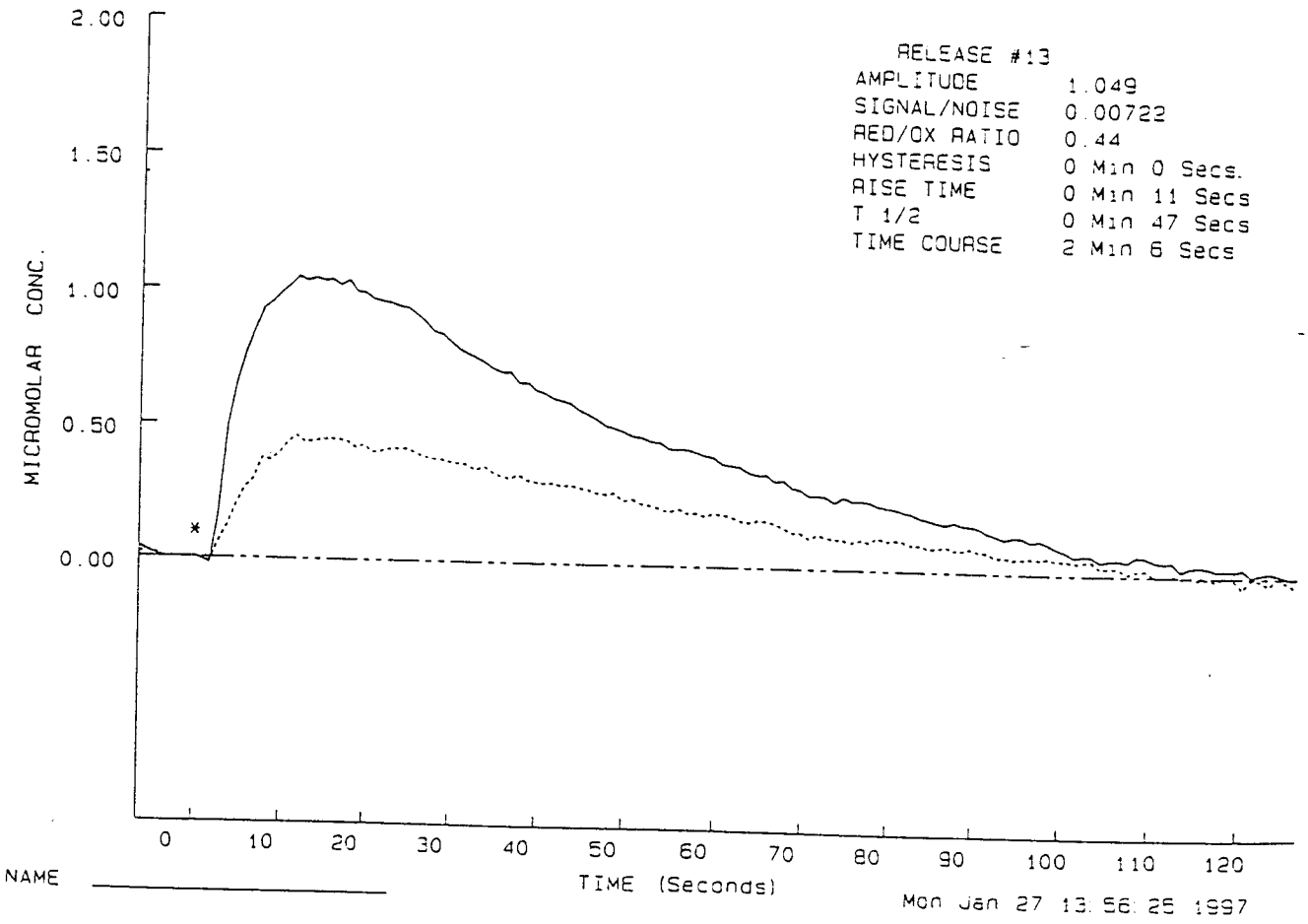
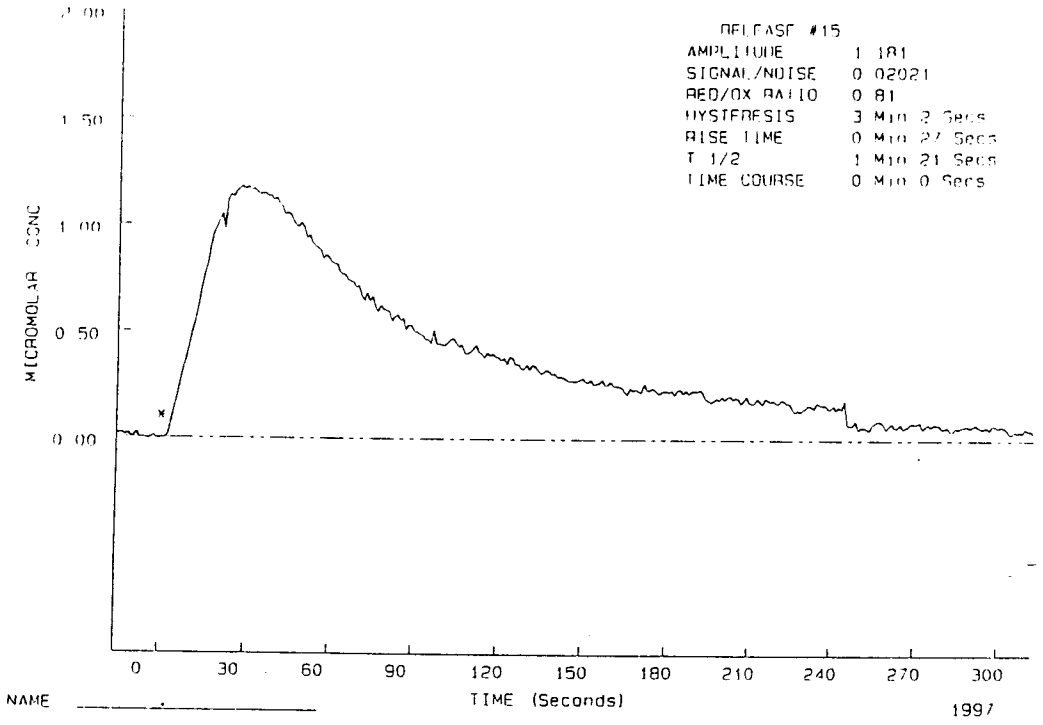

RELEASE #13
AMPLITUDE 1.049
SIGNAL/NOISE 0.00722
RED/OX RATIO 0.44
HYSTERESIS 0 Min 0 Secs.
RISE TIME 0 Min 11 Secs
T 1/2 0 Min 47 Secs
TIME COURSE 2 Min 6 Secs
MICROMOLAR CONC.
2.00
1.50
1.00
0.50
0.00
0
10
20
30
40
50
60
70
80
90
100
110
120
TIME (Seconds)
NAME
Mon Jan 27 13:56:25 1997

Figure 6. An example comparing the oxidation curve of the MPP^+-induced dopamine response (top) verses the K^+-induced (control) dopamine response (bottom). Notice the amplitudes of the responses, micromolar concentrations of dopamine released, and time courses.


RELEASE #15
AMPLITUDE 1 181
SIGNAL/NOISE 0 02021
RED/OX RATIO 0 81
HYSTERESIS 3 Min 2 Secs
RISE TIME 0 Min 27 Secs
T 1/2 1 Min 21 Secs
TIME COURSE 0 Min 0 Secs
MICROMOLAR CONC
1 50
1 00
0 50
0 00
0 30 60 90 120 150 180 210 240 270 300
TIME (Seconds)
NAME
1997

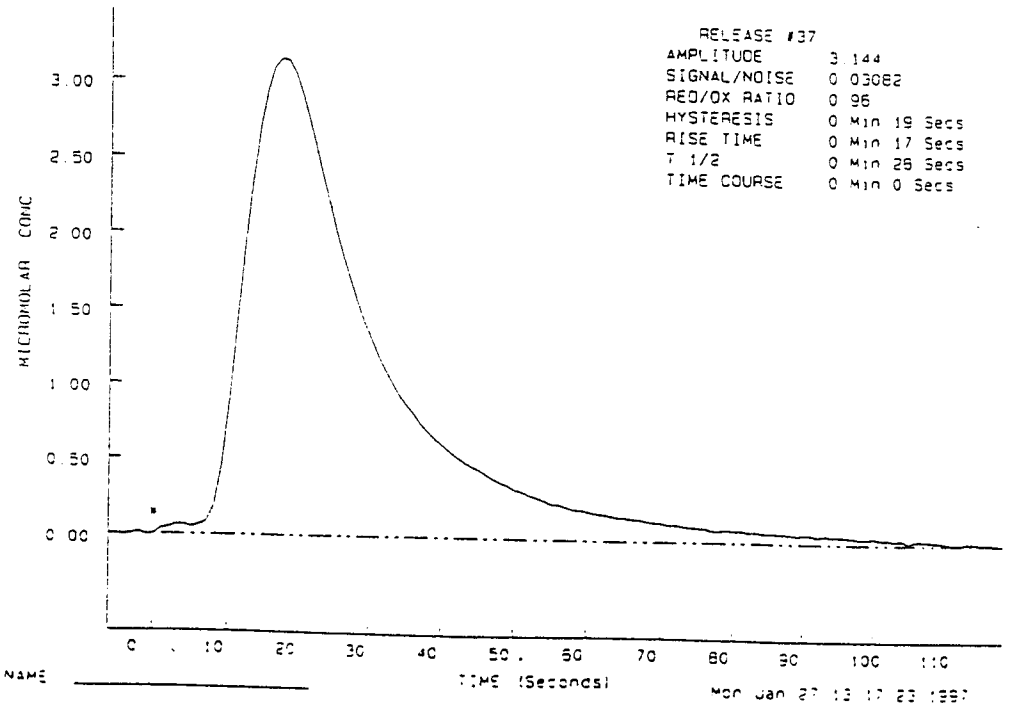

RELEASE #37
AMPLITUDE 3 144
SIGNAL/NOISE 0 03082
RED/OX RATIO 0 96
HYSTERESIS 0 Min 19 Secs
RISE TIME 0 Min 17 Secs
T 1/2 0 Min 28 Secs
TIME COURSE 0 Min 0 Secs
MICROMOLAR CONC
3 00
2 50
2 00
1 50
1 00
0 50
0 00
0 10 20 30 40 50 60 70 80 90 100 110
TIME (Seconds)
NAME

Figure 7. Amplitudes of K^+(control) and MPP^+- induced dopamine responses. For Figures 7-13, the total number of responses (n) for K^+ is 43 while the total number of responses (n) for MPP^+ is 39. The values are given as the mean +/- S.E.M., and the p value is displayed to the right of the graphs for statistical significance.

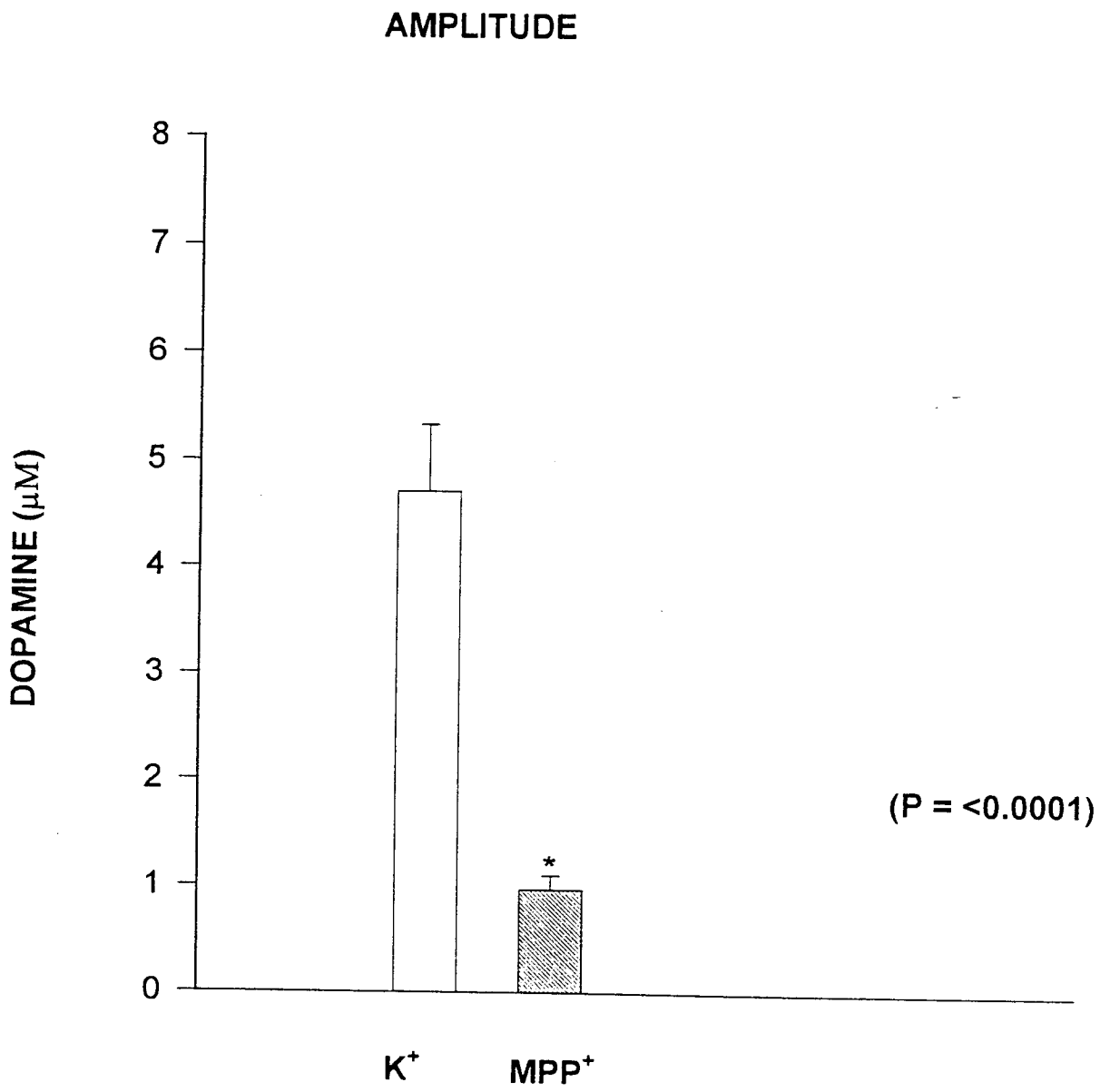

AMPLITUDE
DOPAMINE (μM)
8
7
6
5
4
3
2
1
0
*
(P = <0.0001)
K+
MPP+

Figure 8. Rise times (seconds) shown for K^+ and MPP^+- induced responses.

RISE TIME

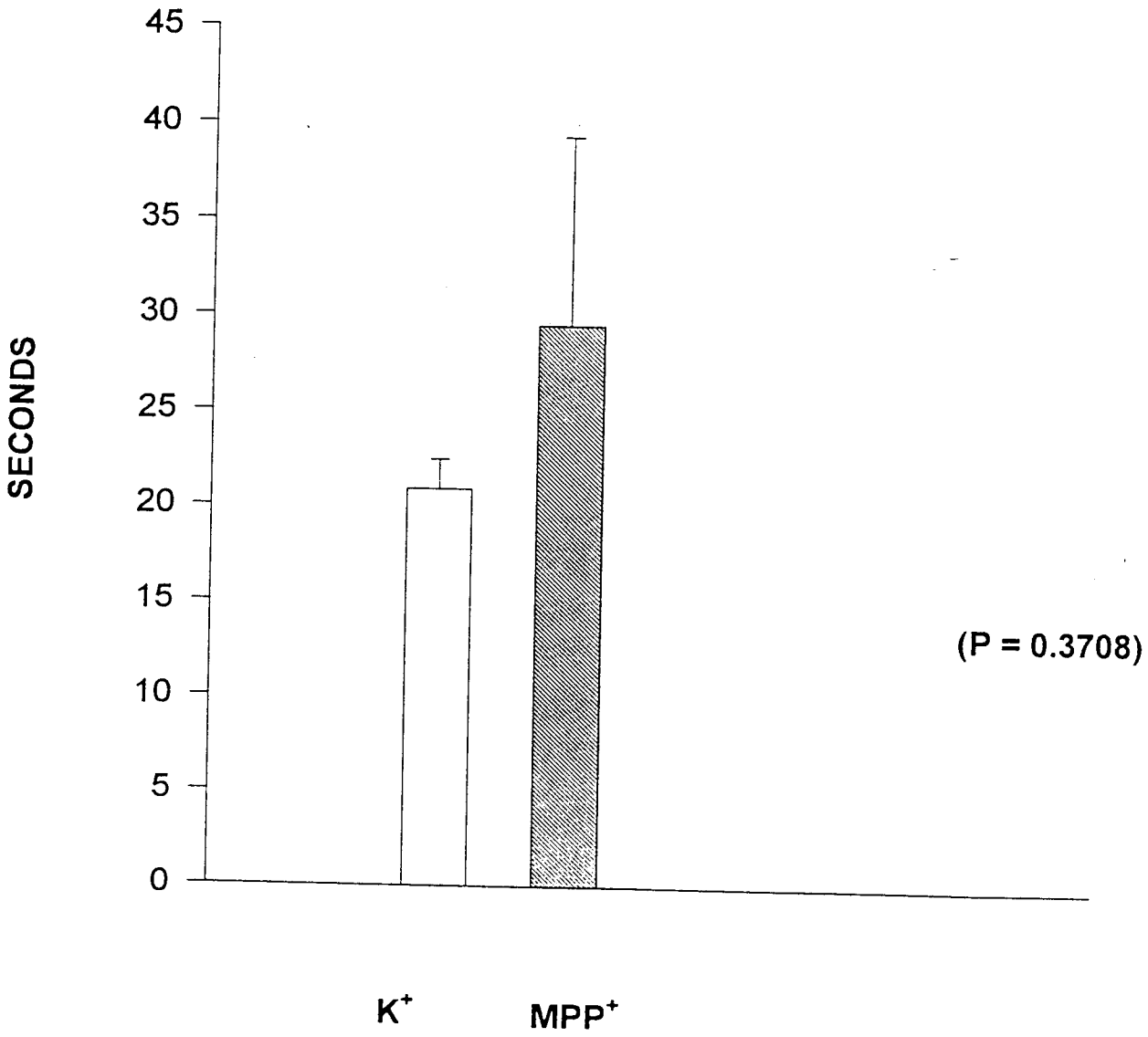


Figure 9. Secretion rates measured in micromolar concentrations of dopamine per second for the two groups - K^+ and MPP^+.

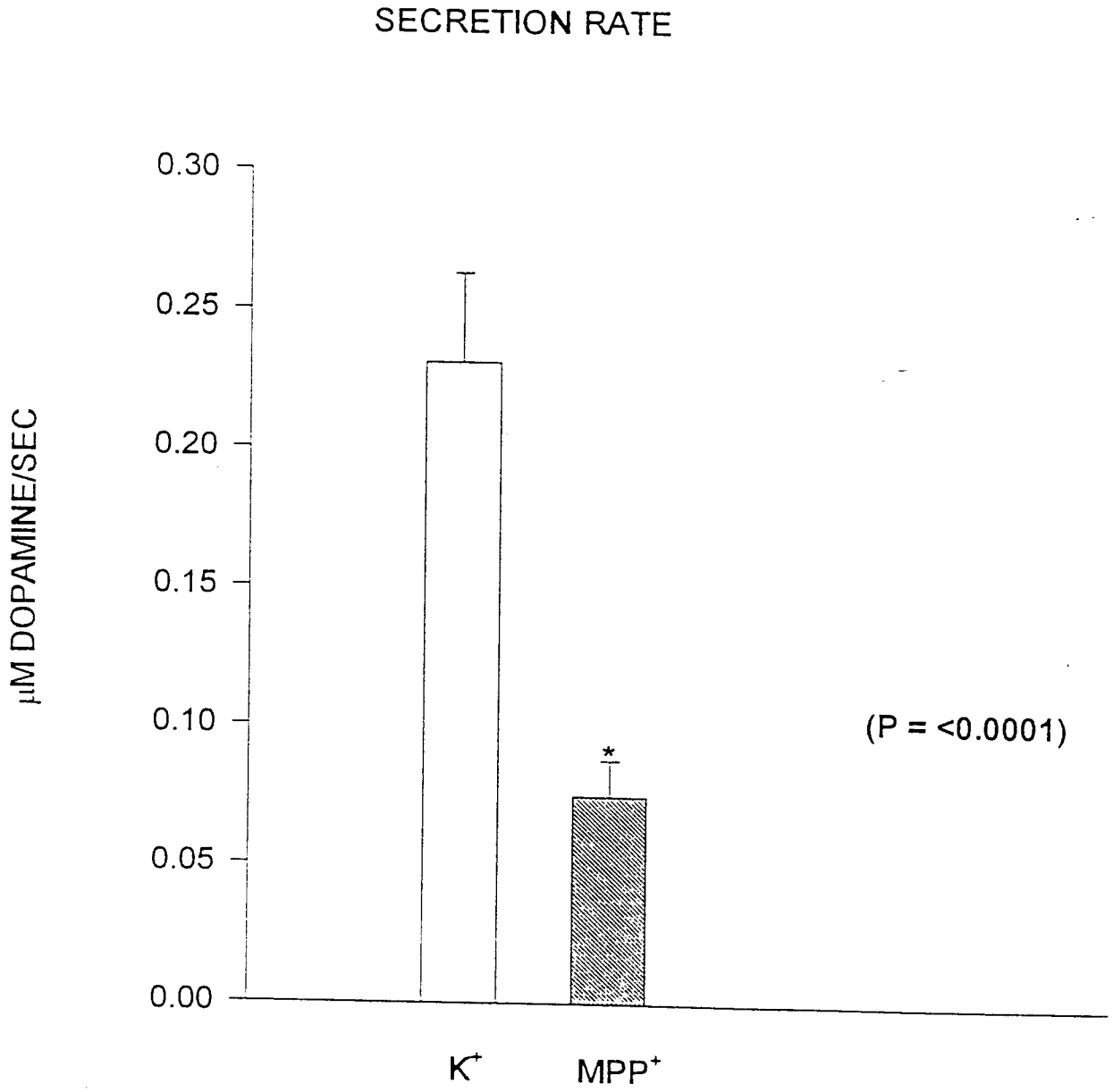

SECRETION RATE
μM DOPAMINE/SEC
0.30
0.25
0.20
0.15
0.10
0.05
0.00
*
(P = <0.0001)
K+
MPP+

Figure 10. T-50, time for the dopamine signal to decay to 50% of peak value is shown for K^+ and MPP^+groups measured in seconds.

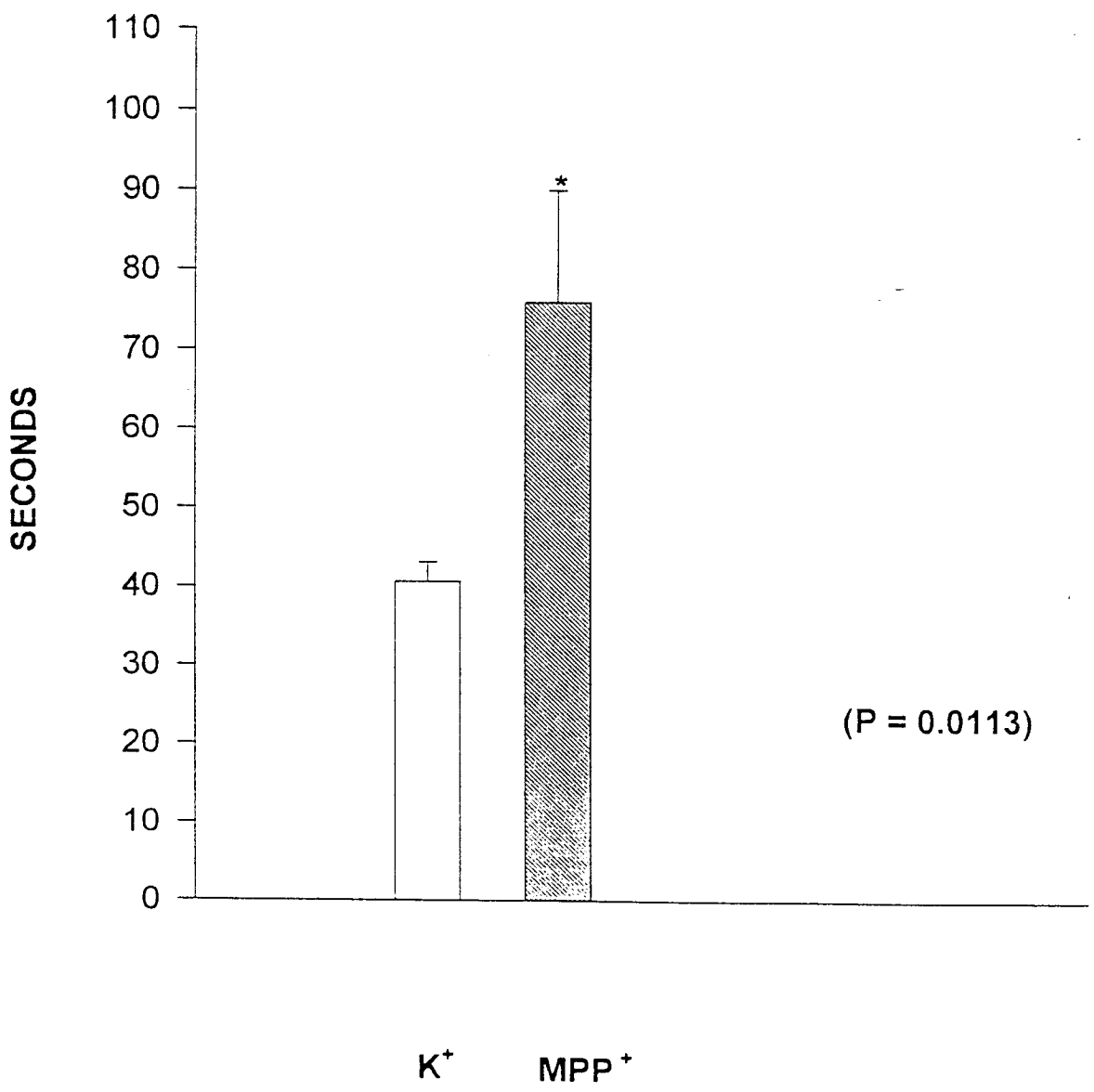

T-50
110
100
90
80
70
60
50
40
30
20
10
0
SECONDS
*
(P = 0.0113)
K+
MPP+

Figure 11. T 40-80, time for the dopamine signal to decay to 40-80% of peak value is shown for K^+ and MPP^+ groups measured in seconds.

T 40-80

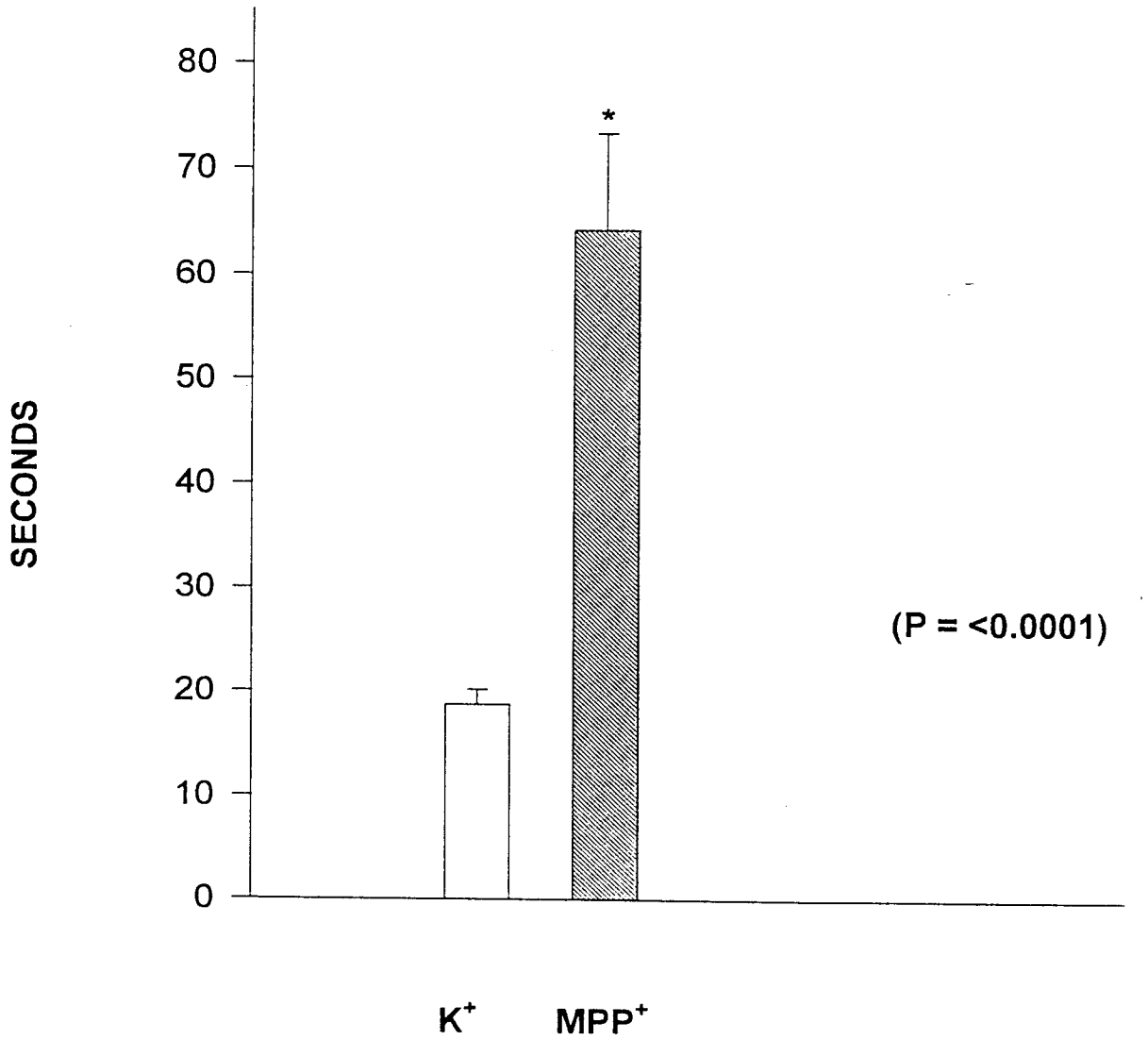


Figure 12. Overall time course for entire dopamine responses for K^+ and MPP^+ groups measured in minutes.

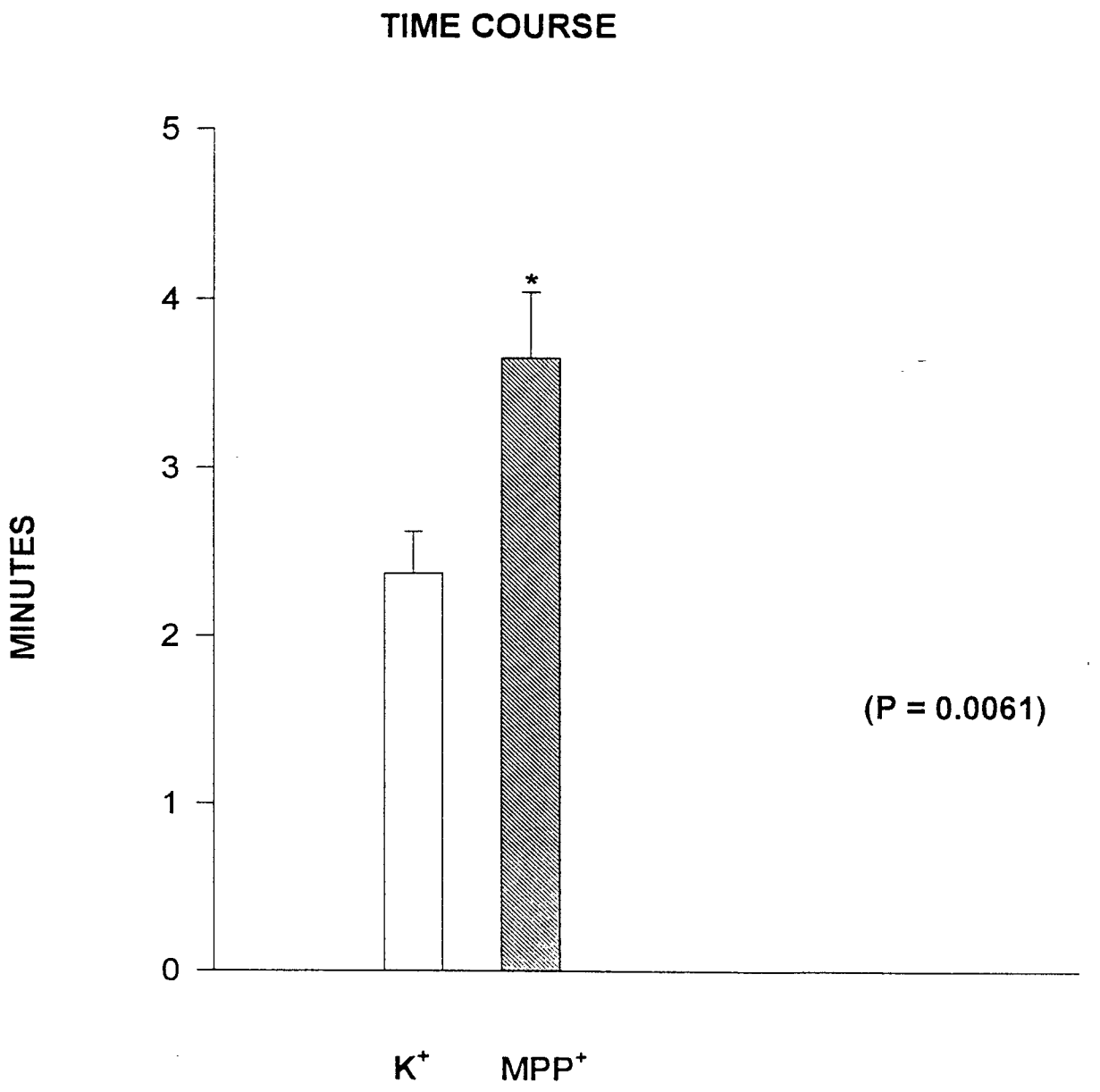
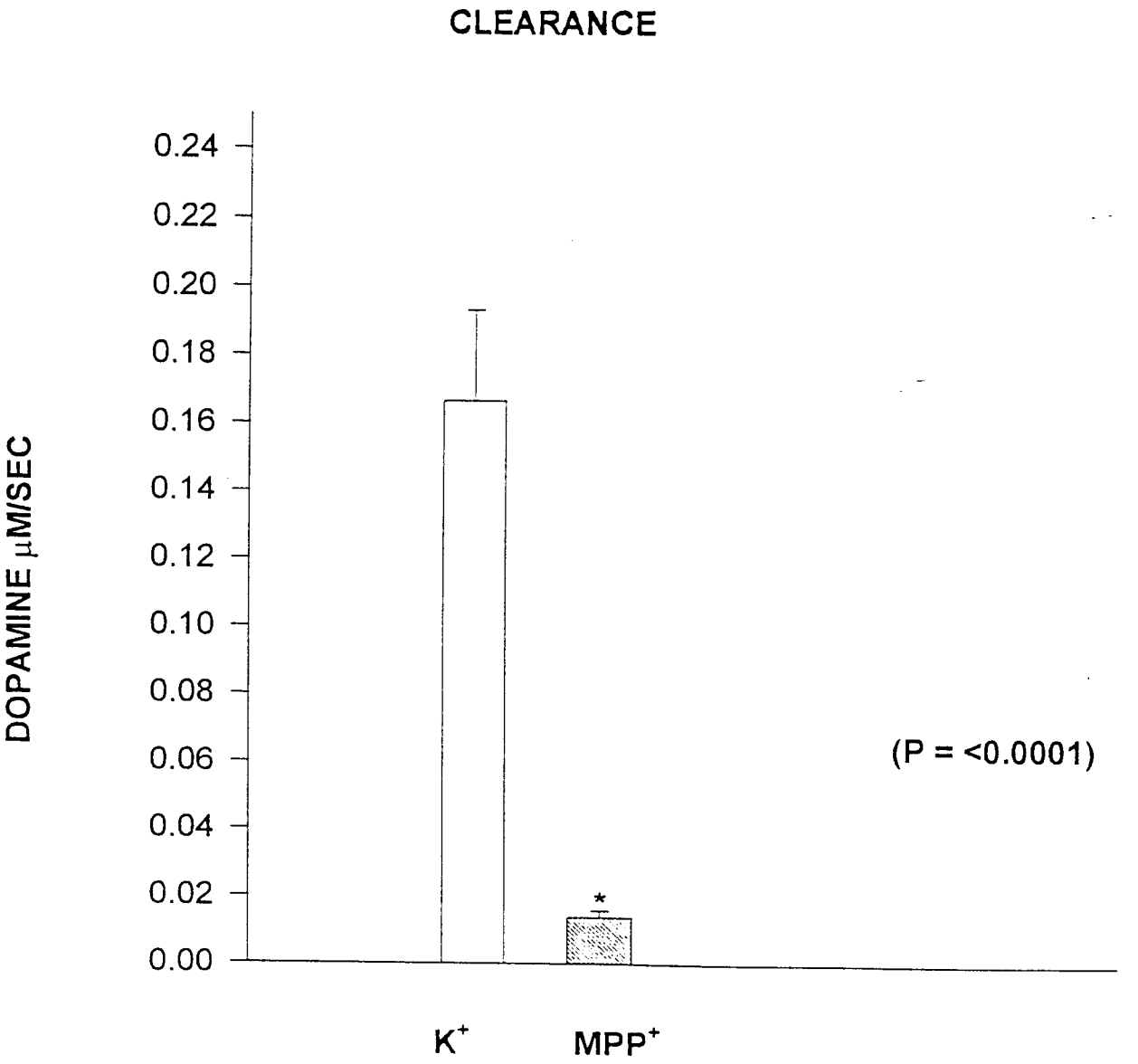

TIME COURSE
MINUTES
5
4
3
2
1
0
*
(P = 0.0061)
K+
MPP+

Figure 13. Clearance rates measured in micromolar concentrations of dopamine per second for K^+ and MPP^+ treated groups.


CLEARANCE
DOPAMINE μM/SEC
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
*
(P = <0.0001)
K+
MPP+

CHAPTER IV

DISCUSSION

This study was conducted to examine the acute effects of MPP^+, a potent neurotoxin, on the dynamics of dopamine release in the corpus striatum of the rat brain. Results demonstrated that MPP^+ - stimulated dopamine responses were markedly different from K^+- induced (control) dopamine releases. Detailed anaylsis of specific parameters of the release and clearance mechanisms provided clear evidence of differences between groups in amplitude, secretion rate, decay times, clearance rate, and overall time course of response.

DOPAMINE RELEASE/CLEARANCE

The amplitude and the secretion rate of the dopamine signal was surpressed by MPP^+ treatment when compared to K^+ stimulation. MPP^+, though, was still inducing dopamine release which is characteristic of this neurotoxin. Disshon and Dluzen (1997) have also reported an immediate release of dopamine by MPP^+ alone using an *in vitro* superfusion technique of rat striatal tissue. Accordingly, Obata et al. (1992) reported MPP^+-stimulated DA release *in vivo* using a microdialysis technique. MPP^+ selects dopaminergic neurons by being actively taken up into the dopaminergic terminal through the dopamine transporter (Chiba et al., 1985). Other interfering actions of MPP^+ might involve mitochondrial respiration. MPP^+

may enter and accumulate in the mitochondria causing a rapid depletion of ATP. This depletion would cause an increase of Ca^{++} in the cytosol which could lead to a substantial increase in dopamine release (Langston, 1995).

The reduction in dopamine release seen by this study could be due to the basic differences between MPP^+ and K^+ in terms of their mechanisms of action in stimulating dopamine release. MPP^+ causes a displacement of dopamine from the storage vesicles by accessing the transporter mechanism, thus, allowing dopamine to be released (Cooper et al. 1986). The acute effects of MPP^+ stimulation, as reported by *in vitro* and *in vivo* experiments, have caused dopamine to be released but in decreased amounts. The mechanisms by which MPP^+ stimulates DA release have not been substantiated but various hypotheses have emerged. MPP^+, through this study, has been shown to potentially inhibit DA reuptake. This inhibitory mechanism could lead to greater enzymatic action by MAO and COMT, and less DA being reused economically by the terminal. Then, DA would be re-synthesized and possibly greater amounts of dopamine could be released from the storage vesicles. Another possible MPP^+ mechanism for DA release is to directly stimulate the DA secretory process. By this mechanism, the storage vesicles containing DA would be disrupted causing further release and plausible dopamine degradation. Furthermore, by MPP^+ stimulation of dopamine release through any one of these mechanisms, the dopamine hypothesis may be relevant. Dopamine produces hydrogen peroxide during its breakdown process which can accumulate and cause damage to dopaminergic neurons. A vicious cycle ensues by the production of hydrogen peroxide and increased release of compensating dopamine neurons (Irwin and Langston, 1995). By MPP^+ stimulating dopamine release, dopamine metabolism could occur at an increased rate which might lead to a higher

amount of hydrogen peroxide in the brain. MPP^+ has also been reported to increase DA auto-oxidation (Forno, 1995). This increase in DA breakdown by MPP^+ could allow even greater amounts of hydrogen peroxide produced. This DA auto-oxidation would compound the vicious cycle already initiated. So, the acute effects of MPP^+ stimulation could potentially cause cell death by the ongoing displacement of the dopamine storage vesicles and the subsequent harmful increased dopamine metabolism.

On the other hand, K^+- stimulated release of dopamine is known to occur by a classical-type mechanism which causes depolarization of the neuron and triggers calcium-induced exocytosis. This is a second-messenger system (Thompson, 1985). As seen by this experiment, potassium-stimulation demonstrated a higher increase of dopamine released plus with an increased secretion rate. K^+ is not known to compete with the dopamine transporter mechanism. Thus, the dopamine responses seen with potassium stimulation are not inhibited and allow a greater amount of dopamine to be released. Therefore, this allowed for a good comparative control for these experiments.

By analyzing decay times, total time courses, and clearance rates, a method of evaluating the effects of MPP^+ on dopamine clearance was also ascertained. The decay times and total time courses for dopamine secretion were lengthened by the stimulation of MPP^+ (figures 10-12). Additionally, MPP^+- treated animals had a much slower clearance rate of dopamine when compared to K^+- stimulated controls (figure 13). There have been suggestions made correlating MPP^+ with the dopamine reuptake mechanism or dopamine clearance . Langston (1995) hypothesized that once MPP^+ gains entrance into the extracellular space of the synapse, it was taken up into the dopaminergic neurons. This reuptake by MPP^+ was referred to as the "trojan

horse" phenomenon. The cells were recognizing MPP^+ as a friendly substrate but were accumulating a substance that would kill them. It was also believed that all catecholamine uptake sites would be vulnerable to MPP^+. This was found not to be the case. MPP^+ was considered to be selectively binding to dopamine, but the reasons for this have not been elucidated (Langston, 1995). Therefore, this particular MPP^+ data might further substantiate Langston's hypothesis.

Again, the clearance rate of the dopamine signals by MPP^+ stimulation was one-eight of the K^+- treated animals (figure 13). This data suggests that this neurotoxin was competing with the dopamine for reuptake into the nerve terminals. The clearance rate data could substantiate earlier reports by Cooper et al.(1985), which claims that MPP^+ disrupts or competes for the dopamine transporter leading to a displacement of dopamine and subsequent release. This competing action of MPP^+ for the transporter is similar to other neurotoxins, such as cocaine or amphetamines. Cocaine is known to block the normal enzymatic actions of monoamine oxidase (MAO) and is known to also inhibit the DA reuptake process. Therefore, dopamine accumulates in the synapse and will cause further stimulation of the postsynaptic cell. Amphetamines stimulate an excess release of dopamine which will overwhelm the processes of reuptake and enzymatic breakdown (Siegal et al., 1989).

SUMMARY

In summary, results of this *in vivo* study suggest that MPP^+ effects dopamine release, as well as, dopamine clearance, presumably by competition for receptor sites on the dopamine transporter. This neurotoxin demonstrated

considerable differences by comparision to the K^+- stimulated dopamine responses. MPP^+- stimulated dopamine releases displayed a decreased amplitude, decreased secretion rate, longer decay times, longer total time course, and a significantly decreased clearance rate. Whether the release of dopamine and slow clearance of this transmitter from the ECF contributes to the subsequent neuronal cell death is still unclear. However, MPP^+ competing with the dopamine transporter could displace dopamine from the storage vesicles, release increased dopamine, metabolize dopamine excessively, and eventually lead to cell death by this damaging dopamine cycle. This exogenous agent (MPP^+) with endogenous consequences (dopamine cycles) could provide a useful model for further investigation concerning dopamine's role in Parkinson's disease.

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