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The Ohio State University Ph.D. 1983

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BEHAVIORAL AND BIOCHEMICAL STUDIES ON THE ROLE OF EXCITATORY AMINO ACIDS IN THE NUCLEUS ACCUMBENS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Bruce A. W. Donzanti, B.S., M.S.

* * * * *

The Ohio State University

1983

Reading Committee: Approved By:
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Dr. Allan M. Burkman                      Advisor
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Dr. Lane Wallace
DEDICATION

Dear Mom and Dad,

There is so much to thank you for, and yet, I really don't know where to begin. Both of you have given me the love, support and encouragement to accomplish one of my major goals in life. For that, I am eternally grateful and indebted. Therefore, as an expression of my immeasurable love for both of you, I dedicate this dissertation to you.
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I wish to express my sincere appreciation to:

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Mr. John M. Hanson

for taking and developing the photomicrographs used in this dissertation.
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Abstracts:


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CHAPTER I

INTRODUCTION

I.1 HISTORICAL PERSPECTIVE

Until the early 1950's it was generally agreed that L-glutamic acid (L-GLU) had no function other than a role in metabolism in the central nervous system (CNS). However, based on a series of experiments by Hayashi (1952, 1954) in the mammalian brain, evidence began to accumulate suggesting that dicarboxylic amino acids influence central neuronal activity by other mechanisms. In these studies, it was shown that the injection of small volumes of L-GLU and L-aspartic acid (L-ASP) directly into the gray matter of dog, ape and human motor cortex produced convulsions which appeared to be caused by a direct chemical stimulation of the neurons. Several years later, it was further demonstrated that the direct application of L-GLU and L-ASP to the brain surface of the cat produced what appeared to be a slowly moving wave of intense neuronal depolarization and inactivation resulting in an abolishment of all spontaneous and evoked electrocortical activity (Purpura et al., 1959). However, at this time the mechanism(s) by which these amino acids stimulated neuronal activity was unclear.
Progress in the study of the central actions of L-GLU and other amino acids was greatly enhanced by the development of the microiontophoretic technique. By applying a variety of amino acids to the extracellular surface membrane of single neurons of the amphibian and mammalian spinal cord, it soon became clear that L-GLU and L-ASP were representative of a new class of neuronal excitants, clearly distinguishable from other neuroexcitatory agents such as cholinomimetics, calcium chelating agents and exogenous convulsants (Curtis et al., 1959, 1960, 1961; Krnjevic and Phillis, 1963; Curtis and Watkins, 1960, 1963). Thus, the dicarboxylic amino acids are commonly referred to as excitatory amino acids.

Intracellular recording studies have demonstrated that L-GLU-induced excitation was the result of a depolarizing action on the neuronal cell membrane similar to that caused by the action of an endogenous transmitter in generating excitatory postsynaptic potentials (Curtis et al., 1960). Since intracellularly injected L-GLU did not produce neuronal excitation (Coombs et al., 1955), the action of this compound, and possibly other excitatory amino acids, appeared to be restricted to external surface membrane sites similar to the sites of action of known neurotransmitters (Krnjevic, 1974).

Based on these initial studies, pharmacological, neurochemical and electrophysiological data continue to accumulate supporting the hypothesis that excitatory amino acids function as neurotransmitters
in the vertebrate CNS (see reviews by Usherwood, 1978; Johnson, 1978; Cotman et al., 1981; Mistri and Costanti, 1979; DeFeudis, 1979; Puil, 1981; Watkins and Evans, 1981). The evidence that these compounds fulfill, at least partially, the criteria for a neurotransmitter will now be discussed below.

I.2 AN OVERVIEW: EVIDENCE FOR EXCITATORY AMINO ACIDS AS NEUROTTRANSMITTERS IN THE VERTEBRATE CNS

In order for a compound to be accepted as a neurotransmitter several criteria must be met (Krnjevic, 1974). The transmitter candidate should: 1) be released upon stimulation in a manner which is dependent on calcium and antagonized by magnesium, and the quantities released must be sufficient to allow its action as a transmitter; 2) act on the postsynaptic cell in a manner that is identical to the action of the natural transmitter. Thus, the time course of its biological action should be the same and the candidate should cause the same permeability changes at the postsynaptic membrane and the same metabolic responses in the postsynaptic neuron. The action of the natural transmitter as well as the candidate should be inhibited by the same antagonists; 3) be inactivated by a mechanism located at the synapse where the compound exerts its pharmacological effects; 4) be synthesized and stored at presynaptic terminals. The machinery to manufacture, store and control the synthesis of the putative transmitter should be located at the nerve terminals. The rate of transmitter synthesis should increase in response to neuronal stimulation so that the tissue can maintain appreciable pools for release under a wide variety of stimulus conditions.
A variety of endogenous amino acids, which possess neuro-
excitatory properties, have been shown to fulfill some of the criteria
listed above. These include: L-Cysteic acid, L-Cysteine sulfinic acid,
L-homocysteic acid, L-homocysteine sulfinic acid, S-sulfocysteine,
and particularly, L-GLU and L-ASP.

A. Properties of Excitatory Amino Acid Release

L-GLU and L-ASP have been shown to be released from
rat brain slices and synaptosomes obtained from a variety of brain
regions (Takagaki, 1976; Cox and Bradford, 1978; Goduklin et al., 1980;
Moroni et al., 1981 a,b; Levi et al., 1981; Dolphim et al., 1982; Levi
and Gallo, 1981; Druce et al., 1981). Depolarization-induced release
of both excitatory amino acids was found to be calcium-dependent and
tetrodotoxin-sensitive. The only other known endogenous excitatory
amino acid which has been shown to be released from brain tissue is
L-cysteine sulfinic acid (Iwata et al., 1982b). This amino acid, similar
to L-GLU and L-ASP, was released from rat cerebral cortex slices in a
calcium-dependent manner by high potassium-induced depolarization.

The most thorough studies on excitatory amino acid
release have come from experiments on rat hippocampal tissue (Cotman
and Hamberger, 1978; Cotman et al., 1981; Cotman and Nadler, 1981). In
these studies, it was demonstrated that endogenous GLU and ASP can be
released from slices of the dentate gyrus (a region of the hippocampus)
and synaptosomes prepared from the whole hippocampal formation. This
release also occurred in a calcium-dependent manner and was antagonized by magnesium. In the presence of calcium, the calcium ionophore, A23187, released endogenous GLU from purified synaptosomes, but not from other subcellular fractions (e.g., mitochondria). A depolarizing agent was not essential for GLU release in the presence of the calcium ionophore suggesting that calcium may be both necessary and sufficient to evoke release. The calcium-dependent release of both GLU and ASP was generally similar with respect to alterations in the ionic composition of the medium. However, their release did differ in two ways: 1) the release of GLU occurred more rapidly and the duration of release was shorter and 2) the release of ASP was more sensitive to variations in the magnesium concentration of the medium. These differences suggest that ASP and GLU may be released by different mechanisms or from different sites.

It has been observed that a small portion of the excitatory amino acid released is not dependent on extracellular calcium (Cotman and Nadler, 1981; Iwata et al., 1982b). The reason for the lack of dependence on external calcium is not clear but may indicate that either calcium is released from intracellular storage sites to mediate exocytosis or that excitatory amino acids can be released by a carrier mechanism which is normally involved in metabolism (Shank and Aprison, 1979; Cotman and Nadler, 1981).

A direct demonstration that release originates from synaptic vesicles would further strengthen the belief that GLU and other
excitatory amino acids are neurotransmitters in the CNS; however, based on studies employing purified synaptosomes obtained from rat cerebral cortex, the release of amino acids, unlike that of acetylcholine and the catecholamines, appears to occur from a cytoplasmic pool rather than a vesicular pool (Cox and Bradford, 1978).

A recent study utilizing cultured neuronal and glial cells has shown that high potassium-induced release of endogenous amino acids is a property unique to neuronal tissue since no such effect could be demonstrated in glial cultures (Ramaharobandrot al., 1982).

B. Postsynaptic Action of Excitatory Amino Acids

Endogenous excitatory amino acids, particularly L-GLU and L-ASP, can depolarize virtually all vertebrate central neurons tested when applied by microiontophoresis or bath administration (Watkins, 1978). Based on electrophysiological studies, these compounds appear to produce changes in membrane conductance and ion permeability similar to that which occurs during evoked synaptic excitation (Davidson, 1976; Freeman, 1976). The stimulatory effects of excitatory amino acids are inhibited by a number of antagonists believed to be relatively specific for this class of compounds (Watkins and Evans, 1981). These putative antagonists also block a variety of excitatory synaptic pathways in the vertebrate CNS (Watkins and Evans, 1981), suggesting that excitatory amino acids may be the natural transmitters at these sites. However, it has been emphasized that the inhibition of the effects of an
exogenous candidate and the endogenous transmitter by an antagonist provides information on the identity of the receptor rather than the transmitter (Watkins and Evans, 1981).

C. Inactivation Mechanisms For Excitatory Amino Acids

One of the major obstacles against accepting excitatory amino acids as neurotransmitters was the apparent lack of a mechanism of inactivation which should terminate their functional effects. Thus, D- and L-GLU are potent neuroexcitants but the D-isomer is not metabolized. It was, therefore, expected that D-GLU would have a much longer duration of action than L-GLU. This prediction was not confirmed from the results of microiontophoretic studies in which it was shown that D- and L-GLU have a similar duration of action (Curtis et al., 1960). Therefore, it was suggested that the functional effects of L-GLU are not terminated by metabolic degradation. Present evidence supports the hypothesis that the inactivation of amino acid transmitters in the vertebrate CNS occurs by a combination of two mechanisms: 1) transport back into the nerve terminal and 2) diffusion out of the synaptic cleft followed by uptake into glial cells (Cox and Bradford, 1978; Johnston, 1981; Currie and Kelly, 1981; Storm-Mathisen, 1981; Duce and Keen, 1983).

Numerous studies using brain slices and synaptosomes have demonstrated the existence of both high and low affinity uptake sites for excitatory amino acids (Johnston, 1981). High affinity uptake appears specific for neuronal and glial cells while the low affinity
uptake mechanism is not restricted to cells in the CNS and may be a
general feature of most animal cells. The high affinity uptake of
excitatory amino acids into brain slices and cultured nerve cells has
been shown to be temperature-, energy- and sodium-dependent (Johnston,
1981; Iwata et al., 1982; Ramaharobandro et al., 1982) while low affinity
uptake, although energy- and temperature-dependent, is much less sensi-
tive to changes in sodium ion concentration and to compounds which modify
sodium ion movements (Johnston, 1981). High affinity uptake into glial
cells was also shown to be temperature-, energy- and sodium-dependent
(Balcar et al., 1977; Ramaharobandro et al., 1982). A recent report
has also shown that L-GLU is accumulated in rat brain synaptic vesicles
by a carrier-mediated uptake mechanism which is distinct from the uptake
systems described for neuronal and glial cells (Disbrow et al., 1982).
However, it remains to be determined whether uptake into synaptic
vesicles occurs under physiological conditions involved in neurotrans-
mission.

Substrate specificity studies indicate that L-GLU,
L-ASP and D-ASP are taken up by the same neuronal high affinity uptake
system while D-GLU and L-homocysteic acid appear to be good substrates
for the low affinity uptake mechanism (Johnston, 1981).

Inhibition of the high affinity uptake system
has been shown to potentiate the in vivo effects of excitants known
to be taken up actively in vitro (Johnston, 1981). This suggests
that reuptake of excitants by the high affinity system may play a functional role in inactivating these amino acids.

D. Biosynthesis and Regulation of Releasable Pools of Excitatory Amino Acids

Of all the known endogenous excitatory amino acids, only GLU has been studied with respect to its biosynthesis and regulation of releasable transmitter pools from nerve terminals. Glutamine and glucose are two potential precursors of GLU which are present in large amounts in the brain (Cooper et al., 1982). Glutamine is converted to GLU by hydrolysis, a reaction catalyzed by the enzyme, glutaminase(s). GLU may also be synthesized by the oxidative metabolism of glucose to α-ketoglutarate which in turn is converted to GLU by the enzyme, GLU dehydrogenase. Nerve terminals appear to contain large quantities of glutaminase (Bradford and Ward, 1976), suggesting that this enzyme may play an important role in the synthesis of transmitter GLU.

Since GLU has been localized in glial and neuronal compartments, both of which may serve a metabolic as well as a neurotransmitter function (Berl and Clarke, 1978; Van den Berg et al., 1978; Hamberger et al., 1982), studies were needed on a system where GLU would be expected to serve as a neurotransmitter. For this purpose Cotman and coworkers (Cotman and Hamberger, 1978; Cotman et al., 1981; Cotman and Nadler, 1981) employed tissue slices from the dentate gyrus
as a model for studying the role of glutamine and glucose as precursors for the synthesis of releasable GLU pools. In this model, high potassium-induced depolarization was found to predominantly release newly synthesized GLU from glutamine and not from glucose. Removal of the entorinal cortex (input to the dentate gyrus) markedly decreased the quantity of both newly synthesized (from glutamine) and endogenous GLU released in response to depolarization. Other investigators (Bradford et al., 1978; Shank and Aprison, 1979) have also suggested that glutamine may be the major precursor for transmitter GLU.

Glutamine is synthesized from GLU predominantly in glial cells in the presence of the enzyme, glutamine synthetase (Martinez-Hernandez et al., 1977). Consequently, it has been suggested that if glutamine is a precursor of transmitter GLU, a dynamic functional relationship must exist between glial cells and nerve terminals (Van den Berg et al., 1978; Berl and Clarke, 1978; Cotman and Nadler, 1981). Such a relationship is schematically represented in Fig. 1. This model shows that GLU is released by depolarization into the synaptic cleft where it can produce its physiological actions by activating specific receptors. The physiological effects of GLU are terminated either by high affinity reuptake into neurons where it is stored into releasable pools or by high affinity uptake into glial cells where it is converted to glutamine in the presence of glutamine synthetase. Glutamine is released from the glial cells and is then
taken up into the neurons where it interacts with glutaminase and is converted to transmitter GLU.

Fig. 1 Schematic representation of the dynamic relationship between glial cells and nerve terminals in regulating transmitter glutamic acid. G, glutamic acid; Gn, glutamine; Ca, calcium ions (Taken from Cotman et al., 1981).
I.3 PHARMACOLOGY OF EXCITATORY AMINO ACIDS

A. Neurophysiology

1. Structure-Activity Relationships

More than 70 α-amino acids (only some of which have been identified endogenously) have been shown to possess excitatory action in the vertebrate CNS (Watkins, 1978), and the list is increasing continually (Krogsgaard-Larsen et al., 1980). Structure-activity studies (Watkins, 1978) indicate that effective excitatory compounds contain two distinct acidic groups, which lose protons and become negatively charged at physiological pH, and at least one basic group which becomes positively charged at physiological pH (see Fig. 2). In all compounds

![General Structure and Ionized Form of Excitatory Amino Acids](image)

**Fig. 2** General structure and ionized form (at physiological pH) of excitatory amino acids. A, acidic group not associated with the asymmetric (α) carbon.

tested thus far, one acidic group is always a carboxyl group which is usually associated with the α-carbon while the second acidic group may
not necessarily be a carboxyl group. The basic group is always a primary or secondary amino group which is usually associated with the α-carboxyl group. The two acidic groups are separated by a distance equivalent to two to three carbons length for optimal activity. Structural modifications which abolish the ionizability of the basic or acidic groups lead to a marked reduction in excitatory activity. Finally, configuration about the asymmetric (α) carbon atom is important for compounds having a chain length greater than GLU.

2. Receptor Types

While investigating the sensitivity of spinal neurons to microiontophoretically-applied amino acid excitants, Duggan (1974) observed that spinal interneurons were more sensitive to L-GLU than to L-ASP, while, in contrast, Renshaw cells were more sensitive to L-ASP than L-GLU. At that time it was suggested that L-GLU and L-ASP might be neurotransmitters, acting on separate receptors which Duggan tentatively termed "GLU-preferring" and "ASP-preferring". In order to test this hypothesis further, the sensitivity of spinal neurons to more potent and more conformationally restricted analogs of GLU and ASP was determined. The compounds chosen for such a study were kainic acid (KA) and N-methyl-aspartic acid (NMA). KA is a naturally occurring antihelmintic isolated from certain kinds of seaweed (Watkins, 1978). It is a conformationally restricted analog of GLU (see Fig. 3) and has
been identified as one of the most potent excitatory amino acids known (Watkins, 1978). NMA is a synthetic analog of ASP (see Fig. 4) which is comparable in potency to KA (Watkins, 1978) and was hypothesized to interact exclusively with the "ASP-preferring" receptor (Johnston et al., 1974). When both of these amino acid excitants were applied onto single spinal neurons, it was observed that KA was relatively more effective on those cells which were previously designated as
"GLU-preferring" while NMA was relatively more effective on those cells which were designated as "ASP-preferring" (McCulloch et al., 1974). The observation that spinal neurons show a differential sensitivity to L-GLU and L-ASP has been verified by other studies (Hutchinson et al., 1978; Biscoe et al., 1976).

Another potent excitatory amino acid, quisqualic acid (QA), has been isolated from some terrestrial plants (Watkins, 1978). Like KA, it is a structural analog of GLU (see Fig. 3) possessing antihelmintic properties (Watkins, 1978). This neuroexcitant,
along with KA and NMA, has played an important role in the discovery of different types of receptors for excitatory amino acids in the vertebrate CNS.

Support for the hypothesis that excitatory amino acids do not function by acting on a homogeneous population of receptors came from microiontophoretic studies on cat and frog spinal cord neurons (Davies et al., 1979). It was observed that low concentrations of magnesium, which normally do not inhibit transmitter release, depressed NMA-induced excitation but did not inhibit neuronal excitation produced by KA or QA. Thus, it was suggested that at least two receptor types were present in the vertebrate spinal cord: 1) a NMA, magnesium-sensitive receptor and 2) a non-NMA, magnesium-insensitive receptor. It is interesting to note that in these studies L-ASP-induced excitation was not significantly reduced by magnesium. Therefore, NMA does not appear to be a selective agonist at the so-called "ASP-preferring" site.

Following these initial studies, a variety of organic compounds were identified as potential antagonists of the excitatory effects produced by the excitatory amino acids (see review by Watkins and Evans, 1981). The microiontophoretic application of these putative antagonists onto spinal neurons which were stimulated by excitatory amino acids revealed the presence of at least three receptors types:

1) NMA-activated receptors which were selectively antagonized by magnesium and a number of organic antagonists, particularly D-amino adipic
acid and 2-amino-5-phosphonovaleric acid; 2) KA-activated receptors which were antagonized, along with NMA receptors, by \( \gamma \)-D-glutamylglycine; and 3) QA-activated receptors which were weakly but selectively antagonized by L-glutamic acid diethyl ester. It was found that L-ASP and L-GLU possess some agonist action on all three receptor types since their neuroexcitatory effects were depressed to some degree by antagonists representative of all three receptor classes. Thus, the terminology of "ASP-preferring" and "GLU-preferring" receptors appears to be oversimplified. Of the other known endogenous excitatory amino acids, L-homocysteic acid, L-homocysteine sulfinic acid and L-cysteine sulfinic acid appear to function predominantly through NMA receptor activation while L-cysteic acid induces its excitatory effects through both NMA and QA receptors.

In order to determine whether a given neuroexcitant is a neurotransmitter, attempts have been made to relate the effects of a specific endogenous neuroexcitant with one of the three proposed excitatory amino acid receptors. The results to date have not been successful, since all of the excitants so far tested can stimulate all three of these receptors (Watkins and Evans, 1981).

The mechanism(s) through which excitatory amino acid antagonists function is still poorly understood. Microiontophoretic studies have suggested that magnesium may act in a non-competitive manner to lower the affinity of the receptor for NMA-type
agonists or may act at the receptor-ionophore coupling process to reduce the effectiveness of the receptor-agonist interaction (Davies et al., 1979). On the other hand, organic antagonists such as D-amino adipic acid appear to act in a competitive manner to reduce agonist-receptor interactions (Davies et al., 1979).

Some of the problems in identifying receptors for potential endogenous excitatory amino acids may be clarified through the development of more potent and selective antagonists. D-Amino adipic acid and 2-amino-5-phosphonovaleric acid are two highly potent and selective antagonists of NMA receptor activation (Watkins and Evans, 1981). However, there are no known antagonists which specifically inhibit KA-induced excitation of central neurons. The most effective antagonist at present is γ-D-glutamylglycine which also blocks NMA receptor activation (Watkins and Evans, 1981). Furthermore, QA-induced excitation is only weakly depressed by L-glutamic acid diethyl ester, an antagonist which, although selective with regard to other excitatory amino acids, also possesses anticholinergic properties (Watkins and Evans, 1981). In addition, L-glutamic acid diethyl ester is ineffective in inhibiting excitatory QA-induced responses when tested in the isolated frog spinal cord preparation (Evans et al., 1979).

3. Configuration of Excitatory Amino Acid Receptors

It was originally suggested by Curtis and Watkins (1960) that the excitatory amino acids possess a three-point attachment
site onto surface membranes involving both of the acidic groups and the single basic group. This concept is still accepted today (Watkins, 1981; McLennan et al., 1981). Despite the possible existence of several types of receptors for excitatory amino acids, evidence suggests that the actual configuration of these receptors may be quite similar. Thus, cis-2,3-piperidine dicarboxylic acid is equally effective as an antagonist against KA-, QA- and NMA-induced excitation without interfering with acetylcholine or substance P responses (Watkins and Evans, 1981). Consequently, it appears that the conformational specificities of the receptors may be very subtle. It has been suggested that the receptors should not be regarded as completely rigid templates to which the agonist molecules must conform but rather that some degree of flexibility in the chemical structure of the receptors themselves is possible (McLennan et al., 1981). By studying the action of a number of conformationally restricted excitatory amino acid agonists and antagonists on spinal neurons, McLennan et al. (1981, 1982) have drawn the following conclusions: 1) the NMA receptor reacts preferentially with substances in a relatively extended configuration, 2) the QA receptor prefers folded molecules, 3) the distance separating the amino group from the distal anionic function is the critical one determining receptor preference, 4) KA receptors, like those of NMA, also appear to be in an extended configuration, but a lack of specific antagonists precludes any analysis similar to that given for NMA or QA receptors.
4. Ionic Mechanisms

Since the initial studies which demonstrated that central neurons could be excited by microiontophoretic application of a range of naturally occurring and synthetic acidic amino acids (Curtis and Watkins, 1960; 1963; Curtis et al., 1960), it was inferred that these compounds produced an increase in membrane conductance due to an increase in membrane permeability to sodium (Curtis and Johnston, 1974). However, more recent studies have indicated that such a concept may be oversimplified since changes in membrane conductance and ion flux across permeable membranes vary among the amino acid excitants. Thus, based on intracellular recordings, excitatory amino acids have been grouped into three categories according to changes induced in membrane conductance, time course of depolarization and the ability to maintain stable repetitive firing (Lambert et al., 1981). Group I consists of L-GLU, L-ASP, QA and L-homocysteic acid. These amino acids produce a small increase in membrane conductance, a time course of depolarization with fast onset and recovery and a short period of repetitive firing. Group II, represented by NMA, D-homocysteic acid and ibotenic acid (another potent excitatory amino acid analog of GLU) show a marked decrease in membrane conductance, a slow onset and offset of depolarization and an ability to maintain stable repetitive firing. Finally, Group III is comprised solely of KA. This potent neuroexcitant displays a very slow onset of depolarization with incomplete recovery, an increase in membrane conductance and repetitive firing which occurs in very short bursts. Interestingly, this
classification of excitatory amino acids is similar to the pharmacological grouping based on receptor antagonist studies (Watkins and Evans, 1981). That is, NMA, KA and QA being representative of three different classes of excitatory amino acid receptors.

There is little information on the ion fluxes that underlie changes in conductance evoked by the amino acid excitants. In general, most studies have suggested that L-GLU, and some related excitatory amino acids, produce an influx of sodium and calcium across neuronal membranes (Engberg et al., 1979; Shapovalov et al., 1978; Retz et al., 1982; Ichida et al., 1982; Berdichevsky et al., 1983). The movement of sodium ions across neuronal membranes appear to play a major role in excitatory amino acid-induced depolarization, and is associated with an increase in membrane conductance, since removal of extracellular sodium from a bath medium containing spinal cord neurons prevented L-GLU-induced depolarization (Hosli et al., 1976). In addition, it has been shown that excitatory amino acids, including NMA, KA, QA and L-GLU, produce a dose-dependent increase in the efflux of sodium from rat brain slices and synaptic membrane vesicles (Chang and Michaelis, 1981; Luini et al., 1981; Goldberg et al., 1981; Teichberg et al., 1980; 1981). Excitatory amino acid antagonists such as 2-amino-5-phosphonovaleric acid, γ-D-glutamylglycine, D,L-amino adipic acid and several lactone derivatives of KA have been shown to inhibit the increase in sodium efflux produced by KA, NMA, QA, L-GLU and L-ASP (Luini et al., 1981; Goldberg et al., 1981). These studies on ion flux
confirm the existence of a heterogeneous population of excitatory amino acid receptors in the vertebrate brain.

A role for potassium in excitatory amino acid-induced depolarization has recently been challenged. Some studies indicate that a decrease in potassium conductance accompanies L-GLU-induced depolarization (Engberg et al., 1979; Shapovalov et al., 1978), but more recent studies claim that potassium ion movement is unaltered (Buhrlé and Sonnhof, 1983). The well established observation that excitatory amino acids produce a marked increase in extracellular potassium is also hard to reconcile with a proposed decrease in potassium membrane conductance (Watkins and Evans, 1981). It has been suggested that changes in potassium conductance, and for that matter, sodium conductance, could vary depending on the type of receptor(s) activated (Watkins and Evans, 1981). Thus, a role for potassium membrane conductance in excitatory amino acid-induced depolarization remains unclear at the present time.

Changes in the movement of chloride ions across neuronal membranes has not been adequately studied. A recent report has indicated that chloride ion conductance is unaltered during L-GLU-induced depolarization of frog motoneurons (Buhrlé and Sonnhof, 1983). However, chloride (as well as potassium) ion fluxes may be important for the action of ibotenic acid, a conformationally restricted analog of GLU (Krogsgaard-Larsen et al., 1980). Unlike all other known GLU
analogues, ibotenic acid possesses inhibitory as well as excitatory effects upon mammalian spinal neurons in vitro (Nistri et al., 1981).

**B. Binding Studies**

The specific binding of radiolabeled excitatory amino acids to vertebrate CNS synaptic membranes has been examined by numerous investigators (see reviews by Roberts, 1981, Briley et al., 1981; Coyle, 1981a; Watkins and Evans, 1981). Binding sites should not be thought of as functional receptors since the receptor in vivo may be coupled to 1 or more transducers, which may vary at different sites (Cooper et al., 1982). However, ligand binding studies can be useful in characterizing neurotransmitter receptor recognition sites (Coyle, 1981a).

1. $^3$H-L-GLU Binding

$^3$H-L-GLU has been shown to bind to a sodium-dependent site, which appears to be associated with the high affinity uptake mechanism of L-GLU and several sodium-independent sites which appear to be associated with pharmacological receptors. The sodium-independent specific binding of $^3$H-L-GLU increases markedly between 2 and 37°C and preincubation with calcium increases the number of binding sites in different brain regions. Binding has been shown to be saturable, reversible and to display a subcellular distribution expected of physiological receptors. Structure-activity studies of the specific binding of $^3$H-L-GLU to synaptic membranes have shown that L-GLU and QA are the most potent displacers of this radioligand, suggesting that
L-GLU, in agreement with pharmacological evidence, may interact with "QA-preferring" receptors. This is further supported by the observation that other non-QA-like agonists such as KA, NMA or D-ASP do not displace $^3$H-L-GLU binding. Stereoselectivity has also been shown since D-GLU is a very weak competitive inhibitor of $^3$H-L-GLU binding. The fact that L-ASP produces a moderate displacement of $^3$H-L-GLU binding suggests that both of these amino acids, in agreement with microiontophoretic studies, share a common site of action. However, more recent studies have shown that L-GLU and L-ASP are also capable of binding to separate sites in the rat brain (Footer et al., 1981).

Putative excitatory amino acid antagonists (based on microiontophoretic studies), such as D-amino adipic acid and 2-amino-4-phosphonobutyric acid, display moderate blocking activity of $^3$H-L-GLU binding. Unexpectedly, L-glutamic acid diethyl ester, a proposed antagonist of L-GLU, did not significantly inhibit $^3$H-L-GLU binding. However, this may be due to the fact that L-glutamic acid diethyl ester is, at best, a weak antagonist of L-GLU-induced excitation (Watkins and Evans, 1981).

The binding of $^3$H-L-GLU to synaptic membranes is enhanced by some ions and inhibited by others. In fact, recent studies have demonstrated that changing the ionic environment will unmask various pharmacologically distinct binding sites for L-GLU, some of which appear to be related to synaptic transmission in the
brain (Larder and McLennan, 1982; Fagg et al., 1982; 1983, Werling and Nadler, 1982).

2. $^3$H-L-ASP Binding

The kinetic parameters for sodium-independent binding of $^3$H-L-ASP are similar to those of L-GLU. Furthermore, L-ASP is a moderately potent competitive inhibitor of $^3$H-L-GLU binding. However, structure-activity studies and ionic manipulations show that a number of differences occur between the binding of these two amino acids. Most notable was the finding that QA, perhaps the most potent inhibitor of $^3$H-L-GLU binding, was only a weak inhibitor of $^3$H-L-ASP binding. Other excitatory amino acids such as KA and D,L-homocysteic acid were also poor inhibitors of $^3$H-L-ASP binding. Several NMA receptor antagonists including D-aminoadipic acid and D-aminosuberic acid were potent inhibitors of $^3$H-L-ASP binding, suggesting a possible interaction at the NMA receptor. One problem with this interpretation is that NMA was found to be a weak inhibitor of $^3$H-L-ASP binding.

3. $^3$H-KA Binding

Specific, saturable and reversible binding of $^3$H-KA has been identified and characterized in the rat brain. The specific binding is enriched in synaptosomal fractions, quite low in white matter and undetectable in membranes from non-neuronal tissues. Two sodium-independent binding sites for $^3$H-KA have been identified with the higher affinity site being restricted to forebrain regions whereas the lower affinity site is found throughout the brain. Potent
inhibitors of the lower affinity site are KA itself, L-GLU and QA, suggesting that KA and QA may share some common recognition site. It was also observed that NMA and several excitatory amino acid receptor antagonists do not displace low affinity $^3$H-KA binding. These data are in close agreement with pharmacological studies which suggest that KA acts on its own receptors with possibly a small interaction with QA receptors. Furthermore, there are no known potent receptor antagonists of KA receptor activation, which agrees with the above binding data on the low affinity site. It has therefore been suggested that the low affinity site labeled by $^3$H-KA represents the receptor mediating its neurophysiologic and neurotoxic effects.

The characteristics of the high affinity site have not been examined at this time.

4. $^3$H-AMPA Binding

D,L-α-AMino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) is a structural analog of L-GLU possessing potent excitatory properties when applied by microiontophoresis onto cat spinal cord neurons (Krogsgaard-Larsen et al., 1980). This neuroexcitant has recently been shown to bind to rat brain synaptic membranes (Honore et al., 1982) in a saturable and reversible manner at physiological pH. Kinetic analysis revealed at least two sodium-independent binding sites with the highest concentration of sites found in striatal membranes. The most potent inhibitors of $^3$H-AMPA binding were AMPA itself, along
with QA and L-GLU. KA had a moderate effect while L- and D-ASP and D-GLU were ineffective. The excitatory amino acid receptor antagonist, D-amino adipic acid, was also ineffective as an inhibitor of $^{3}H$-AMPA binding. These findings have suggested that AMPA is binding to a L-GLU or "QA-preferring" receptor site. It is surprising that L-glutamic acid diethyl ester was a relatively weak inhibitor of $^{3}H$-AMPA binding. However, although L-glutamic acid diethyl ester is a selective inhibitor of the pharmacological effects of QA, its potency is very low (Watkins and Evans, 1981).

5. $^{35}S$- and $^{3}H$-L-Cysteine Sulfinic Acid Binding

Recent studies have revealed that $^{3}H$- or $^{35}S$-cysteine sulfinic acid, an endogenous excitant binds to sodium-dependent and sodium-independent sites throughout the mammalian brain with the highest levels of sodium-independent binding occurring in the cerebellum (Iwata et al., 1982a; Recasens et al., 1983). The subcellular distribution of $^{3}H$-L-cysteine sulfinic acid was found to be predominantly in synaptosomal membrane. The most potent inhibitors of $^{3}H$- or $^{35}S$-cysteine sulfinic acid binding are L- and D- cysteine sulfinic acid, L-GLU, D,L-homocysteic acid and L-ASP while D-GLU, D-ASP, KA and NMA were shown to be ineffective. Although $^{3}H$-cysteine sulfinic acid binding exhibits roughly the same response to ion manipulation and competitive blockers such as $^{3}H$-L-GLU, several quantitative and qualitative differences are apparent. Therefore, it has been postulated that multiple binding sites exist for L-GLU and L-cysteine sulfinic acid, some of which are shared by both amino acids.
Although attempts have been made to compare chemical binding data with agonist potencies of excitatory amino acids in vivo, it is difficult to make such a comparison since excitant responses are a function of efficacy and affinity whereas binding reflects affinity only. This is not a complication in the case of antagonists where chemical binding constants should correlate directly with drug potency. However radiolabeled studies with excitatory amino acid antagonists have not yet been reported.

C. Neurotoxic Effects of Excitatory Amino Acids

Besides possessing a potent neuroexcitatory effect, acidic amino acids have been shown to produce neuronal degeneration after systemic or intracranial administration (Kizer et al., 1978; Olney, 1978; Coyle, 1981b). The peripheral administration of L-GLU to neonatal rodents causes the degeneration of neurons in the inner layers of retina as well as neurons in certain brain regions (the circumventricular organs) lying outside the blood-brain barrier (Olney, 1978; 1981). Cytopathological studies revealed that L-GLU-induced lesions were characterized by rapid swelling of neuronal dendrites and cell bodies followed by acute degenerative changes in intracellular organelles and coarse clumping of the nuclear chromatin. The reaction evolves rapidly with onset of dendritic swelling detectable by 15 to 30 minutes and phagocytosis of cell bodies beginning as early as 3 hours after injection (Olney, 1981). Both biochemical and anatomical evidence has demonstrated that neuronal dendrites and cell bodies are preferentially vulnerable to the neurotoxic effects of GLU and that nerve terminals and axons
appear not to be destroyed until after their parent cell bodies degenerate (Bird, 1981). Thus, this type of cytopathologic reaction has been referred to as "dendrosomatotoxic but axon sparing" (Olney, 1981). The fact that glial and other non-neuronal elements are resistant to GLU-induced lesions indicates that the primary toxic action of L-GLU is specific for neurons (Bird, 1981).

Structure-activity studies have shown a close correlation between the neuroexcitatory affects of a variety of GLU analogs on single neurons and their ability to induce neuronal degeneration after peripheral administration (Olney, 1981; Coyle, 1981b; 1982). This close correlation is the basis for the "excitotoxic hypothesis" initially presented by Olney (1978). According to this hypothesis, excitatory amino acids produce their neurotoxic effects through the persistent stimulation of receptors responsible for their excitatory action. Thus, the sustained increase in neuronal membrane permeability caused by continuous depolarization is thought to be the primary mechanism of the pathological process leading to neuronal degeneration induced by excitatory amino acids. Presumably, a prolonged increase in membrane permeability would lead to a depletion of neuronal energy reserves in an effort to restore ionic balance, with cell death ensuing when energy reserves are exhausted and/or lethal alterations in the internal structure of the cell has occurred. By direct intra-cranial injections, lesions can be produced in discrete areas of the brain which, upon histological examination, show destruction of cell bodies and dendrites
but not degeneration of axons of passage (Olney, 1981). However, structure-activity relationship studies employing intra-striatal injections of neuroexcitants have not verified the observations made after peripheral administration of a close correlation between the neuroexcitatory effects of excitotoxins and their neurotoxic potencies (Zaczek and Coyle, 1982). Furthermore, the intra-hippocampal injection of some excitatory amino acid antagonists, which possess no agonist activity, have also been shown to be potent neurotoxins (Nadler et al., 1981). Therefore, acceptance of the excitotoxic hypothesis will require further investigation.

D. Behavioral Effects of Excitatory Amino Acids

Since excitatory amino acids have a powerful effect upon central neurons, it has become of interest to study the behavioral changes induced by these compounds. Behavioral responses to excitotoxins may be due to their neuroexcitatory and/or neurotoxic properties (Kizer et al., 1978; Coyle et al., 1977). However, based on the "excitotoxic hypothesis" (Olney, 1978) as well as histological (Olney, 1981) and neurochemical (Coyle et al., 1977) changes observed after intra-cranial injections of excitatory amino acids, it is likely that immediate (acute) behavioral responses are the result of neuronal excitation while chronic behavioral changes (i.e., those not occurring until several days to a week after injection) may be due to neurotoxicity. Furthermore, it has recently been shown that subtoxic doses of KA (French et al., 1982), QA (Zaczek and Coyle, 1982) and NMA (Zaczek and

The document discusses the correlation between the neuroexcitatory effects of excitotoxins and their neurotoxic potencies, and highlights the significance of behavioral changes induced by excitatory amino acids. It emphasizes the distinction between immediate acute behavioral responses and chronic changes, attributing the latter to neurotoxicity. The study of subtoxic doses of excitotoxins also sheds light on the manifestation of behavioral effects.
Coyle, 1982) can induce profound behavioral changes (e.g., seizures) after intra-hippocampal injection. Therefore, based on the time of observation or dose of excitotoxin used, it is possible to observe behavioral changes due solely to neuronal excitation.

Behavioral responses to the intra-cranial injection of excitatory amino acids have been confined, for the most part, to basal ganglia and limbic structures. The unilateral injection of KA and NMA, but not L-GLU or QA, into the rat striatum produce postural changes and rotation which occur immediately upon injection (Coyle et al., 1977; Jenner et al., 1980; Taylor et al., 1981). The rotational response is blocked by haloperidol (Taylor et al., 1981) but not by putative excitatory amino acid antagonists (Jenner et al., 1980). Long term behavioral effects of unilateral or bilateral intra-striatal injections of KA include various directional changes in rotation (Dunnet and Iversen, 1982a; Schwarcz et al., 1979; Taylor et al., 1981), a decrease in sensorimotor performance (Dunnet and Iversen, 1982b), and changes in locomotion which may be analogous to the locomotion pattern in patients with Huntington's chorea (Hruska and Silbergeld, 1979; Coyle et al., 1977; Mason and Fibiger, 1978), a hereditary degenerative disorder of the nervous system characterized by dementia and movement abnormalities (Hayden, 1981).

Both acute and chronic motor effects of excitatory amino acids have been studied after injection into the substantia nigra.
Unilateral injections of KA (Olianas et al., 1978 a, b; Proceddu et al., 1979; Arnt, 1981a) into the substantia nigra have been shown to produce an acute stimulation of contraversive circling while bilateral injections of KA and NMA into the substantia nigra, pars compacta enhance locomotor activity (Pycock and Dawbarn, 1980). This latter finding was shown to be blocked by fluphenazine, a dopamine receptor antagonist. In contrast to the above findings, bilateral injections of KA and NMA into the nigra, pars reticulata caused sedation and catalepsy (Pycock and Dawbarn, 1980). Unilateral KA lesions of the substantia nigra, pars reticulata result in ipsilateral turning after systemic administration of apomorphine, a dopamine receptor agonist (DiChiara et al., 1979). Destruction of postsynaptic structures in the striatum of the side contralateral to that injected with KA results in a loss of the turning effects elicited by apomorphine (DiChiara et al., 1979). Such a finding has led to the suggestion that the substantia nigra, pars reticulata is an out-put station for the expression of striatal motor function (DiChiara et al., 1979).

Other areas of the mammalian brain which have been studied with regard to excitatory amino acid-induced behavioral changes have been the ventral tegmental area (Pycock and Dawbarn, 1980), midbrain reticular formation (Kitsikio and Steriade, 1981; Imperato et al., 1981; Garcia-Munoz et al., 1982), superior colliculus (Imperato et al., 1981; Garcia-Munoz et al., 1982) and nucleus accumbens (Arnt, 1981b). The bilateral injection of KA and NMA into the ventral tegmental area
produces an immediate increase in locomotor activity at low doses and running seizures at high doses. Both responses are inhibited by fluphenazine. Bilateral injections of KA into the midbrain reticular formation produce a variety of acute anomalous behaviors (Kitsikis and Steriade, 1981) while long term effects include a decrease in rotational behavior elicited by a unilateral KA injection into the substantia nigra, pars compacta (Imperato et al., 1981). Bilateral injections of AMPA into the nucleus accumbens produce an immediate and intense increase in locomotor activity which is inhibited by a dopamine receptor antagonist, reserpine and a GABA agonist (Arnt, 1981b); however, L-glutamic acid diethyl ester, an excitatory amino acid antagonist, did not block AMPA-induced hypermotility.

Intra-ventricular (Lanthorn and Isaacoon, 1978; Kleinrok et al., 1980) and systemic (Worms et al., 1981; Lenique et al., 1981) injections of excitatory amino acids have been shown to produce a variety of behavioral effects including wet dog shakes, convulsions, hyperexcitability, aggressiveness and aphagia. It is indeed interesting that ingestion of monosodium glutamate has been reported to produce shuddering in children (Reif-Lehrer and Stemmermann, 1975).

A few reports have been published on the behavioral effects of excitatory amino acid antagonists. Dawbarn and Pycock (1981) first reported that 2-amino-5-phosphonovaleric acid and γ-D-glutamyl-glycine produced an intense increase in motor function after unilateral
or bilateral injections into the substantia nigra, pars compacta or ventral tegmental area. These effects were inhibited by fluphenazine. Unilateral injections of these antagonists into the substantia nigra, pars reticulata produce sedation. These results are identical to those reported for excitatory amino acids (Pycock and Dawbarn, 1980).

L-Glutamic acid diethyl ester has been found to produce catalepsy after intraventricular injections and has been proposed as a model for schizophrenia (Kornhuber and Fischer, 1982).

Since excitatory amino acids produce convulsions when injected intracranially or systemically, it has been investigated whether excitatory amino acid antagonists possess anticonvulsant properties. Convulsions induced by intracerebral L-GLU were not blocked by L-glutamic acid diethyl ester (Stone and Javid, 1983), a proposed L-GLU antagonist; however, classical anticonvulsants did inhibit L-GLU-induced seizures (Stone and Javid, 1983). Pentylenetetrazol-, strychnine- or sound-induced seizures were either delayed in onset (Abdul-Ghani et al., 1982) or completely blocked (Croucher et al., 1982) by several putative excitatory amino acid antagonists.

1.4 IDENTIFICATION OF SPECIFIC EXCITATORY AMINO ACID-CONTAINING PATHWAYS

Excitatory amino acids may function as transmitters in the vertebrate CNS. The fact that virtually all neurons in the CNS are stimulated by excitatory amino acids indicate that these neuroexcitants
may be utilized as transmitters in a wide variety of neuronal pathways. It has been recently suggested that there are indeed many areas of the vertebrate brain and spinal cord which may use excitatory amino acids as transmitters in both interneurons and projection pathways (see reviews by Watkins and Evans, 1981; Fonnum and Malthe-Sorensen, 1981). However, in most cases the endogenous excitatory transmitter is unknown but is assumed to be L-GLU or L-ASP.

I.5 THE NUCLEUS ACCUMBENS

The nucleus accumbens is a forebrain region which has received much anatomical and physiological attention over the past few years. Interest in this structure is by no means new (see Chronister and DeFrance, 1981) but its strategic location between limbic and basal ganglia structures has recently caused a sudden outburst of studies on its functional significance.

A. Neuroanatomy and Neurochemistry

In the rodent brain (see Fig. 5), the nucleus accumbens is shown to be a fairly large area of the forebrain lying ventral to the anterior horn of the lateral ventricle. In most mammals, it appears morphologically distinct because of the relative absence of perforating fiber bundles. The nucleus is transversed by the anterior part of the anterior commissure. Postrally, the nucleus is bordered by the posterior part of the nucleus olfactorius and tractus olfactorius intermedius, and caudally, it is continuous with the bed nucleus of the stria terminalis. Ventrally, it faces areas such as the olfactory
Fig. 5  Location and boundaries of the nucleus accumbens (a) in the rat brain (taken from König and Klippel, 1963). Abbreviations: FLC, Fissura longitudinalis cerebri; SLM, Stria longitudinalis medialis; HIA, Hippocampus, pars anterior; SLL, Stria longitudinalis lateralis; C, Cingulum; VL, Ventriculus lateralis; TSC, Tractus septocorticalis; TSHT, Tractus septohypothalamicus; sm, Nucleus septi medialis; CAE, Capsula externa; SR, Sulcus rhinalis; CPF, Cortex piriformis; TCL, Tractus corticohypothalamicus lateralis; CAA, Commissura anterior, pars anterior; FMP, Fasciculus medialis prosencephali; IC, Insulae Calleja; TULI, Tuberculum olfactorium, pars interna, lamina polymorphica; TULC, Tuberculum olfactorium, pars corticalis, lamina; TULP, Tuberculum olfactorium, pars corticalis, lamina' plexiformis; TST, Tractus septotubercularis + Tractus tuberculoseptalâ; ICM, Insula Calleja magna; POP, Fasciculus opticus; TSTH, Tractus striohypothalamicus; TOL, Tractus olfactorius lateralis; CL, Claustrum; GCC, Genu corporis callosi; RCC, Radiatio corporis callosi; sl, Nucleus septi lateralis; cp, Nucleus caudatus putamen.
tubercle and the medial forebrain bundle; dorsally, the nucleus caudatus and the septum; medially, the nucleus septi medialis, the nucleus tractus diagonalis (Broca) and the tractus diagonalis (Broca) and laterally the caudate nucleus (also by frontal cortical areas more postally). Based on its efferent connections as well as the pattern of its neurogenesis, the nucleus accumbens is considered by some to be a subdivision of the ventral striatum (Domesick, 1981).

The nucleus accumbens receives afferent fibers from the neocortex, hippocampus, amygdala, several nuclei of the thalamus, cell group A10 of Dahlstrom and Fuxe (1964) in the ventral tegmental area and the dorsal raphe nucleus (Domesick, 1981; Groenewegen et al., 1981; 1982; Krayniak et al., 1981; Kelley and Domesick, 1982; Hemphill et al., 1981). In general, cortical and amygdala afferents terminate in the lateral portion of the nucleus accumbens while hippocampal afferents, which travel by way of the precommissural fornix, terminate in the medial part of the nucleus accumbens adjacent to the septum (Domesick, 1981; Groenewegen et al., 1981; 1982; Krayniak et al., 1981; Kelley and Domesick, 1982). Biochemical evidence has suggested that both frontal neocortical and limbic inputs to the nucleus accumbens may use GLU as their transmitter (Fonnum and Walaas, 1981). The afferent input from the ventral tegmental area has been shown to be dopaminergic and that of the dorsal raphe is serotonergic (Fonnum and Walaas, 1981). Both serotonin and dopamine have a homogeneous distribution throughout the nucleus accumbens (Fonnum and Walaas, 1981; Johansson and Hokfelt, 1981; DeFrance et al., 1983).
The nucleus accumbens projects to the ventral pallidum (substantia innominata), septum, preoptic area, hypothalamus, mesencephalic tegmentum, ventral tegmental area, substantia nigra (pars compacta and pars reticulata), thalamus, cell group A8 of Dahlstrom and Fuxe (1964), central gray matter and medial raphe nuclei (Domesick, 1981). All of these structures, except the ventral pallidum and septum, appear to receive their accumbal input by way of the medial forebrain bundle (Domesick, 1981). Projections from the nucleus accumbens to the ventral pallidum, substantia nigra and ventral tegmental area appear to be GABAergic in nature (Fonnum and Walaas, 1981).

Lesioning studies have indicated that the nucleus accumbens contains cholinergic, GABAergic and glutamatergic/aspartatergic interneurons (Fonnum and Walaas, 1981). Both cholinergic and GABAergic interneurons appear to be concentrated in the medial portion of the nucleus (Fonnum and Walaas, 1981).

The nucleus accumbens also contains high levels of norepinephrine which presumably is contained in fibers originating from the locus coeruleus (Fonnum and Walaas, 1981; DeFrance et al., 1983). In addition, histochemical studies have revealed the existence of numerous peptide-containing neurons (Johansson and Hokfelt, 1981).

**B. Functional Significance**

The nucleus accumbens has been linked with various functions, such as olfaction, milk ejection, prolactin release, conditioned
avoidance behavior and narcotic analgesia. However, from recent studies of afferent and efferent connections (see above) the nucleus accumbens is now viewed as a bridge linking limbic and motor systems. This concept has led to suggestions about the possible functional role of the nucleus accumbens in translating emotional to motivational determinants of behavior (Mogenson and Yim, 1981). Indeed, this nucleus has been implicated in the pathophysiology of psychomotor disorders such as Parkinson's disease, Huntington's chorea and schizophrenia (Palmer and Chronister, 1981).

Both limbic and motor components of the nucleus accumbens have been studied through electrophysiological, pharmacological and anatomical techniques (Mogenson et al., 1980; 1983; Mogenson and Yim, 1981; Groenewegen et al., 1981; Yim and Mogenson, 1982; Scarnati et al., 1983). However, based on the profound change in motor performance after intra-accumbal injection of drugs and putative neurotransmitters, it has been the motor component which has received a great deal of attention (Koob et al., 1981; Makanjuola and Ashcraft, 1982; Jones et al., 1981; Slater et al., 1982; Costall and Naylor, 1979; Kelly, 1977; Scheel-Kruger, 1982).

Limbic input to the nucleus accumbens has been shown to be predominantly excitatory (Mogenson and Yim, 1981; Groenewegen et al., 1981). Biochemical evidence suggests that GLU is the excitatory transmitter of these afferents (Fonnum and Walaas, 1981). It is
therefore possible that GLU or some related excitatory amino acid may serve as a limbic transmitter in the nucleus accumbens to influence motor function. There is, however, very little information on the behavioral or biochemical effects of excitatory amino acids in the nucleus accumbens. The only behavioral study was a recent report by Arnt (1981b) who showed AMPA, a GLU analog, to produce a dose-dependent increase in locomotor activity. This hypermotility response, like that produced by dopamine, is inhibited by a dopamine receptor antagonist and a GABA agonist. In addition, AMPA-induced hypermotility, is also inhibited by catecholamine depletion, suggesting that AMPA is producing its effect through the release of dopamine and subsequent stimulation of dopamine receptors within the nucleus accumbens. Biochemical studies have shown that L-GLU in very high concentrations is capable of releasing dopamine from accumbal slices (Roberts and Anderson, 1979; Marien et al., 1983). This response is antagonized by Magnesium and L-glutamic acid diethyl ester, two putative excitatory amino acid antagonists (Marien et al., 1983). Thus, both biochemical and behavioral studies are in agreement that excitatory amino acids may release dopamine in the nucleus accumbens by activating a GLU-type receptor on dopaminergic nerve terminals. However, it may be significant that in contrast to biochemical studies, L-glutamic acid diethyl ester was ineffective as an antagonist of AMPA-induced hypermotility (Arnt, 1981b). Thus, it remains to be shown in vivo that specific receptors exist for excitatory amino acids in the nucleus accumbens which can influence motor function.
1.6 STATEMENT OF THE PROBLEM

The objective of this dissertation is to determine the function of excitatory amino acid transmitters in the nucleus accumbens. In my initial studies, I observed, in agreement with Arnt (1981b), that the acute administration of excitatory amino acids into the nucleus accumbens produced a marked stimulation of locomotor activity. Therefore, the specific aims of these studies were:

1) to determine the role of dopamine in the stimulation of locomotor activity induced by the excitatory amino acids. This was done by studying the interaction between dopamine antagonists and drugs that interfere with dopaminergic neurotransmission on excitatory amino acid-induced hypermotility. In addition, the effect of excitatory amino acids on dopamine turnover in the nucleus accumbens was investigated.

2) to determine whether the locomotor activity stimulation induced by the excitatory amino acids was mediated through specific receptors. In these studies, the effect of a variety of putative excitatory amino acid antagonists on the hypermotility response induced by excitatory amino acids was examined.

By studying such mechanisms, it is hoped that a better understanding will emerge on the functional role of the nucleus accumbens in motor behavior.
CHAPTER II

EFFECTS OF EXCITATORY AMINO ACIDS ON LOCOMOTOR ACTIVITY AFTER BILATERAL MICROINJECTION INTO THE NUCLEUS ACCUMBENS

1.1 INTRODUCTION

The nucleus accumbens is a forebrain region currently believed to be involved in the initiation and regulation of spontaneous locomotor activity (Mogenson, Jones and Yim, 1980). It has been implicated in the pathophysiology of schizophrenia (Stevens, 1979; Mackay, et al., 1980). Parkinson's disease (Price et al., 1978) and Huntington's chorea (Hayden, 1981; Bots and Bruyn, 1981), and also in the antipsychotic action of neuroleptics (Bartholini, 1977; Costa, 1977). It is well known that this nucleus receives a prominent dopaminergic input from the ventral tegmental area (Lindvall and Bjorklund, 1978) and that bilateral injections of dopamine into the nucleus accumbens produce a marked increase in locomotor activity (Pijnenburg and Van Rossum, 1973; Costall and Naylor, 1975; Jackson, et al., 1975; Pijnenburg et al., 1975b; Pijnenburg et al., 1976; Makanjuola et al., 1980; Jones et al., 1981), which can be blocked by neuroleptic drugs (Pijnenburg et al., 1975a; Jackson et al., 1975; Pijnenburg et al., 1976; Costall et al., 1979; Makanjuola et al., 1980). The nucleus accumbens also contains a variety of other neurotransmitters and
neurotransmitter candidates, such as the excitatory amino acid, GLU (Fonnum and Walaas, 1981). Biochemical evidence suggests that the glutamatergic input into this forebrain area is derived from both the allocortex (Walaas and Fonnum, 1979; Walaas, 1981) and frontal neocortex (Carter, 1980; Walaas, 1981), and that GLU-containing interneurons may also be present (Walaas and Fonnum, 1979).

The functional significance of the excitatory amino acids in the nucleus accumbens is unclear at the present time. However, in vitro studies have shown L-GLU to release dopamine from accumbal slices (Roberts and Anderson, 1979). Thus, GLU might play a role in the regulation of locomotor activity by influencing dopaminergic mechanisms. This hypothesis has recently been supported by the observation that bilateral injections of AMPA, a potent excitatory amino acid agonist (Krogsgaard-Larsen et al., 1980), into the nucleus accumbens produced an increase in locomotor activity which was antagonized by the systemic administration of cis-Z-flupenthixol, a dopamine receptor blocking agent (Arnt, 1981b).

Based on results obtained from studies of mammalian spinal neurons, excitatory amino acid receptors have been classified into three types, using the potent receptor agonists NMA, KA and QA (Watkins, 1981; McLennan et al., 1981). The endogenous excitatory amino acids, ASP and GLU seem to have mixed agonist actions at these proposed receptor sites (Watkins and Evans, 1981), while AMPA is
believed to be a selective agonist at QA receptors (Evans, 1981).
However, glutamic acid diethyl ester, a putative QA receptor
blocking agents (McLennan and Lodge, 1979; Davies and Watkins, 1979)
did not antagonize AMPA-induced hypermotility (Arnt, 1981). In order
to determine whether the hypermotility response to excitatory amino
acids was selective for a given receptor agonist the effects of various
excitatory amino acids were studied after their direct administration
into the nucleus accumbens. In addition, experiments were performed
to determine whether the response to these compounds was mediated
through the release of dopamine and subsequent stimulation of dopamine
receptors within the nucleus accumbens.

II.2 METHODS
A. Surgical Procedure
Male Sprague-Dawley rats (Harlan-Sprague-Dawley,
Indianapolis, IN) weighing 175-200 g at the time of surgery were
anesthetized with chloral hydrate (420 mg/kg, i.p.) and placed in a
stereotaxic frame (David Kopf Inst., CA). Holes were then drilled on
each side of the skull for injection into the nucleus accumbens
(coordinates: A 9.4; V -1.0; L ± 2.4), striatum (coordinates: A 9.4;
L ± 2.0; V +0.8) or olfactory tubercles (coordinates: A 9.4; L ± 1.7;
V -2.2) using the atlas of König and Klippel (1963). The animals were
then returned to their home cage. On the following day, the rats
were reanesthesized with a halothane/oxygen mixture and returned to
the stereotaxic frame for the intra-cranial injection of drugs.
During the injection of the drug, the rats received halothane and oxygen continuously. After exposing the skull, the needle (o.d. = 0.46 mm) of a 5 µl Unimetrics syringe (Kew Scientific, Inc., OH) was inserted at a 10° angle (to avoid puncturing the ventricles) into the holes previously drilled in the skull and a 0.5 or 1.0 µl vol. of solution was injected bilaterally at a rate of 0.5 µl/min. The micro-syringe was left in place for an additional minute to allow diffusion of the solution away from the injection needle. After the injections, the skin incision was closed with wound clips and covered with lidocaine ointment (5%) to relieve any pain which might interfere with motility. The rats recovered from anesthesia within 5 min after the removal of halothane. Each rat was injected only once.

B. Monitoring Locomotor Activity

After the intra-cranial injections, the rats were placed in motor activity cages (Opto-Varimex-Minor, Columbus Inst., OH) and allowed 10 min to adapt to the environment. The activity cages were designed to measure ambulatory movements, but not total horizontal (e.g. tail flicking) or total vertical (e.g. rearing) movements. The cages contained 12 x 12 infrared beams passing at a height of 5 cm from the bottom of the cage through a ventilated plexiglass box measuring 42 cm square and 20 cm high. Locomotor activity (i.e. ambulatory movement) was recorded as the number of times two consecutive beams, 3.5 cm apart, were interrupted per hour. The data were printed out by a digital counter (Columbus Inst., Columbus, OH). The animals were
observed visually for convulsion, rearing or any other non-ambulatory behavior.

All observations were made between 10 a.m. and 5 p.m. in an isolated environmental room maintained at a temperature of 22 ± 1°C.

C. Drugs

The following compounds were purchased from Sigma Chemical Co. (St. Louis, MO): N-methyl-DL-aspartic acid, quisqualic acid and folic acid were dissolved in 0.1 M Na$_2$HPO$_4$ (pH 7.4); D-glutamic acid (free acid; was dissolved in 0.25 M Na$_3$PO$_4$ (pH 6.0); L-aspartic acid (monosodium salt), L-glutamic acid (monosodium salt), kainic acid, quinolinic acid, L-pyroglutamic acid, D, L-threo-ß-Hydroxy-aspartic acid (Calbiochem., LaJolla, CA) and γ-L-glutamylglycine (ICN Pharmaceuticals, Plainsview, NY) were dissolved in saline and adjusted, if necessary, to physiological pH with IN NaOH. Fluphenazine hydrochloride (Prolixin®, Squibb), haloperidol (Haldol®, McNeil) and reserpine (Serpasil®, Ciba) were used as commercially prepared.

D. Statistics

Data were expressed as the mean and standard error of the mean (SEM). Significant differences were evaluated using the non-parametric two-tailed Mann-Whitney U-test (Sokal and Rohlf, 1969), with a level of P < 0.05 being considered significant. Percent inhibitions were calculated as follows: [control-drug treatment/control] x 100.
E. Histology

The injection coordinates were verified by injection of dye into the nucleus accumbens (Fig. 6). Frozen sections (40 µ thick) were sliced using a Cryo-Cut Microtome (American Optical Corp., Buffalo, NY) and prepared and photographed as described previously (Skinner, 1971). The spread of the dye showed an anterior-posterior range from 8900 to 9800 µ (based on the atlas of König and Klippel, 1963) and was localized exclusively within the nucleus accumbens.

II.3 RESULTS

A. Effect of KA, QA and NMA on Locomotor Activity in the Rat

After bilateral injection into the nucleus accumbens, KA (15-125 ng injected on each side) produced a dose-dependent increase in locomotor activity (Fig. 7). In larger doses (250 and 500 ng), the hyperactivity response was substantially masked by intermittent tonic/clonic convulsive episodes. Similarly, QA (0.15 - 5 µg) also induced a dose-dependent increase in locomotor activity (Fig. 8), with no apparent generalized convulsions even at a dose of 10 µg. Instead, at a dose of 2.5 - 10 µg, some animals exhibited a "praying" response (Zaczek and Coyle, 1982) in which they reared back on their hind limbs with their forepaws extended and crossed. This behavior produced much less impairment of motor function than generalized convulsions, possibly accounting for the greater maximum locomotor activity response to QA as compared to all of the other excitatory amino acids tested. Bilateral injections of NMA (1.25 and 2.5 µg) also elicited
Fig. 6 Photomicrograph showing a unilateral injection of dye (0.5 μl) into the rat nucleus accumbens. Note that the dye is deposited around the anterior portion of the anterior commissure.
Fig. 7 Hypermotility induced by kainic acid after bilateral injection into the nucleus accumbens. Kainic acid or saline (SAL) was injected in a 0.5 μl vol, and the rats were placed in motor activity cages for 1 hr. Each point represents the mean and SEM. N = number of observations. *P < 0.05 with respect to SAL.
Fig. 7
Fig. 8  Hypermotility induced by quisqualic acid after bilateral injection into the nucleus accumbens. Quisqualic acid or vehicle (VEH; phosphate buffer) was injected in a 0.5 μl vol. and the rats were placed in motor activity cages for 1 hr. Each point represents the mean and SEM. N = number of observations. *P < 0.05 with respect to VEH.
Fig. 8

MOTILITY/HOUR

QUISQUALIC ACID [μg]

VEH
N = 7

0.15
0.31
0.62
1.25
2.5
5
10
a dose-dependent increase in locomotor activity but the maximal response was much less than that of KA or QA (Table 1). A 2.5 μg dose produced a brief period (approx. 5-10 min) of immobility followed by an enhancement of locomotion while a 5 μg dose produced immediate and continuous motor convulsions with no ambulatory movements. The doses of the three excitatory amino acids that stimulated locomotor activity were smaller than those previously found to produce neurotoxicity (Table 3).

Striatal mechanisms do not appear to be important for the locomotor effects of KA since bilateral intra-striatal injections of KA (15 ng/0.2 μl) did not produce an increase in locomotor activity as seen after bilateral injections into the nucleus accumbens or olfactory tubercles (Table 2).

B. Effect of Pretreatment with Reserpine on KA-Induced Hypermotility in the Rat

In order to determine the role of endogenous stores of dopamine on the hypermotility induced by KA, rats were pretreated with reserpine (5 mg/kg, i.p.) 4 hr prior to the bilateral administration of saline or KA (31 ng) into the nucleus accumbens (Fig. 9). Pretreatment with reserpine inhibited KA-induced stimulation of locomotor activity by 95% and that of control animals by 87%. 
TABLE 1. Hypermotility Induced by NMA

<table>
<thead>
<tr>
<th>NMA (μg/0.5 μl)</th>
<th>N</th>
<th>Motility/hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Vehicle 0.5 μl)</td>
<td>6</td>
<td>218 ± 57</td>
</tr>
<tr>
<td>0.62</td>
<td>3</td>
<td>447 ± 83</td>
</tr>
<tr>
<td>1.25</td>
<td>3</td>
<td>604 ± 59*</td>
</tr>
<tr>
<td>2.5</td>
<td>3</td>
<td>2310 ± 740*</td>
</tr>
<tr>
<td>5.0</td>
<td>4</td>
<td>Convulsions (no coordinated motor activity)</td>
</tr>
</tbody>
</table>

*Rats were injected bilaterally in the nucleus accumbens with vehicle (phosphate buffer) or N-methyl-aspartic acid (NMA; 0.62-5.0 μg) in a 0.5 μl vol. and placed in motor activity cages for 1 hr. N = number of observations. *P < 0.05 with respect to vehicle.

TABLE 2. Effects of KA on Locomotor Activity After Injection Into Various Brain Regions

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Motility/hour</th>
<th>Duration(Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleus Accumbens</td>
<td>3192 ± 442(3)</td>
<td>90 - 110</td>
</tr>
<tr>
<td>Striatum</td>
<td>475 ± 108(3)</td>
<td>60 - 70</td>
</tr>
<tr>
<td>Olfactory Tubercles</td>
<td>5540 ± 896(4)</td>
<td>100 - 140</td>
</tr>
</tbody>
</table>

Rats were injected bilaterally in the nucleus accumbens, striatum or olfactory tubercles with kainic acid (KA 15 ng/0.2 μl) and placed in motor activity cages for 1 hr. The number of observations are shown in parentheses.
TABLE 3. Comparison Of The Threshold Doses For Hypermotility and Seizure-like Behavior to the Neurotoxic Doses of Excitatory Amino Acids

<table>
<thead>
<tr>
<th></th>
<th>Hypermotility Threshold Dose (nmol)</th>
<th>Seizure-like Behavior Threshold Dose (nmol)</th>
<th>Neurotoxic dose (nmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kainic acid</td>
<td>0.07 (0.015)</td>
<td>1.17</td>
<td>1.2*</td>
</tr>
<tr>
<td>Quisqualic acid</td>
<td>0.8 (0.15)</td>
<td>13.2</td>
<td>110*</td>
</tr>
<tr>
<td>N-methyl-DL-aspartic acid</td>
<td>8.5 (1.25)</td>
<td>34</td>
<td>640†</td>
</tr>
</tbody>
</table>

The neurotoxic doses shown above produced a significant reduction in the activity of choline acetyl-transferase and glutamate decarboxylase in the rat after intra-striatal injections (*taken from Zaczek and Coyle, 1982; †taken from Coyle, 1981). The hypermotility threshold dose produced increases in locomotor activity significantly greater than controls. The seizure-like behavior threshold dose for kainic acid produced tonic/clonic convulsive episodes while the seizure-like behavior threshold dose for N-methyl-DL-aspartic acid produced immediate and continuous motor seizures with no ambulatory movement. Quisqualic acid produced seizure-like behavior that was different from the convulsions produced by either kainic acid or N-methyl-DL-aspartic acid. This behavior, previously termed "praying" (Zaczek and Coyle, 1982) consisted of persistent rearing with the forepaws apposed. The numbers in parentheses refer to the dose (μg) injected on each side of the nucleus accumbens.
Fig. 9 Effect of reserpine (RES) on kainic acid (KA)-induced hypermotility. Rats were pretreated with RES (5 mg/kg, i.p.) 4 hr prior to the intra-accumbens administration of KA (31 ng/0.5 μl) or saline (SAL, 0.5 μl). Each bar represents the mean and SEM for the number of observations indicated on the top of each bar. *P < 0.05 with respect to SAL. **P < 0.05 with respect to KA alone.
Fig. 9
C. Effect of Neuroleptics on Hypermotility in the Rat Induced by KA, QA and NMA

In order to determine the effects of blockade of dopamine receptors on the hypermotility induced by KA or QA, rats were pretreated with haloperidol (0.8 mg/kg, i.p.) 30 min prior to the administration of these excitants into the nucleus accumbens (Table 4). Haloperidol, which markedly decreased the locomotor activity of control animals, inhibited the effect of KA, 31 and 125 ng, by 84 and 75% respectively. In contrast, haloperidol did not block the convulsive effects of KA (500 ng) (data not shown). Pretreatment with haloperidol also decreased hypermotility induced by QA (2.5 μg) by 75%.

In order to determine whether all three of these excitatory amino acids act through the stimulation of dopamine receptors within the nucleus accumbens, fluphenazine, a potent dopamine receptor blocking agent, was injected directly into this nucleus along with each of the excitants (Fig. 10). Fluphenazine decreased the hypermotility induced by KA (31 ng), QA (0.31 μg) and NMA (2.5 μg) by 75, 82 and 74% respectively and decreased the locomotor activity of control animals by 74%.

D. Effect of Endogenous Excitatory Amino Acids, Other Endogenous Compounds and D-Glutamic Acid on Locomotor Activity in the Rat

L-GLU (up to 200 μg), injected bilaterally into the nucleus accumbens, did not produce an increase in locomotion; however,
TABLE 4. Effects Of Pretreatment With Haloperidol On
Hypermotility Induced by KA and QA

<table>
<thead>
<tr>
<th>KA (ng/0.5 µl)</th>
<th>Pretreatment</th>
<th>N</th>
<th>Motility/hour (Mean ± SEM)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Saline 0.5 µl)</td>
<td>-</td>
<td>10</td>
<td>245 ± 49</td>
<td>-</td>
</tr>
<tr>
<td>0 (Saline 0.5 µl)</td>
<td>Haloperidol</td>
<td>5</td>
<td>48 ± 14*</td>
<td>80</td>
</tr>
<tr>
<td>31</td>
<td>-</td>
<td>8</td>
<td>4612 ± 875*</td>
<td>-</td>
</tr>
<tr>
<td>31</td>
<td>Haloperidol</td>
<td>4</td>
<td>751 ± 210**</td>
<td>84</td>
</tr>
<tr>
<td>125</td>
<td>-</td>
<td>4</td>
<td>8707 ± 1900*</td>
<td>-</td>
</tr>
<tr>
<td>125</td>
<td>Haloperidol</td>
<td>4</td>
<td>2210 ± 856**</td>
<td>75</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>QA (µg/0.5 µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Vehicle 0.5 µl)</td>
</tr>
<tr>
<td>0 (Vehicle 0.5 µl)</td>
</tr>
<tr>
<td>2.5</td>
</tr>
<tr>
<td>2.5</td>
</tr>
</tbody>
</table>

Rats were pretreated with haloperidol (0.8 mg/kg, i.p.) 30 min prior to the administration of saline, kainic acid (KA), vehicle (phosphate buffer) or quisqualic acid (QA) into the nucleus accumbens. There was no difference in motility between saline-or phosphate buffer-treated animals. N = number of observations. Percentage inhibitions were calculated as described in the Methods section. *P < 0.05 with respect to saline or vehicle. **P < 0.05 with respect to KA or QA alone.
Fig. 10 Effect of fluphenazine (FLU) on hypermotility induced by excitatory amino acids. Rats were injected with FLU into the nucleus accumbens (2.5 μg/μl) alone or simultaneously with kainic (KA; 31 ng/0.5 μl), quisqualic (QA; 0.31 μg/0.5 μl) or N-methyl-aspartic acids, (NMA; 2.5 μg/0.5 μl). Motility was recorded for 1 hr. Each bar represents the mean and SEM for the number of observations indicated on the top of each bar. *P < 0.05 with respect to saline (SAL). **P < 0.05 with respect to KA, QA or NMA alone.
Fig. 10
both D-GLU acid (100 μg) and L-ASP (200 μg) significantly enhanced locomotor activity (Fig. 11). Combining both the D- and L-isomers of GLU (100 μg each) did not increase motility above that seen with D-GLU alone (Fig. 11); however, combining L-GLU (50 μg) with β-hydroxy-ASP (10 μg), a specific inhibitor of high affinity L-GLU uptake, did produce a slight but significant increase in locomotor activity (Table 5).

Pretreating rats with haloperidol (0.8 mg/kg, i.p.) for 30 min produced an 84% inhibition of the stimulatory effect of D-GLU on locomotor function (Fig. 12), indicating a mechanism of action similar to that of the more potent excitatory amino acid analogs, KA, QA and NMA.

Other endogenous compounds such as quinolinic acid (10 μg), γ-L-glutamylglycine (10 μg) and folic acid (5 μg), but not L-pyroglutamic acid (50 μg), produced a significant increase in locomotor activity after bilateral injection into the nucleus accumbens (Table 5).

II.4 DISCUSSION

The excitatory amino acid receptor agonists, KA, QA and NMA have been used to classify three types of receptors for excitatory amino acid in the mammalian central nervous system (Watkins, 1981; McLennan et al., 1981). The effects of these excitants were studied after administration into the nucleus accumbens. The results indicate that all three compounds, when injected bilaterally into the nucleus
Fig. 11 Effect of glutamic acid (GLU) and aspartic acid (ASP) on locomotor activity in the rat. Saline (SAL; 0.5 μl), L-GLU, D-GLU, D-GLU + L-GLU and L-ASP were injected bilaterally into the nucleus accumbens and motility was recorded for 1 hr. Each bar represents the mean and SEM for the number of observations indicated on the top of each bar. *P < 0.05 with respect to saline.
Fig. 11
### TABLE 5. Effects Of Endogenous Compounds on Locomotor Activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Motility/Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Vehicle 0.5 µl)</td>
<td>5</td>
<td>171 ± 48</td>
</tr>
<tr>
<td>L-Pyroglutamic acid (50 µl)</td>
<td>3</td>
<td>134 ± 21</td>
</tr>
<tr>
<td>Quinolinic acid (10 µg)</td>
<td>4</td>
<td>2635 ± 500*</td>
</tr>
<tr>
<td>L-GLU (50 µg) + β-OH-ASP (10 µg)</td>
<td>3</td>
<td>278 ± 13*</td>
</tr>
<tr>
<td>γ-L-Glutamylglycine (10 µg)</td>
<td>4</td>
<td>711 ± 177*</td>
</tr>
<tr>
<td>Folic Acid (5 µg)</td>
<td>3</td>
<td>6724 ± 1442*</td>
</tr>
</tbody>
</table>

Rats were injected bilaterally in the nucleus accumbens with vehicle (phosphate buffer) or one of the above endogenous compounds in a 0.5 µl vol. and placed in motor activity cages for 1 hr. N = number of observations. *P < 0.05 with respect to vehicle.
Fig. 12 Effect of haloperidol (HAL) on D-glutamic acid (D-GLU)-induced hypermotility. Rats were pretreated with HAL (0.8 mg/kg, i.p.) 30 min prior to the intracaccumbens administration of saline (SAL, 1 μl) or D_GLU (100 μg/μl). Motility was recorded for 1 hr. Each bar represents the mean and SEM for the number of observations indicated on the top of each bar. *P < 0.05 with respect to SAL. **P < 0.05 with respect to D-GLU alone.
Fig. 12

MOTILITY/HOUR

SAL  SAL + HAL  D-GLU  D-GLU + HAL

200  5  *  1500  10  **

★ ★
accumbs in small doses, produced an increase in locomotor activity. In larger doses, these acidic amino acids produced seizure-like behavior which interfered with or abolished motility. An increase in locomotor activity was also produced by D-GLU and L-ASP. The effects of the excitatory amino acids on locomotor activity appear to be mediated through mesolimbic dopaminergic mechanisms. This concept is supported by the observation that the excitatory amino acids, like dopamine (Costall and Naylor, 1975; Pijnenburg et al., 1976), elicited hypermotility after injection into the nucleus accumbens and olfactory tubercles but not the striatum. In addition, the activity response was antagonized by drugs that impair dopaminergic neurotransmission.

It is difficult to determine which type of excitatory amino acid receptor in the nucleus accumbens was responsible for the stimulation of locomotor activity. Studies on mammalian spinal cord suggest that each of the three excitatory amino acids can act as a partial agonist on other types of reception for excitatory amino acid (Watkins, 1981). In particular, the effects of KA and QA may overlap. Nevertheless, of the excitatory amino acids tested, KA was the most potent in stimulating locomotor activity followed by QA and lastly, NMA. In addition, the maximum intensity of locomotor activity stimulation was much greater for both KA and QA than for NMA, although these differences might be due in part to the onset of seizures which interfere with locomotor activity. These results suggest that receptors in the nucleus accumbens, sensitive to KA and QA play a more important role in
in regulating locomotor activity than receptors for NMA. This hypothesis is being tested by investigating the effect of putative receptor antagonists on the stimulation of locomotor activity produced by these three "prototype" excitants.

The stimulation of locomotor activity produced by either of the three excitatory amino acids appears to be mediated by a dopaminergic mechanism. Thus, the stimulation of locomotor activity produced by KA was markedly inhibited by pretreatment with reserpine, which depletes stores of catecholamine. In addition, the systemic administration of haloperidol, which has been shown to inhibit the hypermotility induced by the administration of dopamine into the nucleus accumbens (Pijnenburg et al., 1976; Makanjuola et al., 1980), significantly attenuated the hypermotility produced by KA, QA and D-GLU. Consistent with the concept that normal motor function is regulated by mesolimbic dopaminergic systems, both reserpine and haloperidol also inhibited locomotor activity after the injection of vehicle into the accumbens. Similarly, a decrease in spontaneous locomotor activity has also been observed after the systemic administration of spiroperidol and thioridazine (Bentall and Herberg, 1980) and after destruction of dopamine nerve terminals in the nucleus accumbens following the administration of 6-hydroxydopamine (Iversen and Koob, 1977; Koob et al., 1978). This data is in agreement with a recent study showing that the systemic administration of either reserpine or flupenthixol could inhibit the hyperactivity induced by AMPA (Arnt, 1981b), a proposed QA receptor agonist (Evans, 1981).
In order to determine whether dopamine receptors located in the nucleus accumbens mediate the hypermotility induced by the excitatory amino acids, the dopamine receptor blocking agent, fluphenazine, was injected directly into the nucleus accumbens together with the excitatory amino acids. Similar to the effect produced by systemically-administered haloperidol, intra-accumbens injections of fluphenazine, at a dose that blocked dopamine-induced hypermotility (Costall et al., 1979), antagonized the hypermotility produced by KA, QA and NMA. These observations suggest that the stimulation of dopamine receptors within the nucleus accumbens, itself, is involved in the stimulatory effects of excitatory amino acids on locomotor activity. This hypothesis is consistent with in vitro experiments showing that several excitatory amino acids can cause the release of \(^{3}\text{H}\)dopamine from slices obtained from both the striatum and nucleus accumbens (Giorguieff, Kemel and Glowinski, 1977; Roberts and Anderson, 1979).

Excitatory amino acids have been shown to be potent neurotoxins, predominantly causing the destruction of neuronal cell bodies and dendrites (Bird, 1981). However, it seems unlikely that the ability of the excitatory amino acids to produce an acute stimulation of locomotor activity is related to their neurotoxic effects, since: 1) the doses required for neurotoxicity are much greater than those which cause the stimulation of locomotor activity (Table 3); 2) the neurotoxic effects of excitatory amino acids are not readily apparent until several days to a week after intra-cranial injection (Coyle et al., 1977); 3) the
nucleus accumbens, particularly the medial portion, is relatively insensitive to the neurotoxic effects of KA after intra-cranial injection (Zaczek et al., 1980); 4) folic acid, a neuroexcitatory compound (Davies and Watkins, 1973) which does not produce local cell damage after intra-cranial injection (Olney et al., 1981), caused a marked stimulation of locomotor activity.

Large doses of the excitatory amino acids produced either tonic/clonic convulsive episodes (KA), continuous generalized convulsions (NMA), or seizure-like behavior (QA) termed "praying" (Zaczek and Coyle, 1982). Similar types of seizure activity have been observed after the administration of excitatory amino acids into other areas of the brain, particularly the hippocampus (Zaczek and Coyle, 1982). The seizures impaired locomotor activity, the effect being marked for KA. As shown in a previous study (Worms et al., 1981), pretreatment with haloperidol did not prevent the convulsive episodes seen with KA suggesting that the neuronal mechanisms involved in the production of convulsions are distinct from those causing the stimulation of locomotor activity.

The endogenous amino acid, L-GLU, after injection into the nucleus accumbens, did not produce an increase in locomotor activity. This observation is similar to the recent finding that L-GLU does not cause circling in the rat even though KA caused an intense circling response (Taylor, et al., 1981). The reason for the lack of
behavioral effects of L-GLU is unclear. D-GLU, which is also
an excitant of spinal neurons (Watkins, 1978), produced an increase in
motility in large doses. In this respect, D-GLU may be more effective
than L-GLU because it is not a substrate for the high affinity trans-
port mechanism and therefore it may not be as efficiently removed from
the extracellular space as is L-GLU (Currie and Kelly, 1981; Storm-
Mathisen, 1981). Alternatively, D-GLU may produce its effects by in-
creasing the concentration of endogenous L-GLU at synaptic sites,
possibly by inhibiting the uptake and/or metabolism of L-GLU (Balcar
and Johnston, 1973; Davidoff and Adair, 1975). According to this
hypothesis, the simultaneous application to the nucleus accumbens of
both D- and L-GLU should greatly enhance locomotor activity over that
seen with D-GLU alone. However, this was not observed, suggesting that
the effects of D-GLU are not mediated through the accumulation of
endogenous L-GLU.

L-ASP, which is similar to L-GLU in that it is capable of
stimulating all three types of receptors for excitatory amino acid,
also produced a hypermotility response. Since L-ASP and L-GLU have
similar kinetic properties for high and low affinity uptake sites
(Balcar and Johnston, 1973; Davidoff and Adair, 1975), it is unlikely
that the lack of effect of L-GLU on locomotor activity is due to the
rapid uptake and removal of this amino acid from the extracellular space.
In agreement with this concept is the finding that the intra-accumbens
injection of L-GLU, in combination with β-hydroxy-ASP, a high affinity
uptake inhibitor of L-GLU, produces only a slight enhancement of spontaneous locomotor activity.

Other endogenous neuroexcitatory compounds such as folic acid (Davies and Watkins, 1973) and quinolinic acid (Stone and Perkins, 1981) also produce a hypermotility response after bilateral intracaccumbens injection. In addition, \( \gamma \)-L-glutamylcysteine, an endogenously-identified dipeptide (Sano et al., 1966), also produced an increase in locomotion. These findings would suggest that some peptide or molecule containing L-GLU (or some related amino acid) may be the endogenous transmitter and not L-GLU itself.

There is little information available on the functional role of excitatory amino acids in the nucleus accumbens. Dopaminergic neurotransmission in this nucleus has been associated with the regulation of motor function under normal conditions (Kelly and Moore, 1976; Iversen and Koob, 1977; Koob et al., 1978) and after the administration of such drugs as neuroleptics (Pijnenburg et al., 1975a; Costall et al., 1979; Lai et al., 1981), amphetamine (Jackson et al., 1975; Pijnenburg et al., 1976; Costall et al., 1980) and apomorphine (Jackson et al., 1975; Pijnenburg et al., 1976; Costall et al., 1980; Van Ree and Wolterning, 1981). In addition, dopaminergic mechanisms in this area of the brain have been implicated in abnormal behavior associated with schizophrenia (Stevens, 1979; Mackay et al., 1980), Parkinson's disease (Price et al., 1978) and Huntington's chorea (Hayden, 1981).
Previous evidence suggests that excitatory amino acids in the nucleus accumbens may act as neurotransmitters or neuromodulators (Roberts and Anderson, 1979). The present results indicate that excitatory amino acids, by facilitating dopaminergic neurotransmission, can enhance the activity of dopamine receptors in the nucleus accumbens and, thus, may be involved in the regulation of both normal locomotor activity and higher mental functions.
CHAPTER III

ANTAGONISM OF THE HYPERMOTILITY RESPONSE INDUCED BY EXCITATORY AMINO ACIDS IN THE NUCLEUS ACCUMBENS

III.1 INTRODUCTION

Biochemical evidence suggests that excitatory amino acid-containing neurons are present in a number of limbic and basal ganglia structures, including the nucleus accumbens, a brain region which has been implicated in the initiation and regulation of locomotor activity (Mogenson and Yim, 1981). The nucleus accumbens appears to receive glutamatergic inputs from both frontal neocortex (Carter, 1980; Walaas, 1981) and allocortex (Walaas and Fonnum, 1979; Walaas, 1981) and may also contain glutamatergic/aspartatergic interneurons (Walaas and Fonnum, 1979). In addition, it has been shown to contain a high density of binding sites for KA (Monaghan and Cotman, 1982; Unnerstall and Wamsley, 1983), a conformationally restricted analog of GLU.

The nucleus accumbens also contains dopaminergic terminals of neurons originating in the ventral tegmental area (Lindvall and Bjorklund, 1978). Recent studies indicate that excitatory amino acids may play a role in the regulation of accumbal dopaminergic mechanisms.
Bilateral injections of dopamine (Pijnenburg and Van Rossum, 1973; Costall and Naylor, 1975; Jackson et al., 1975; Pijnenburg et al., 1975b; Pijnenburg et al., 1976; Makanjuola et al., 1980; Jones et al., 1981), as well as several excitatory amino acids (Arnt, 1981b; see Chapter II), into the nucleus accumbens produce an increase in locomotor activity which is blocked by dopamine receptor antagonists (Pijnenburg et al., 1975a; Jackson et al., 1975; Pijnenburg et al., 1976; Costall et al., 1979a,b; Makanjoula et al., 1980; Arnt, 1981b; see Chapter II). Consequently, it has been suggested that the hypermotility response produced by excitatory amino acids is occurring through the release of dopamine and subsequent stimulation of dopamine receptors within the nucleus accumbens (Arnt, 1981b; see Chapter II).

The excitatory amino acid receptors(s) in the nucleus accumbens responsible for mediating the increase in motility has not been identified. However, the hypermotility response can be produced by NMA, QA or KA (see Chapter II). Based on microiontophoretic studies of mammalian central neurons (Watkins, 1981; McLennan and Liu, 1982), these excitatory amino acids appear to produce their effects by activating three different receptors. Thus, it is possible that all three of these receptors could mediate the hypermotility response produced by the intra-accumbens administration of excitatory amino acids.

Excitatory amino acid antagonists have played an important role in the classification of multiple excitatory amino acid receptors.
within the CNS (Evans and Watkins, 1981; Davies et al., 1982; McLennan and Liu, 1982; McLennan et al., 1981). When many of these antagonists were tested in behavioral models of motor function, they either were ineffective in inhibiting the response to excitatory amino acids (Jenner et al., 1980; Arnt, 1981b) or they produced behavioral effects by themselves which were similar to those elicited by excitatory amino acids (Pycock and Dawbarn, 1980; Dawbarn and Pycock, 1981). However, a number of potential antagonists remain to be examined in these behavioral models. Therefore in the present study, we have evaluated several of these putative antagonists on excitatory amino acid-induced hypermotility when injected directly into the nucleus accumbens.

III.2 METHODS

A. Surgical Procedure

Male Sprague-Dawley rats weighing 175-200 g were used throughout this study. Injections into the nucleus accumbens were performed as described in Chapter II.

B. Monitoring Locomotor Activity

Locomotor activity was monitored as described in Chapter II.
C. **Drugs**

The following compounds were dissolved in 0.5 M Na$_2$HPO$_4$ (pH 7.4): D-α-aminoacidic acid, L-glutamic acid diethyl ester (Sigma, St. Louis, MO), D,L-2-amino-4-phosphonobutyric acid, D,L-α,ε-diaminopimelic acid (Calbiochem, La Jolla, CA), γ-D-glutamyglycine, D,L-cis-2,3-piperidinedicarboxylic acid, D,L-2-amino-5-phosphonovaleric acid (CRB, Cambridgeshire, UK).

D. **Statistics**

Data were expressed as the mean and standard error of the mean (SEM). Significant differences were evaluated using the non-parametric two-tailed Mann-Whitney U-Test with a level of P < 0.05 being considered significant. Since many of the compounds being evaluated as potential excitatory amino acid antagonists (e.g. diaminopimelic acid) produced significant changes in motility by themselves when compared to vehicle-treated controls, it was necessary to take this effect into account when determining the percent change produced by these agents on agonist (e.g. KA)-induced hypermotility. Therefore, the following equation was utilized for calculating this parameter:

$$\left[ \frac{(\text{AGONIST-CONTROL}) - ([\text{ANTAGONIST + AGONIST}] \div \text{ANTAGONIST})}{(\text{AGONIST - CONTROL})} \right] \times 100$$

E. **Histology**

After the experiments were completed, the animals were killed by decapitation and their brains rapidly removed and fixed in a
10% formalin solution for 48 hours. Frozen sections (80 μ thick) were then prepared using a Cryo-Cut Microtome (American Optical Corp., Buffalo, NY, USA) to verify the location of the injection sites (Fig. 13).

III.3 RESULTS

A. Effects of Excitatory Amino Acids on Locomotor Activity in the Rat

As shown previously (see Chapter II), NMA (1.25 and 2.5 μg/0.5 μl), KA (15 ng/0.5 μl) and QA (0.5 μg/0.5 μl) produced a significant increase in motility after bilateral administration into the nucleus accumbens which lasted for at least 1 hour after injection (Fig. 14-16, Table 6). This increase in motility was not accompanied by other behavioral effects (e.g. convulsions, gnawing, sniffing or circling) except for an infrequent rearing response produced by QA.

B. Effects of D-Aminoadipic Acid, Diaminopimelic Acid and Glutamic Acid Diethyl Ester on Excitatory Amino Acid-Induced Hypermotility

Bilateral injections of D-aminoadipic acid (5 and 10 μg/0.5 μl) into the nucleus accumbens did not significantly alter locomotor activity compared to that produced by vehicle (Fig. 14). However, D-aminoadipic acid (5 and 10 μg) did produce a dose-dependent inhibition (37 and 68%, respectively) of NMA-induced hypermotility over the 1 hour test period which was significant at the 10 μg dose (Fig. 14). D-Aminoadipic acid also produced a slight decrease (36%) in KA-induced
Fig. 13 Photomicrograph showing bilateral needle tracks with tips located in the nucleus accumbens.
Fig. 14 Effect of D-aminoacidic acid (DAA) on excitatory amino acid-induced hypermotility. Rats were injected into the nucleus accumbens on each side with DAA (5 or 10 µg/0.5 µl) alone or in combination with NMA (2.5 µg/0.5 µl), KA (15 ng/0.5 µl) or QA (0.5 µg/0.5 µl). Motility was recorded for 1 hour, starting at 10 minutes after injection. Each bar represents the mean and SEM for the number of observations indicated in the parentheses. *P < 0.05 with respect to control. **P < 0.05 with respect to NMA, KA or QA alone.
Figure 14

D-α-AMINOADIPIC ACID

Motility/Hour

Control
DAA (5 μg)
DAA (10 μg)
NMA (2.5 μg)
NMA + DAA (5 μg)
NMA + DAA (10 μg)
KA (15 ng)
KA + DAA (5 μg)
KA + DAA (10 μg)
QA (0.5 μg)
QA + DAA (10 μg)

(18) (4) (15) (5) (6) (6) (5) (7) (7) (10)
Fig. 15 Effect of diaminopimelic acid (DAP) on excitatory amino acid-induced hypermotility. Rats were injected into the nucleus accumbens on each side with DAP (20 µg/0.5 µl) alone or in combination with NMA (2.5 µg/0.5 µl), KA (15 ng/0.5 µl) or QA (0.5 µg/0.5 µl). Motility was recorded for 1 hour, starting 10 minutes after injection. Each bar represents the mean and SEM for the number of observations indicated in the parenthesis. *P < 0.05 with respect to control. **P < 0.05 with respect to NMA, KA or QA alone.
D, L-α, E-DIAMINOPIMELIC ACID

Fig. 15
Fig. 16 Effect of glutamic acid diethyl ester (GDEE) on excitatory amino acid-induced hypermotility. Rats were injected into the nucleus accumbens on each side with GDEE (125 μg/0.5 μl) alone or in combination with NMA (2.5 μg/0.5 μl), KA (15 ng/0.5 μl) or QA (0.5 μg/0.5 μl). Motility was recorded for 1 hour, starting 10 minutes after injection. Each bar represents the mean and SEM for the number of observations indicated in the parentheses. *P < 0.05 with respect to control. **P < 0.05 with respect to NMA, KA or QA alone.
Fig. 16
hypomotility which was not statistically significant. D-Aminoadipic acid had virtually no effect (9% inhibition) on QA-induced hypomotility.

Bilateral injections of diaminopimelic acid (20 μg/0.5 μl) into the nucleus accumbens produced a small but significant increase (64%) in locomotor activity when compared to that produced by vehicle (Fig. 15). No other behavioral changes were observed with this compound. Despite the significant increase in motility caused by diaminopimelic acid alone, a marked inhibition of both NMA (76%) and KA (90%)-induced hypomotility was produced by this compound (Fig. 15). In contrast, QA-induced hypomotility was unaffected by diaminopimelic acid.

Bilateral injections of glutamic acid diethyl ester (125 μg/0.5 μl) into the nucleus accumbens did not influence normal motor function (Fig. 16) nor did it produce any other apparent behavioral effects. This compound, however, produced a highly significant inhibition of both QA (81%)- and KA (70%)-induced hypomotility while the increase in motility due to NMA was not significantly affected (Fig. 16).

C. Effects of Other Putative Antagonists on Excitatory Amino Acid-Induced Hypomotility

The bilateral administration of γ-D-glutamylglycine (10 μg/0.5 μl), 2-amino-4-phosphonobutyric acid (25 μg/0.5 μl), 2-amino-5-phosphonovaleric acid (5 μg/0.5 μl) or cis-2,3-piperidine dicarboxylic acid (2.5 μg/0.5 μl) into the nucleus accumbens produced significant
increases in locomotor activity which lasted for at least 1 hour after injection (Table 6). No other behavioral effects of these compounds were observed when used alone at the above doses.

The activity produced by these putative antagonists when administered together with an agonist was never less than that produced by the administration of the agonist alone (Table 6). Unexpectedly, γ-D-glutamylglycine markedly enhanced the locomotor activity produced by both NMA and KA. The hypermotility response elicited by the combination of γ-D-glutamylglycine and KA was similar to the sum of the responses produced by KA and γ-D-glutamylglycine alone. On the other hand, the hypermotility response produced by the combination of γ-D-glutamylglycine and NMA was much greater than that produced by NMA and γ-D-glutamylglycine alone, suggesting a synergistic action between these latter two compounds. The combination of 2-amino-5-phosphonovaleric acid and NMA also produced a statistically significant increase in locomotor activity over that seen with NMA alone.

2-Amino-4-phosphonobutyric acid and cis-2,3-piperidine dicarboxylic acid did not significantly change the hypermotility response induced by NMA or KA. Higher doses of either 2-amino-4-phosphonobutyric acid (50 µg) or cis-2,3-piperidine dicarboxylic acid (5 µg) were not tested for antagonistic activity since both of these compounds at higher doses precipitated convulsive episodes. cis-2,3-Piperidine dicarboxylic acid (2.5 µg) produced variable responses either when used alone or in combination with excitatory amino acids.
## TABLE 6. Effects of Putative Excitatory Amino Acid Antagonists on NMA- and KA-Induced Hypermotility

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Alone</th>
<th>+NMA</th>
<th>+KA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>155 ± 37(8)</td>
<td>513 ± 26(4)</td>
<td>1885 ± 81(4)</td>
</tr>
<tr>
<td>DGG (10 µg)</td>
<td>2161 ± 315(4)*</td>
<td>4212 ± 417(5)**</td>
<td>4944 ± 877(4)**</td>
</tr>
<tr>
<td>Control</td>
<td>156 ± 38(5)</td>
<td>580 ± 46(5)</td>
<td>-</td>
</tr>
<tr>
<td>APV (5 µg)</td>
<td>5277 ± 1112(4)*</td>
<td>3549 ± 461(4)**</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>152 ± 33(9)</td>
<td>5155 ± 883(6)b</td>
<td>2251 ± 251(6)</td>
</tr>
<tr>
<td>PDA (2.5 µg)</td>
<td>873 ± 306(7)*</td>
<td>6147 ± 2304(6)</td>
<td>1979 ± 638(6)</td>
</tr>
<tr>
<td>Control</td>
<td>152 ± 36(7)</td>
<td>3749 ± 613(5)b</td>
<td>3468 ± 585(5)</td>
</tr>
<tr>
<td>APB (25 µg)</td>
<td>734 ± 191(5)*</td>
<td>5196 ± 1144(4)</td>
<td>3188 ± 854(6)</td>
</tr>
</tbody>
</table>

Rats were injected into the nucleus accumbens on each side with NMA (1.25a or 2.5b µg/0.5 µl) or KA (15 ng/0.5 µl) alone or in combination with one of the following putative excitatory amino acid antagonists: γ-D-glutamylglycine (DGG, 10 µg/0.5 µl); 2-amino-5-phosphonovaleric acid (APV, 5 µg/0.5 µl); 2-amino-4-phosphonobutyric acid (APB, 25 µg/0.5 µl) cis-2,3-piperidine dicarboxylic acid (PDA, 2.5 µg/0.5 µl); 2-amino-4-phosphonobutyric acid (APB; 25 µg/0.5 µl). Motility was recorded for 1 hour, starting 10 minutes after injection. Values represent the mean ± SEM for the number of observations shown in parentheses. *P < 0.05 with respect to control. **P < 0.05 with respect to NMA or KA alone.
III.4 DISCUSSION

The present study demonstrates that the putative excitatory amino acid antagonists, diaminopimelic acid, D-aminoadipic acid and glutamic acid diethyl ester were able to antagonize the hypermotility responses induced by certain excitatory amino acids after bilateral injection into the rat nucleus accumbens (Table 7). Diaminopimelic acid markedly decreased NMA- and KA-induced hypermotility at a dose that had no effect on QA-induced hypermotility. D-Aminoacidipic acid significantly inhibited the hypermotility response induced by NMA but not that induced by KA or QA. In contrast to D-aminoacidipic acid, glutamic acid diethyl ester inhibited the increase in motility produced by KA and QA but not that produced by NMA. These results suggest that the hypermotility response induced by the various excitatory amino acids is mediated through the stimulation of multiple excitatory amino acid receptors located within the nucleus accumbens.

Based on the relative sensitivity of NMA, KA and QA to a variety of organic antagonists when applied microiontophoretically onto amphibian and mammalian CNS neurons, at least three types of excitatory amino acid receptors have been identified:

1) NMA receptors which are selectively blocked by a number of compounds including D-aminoacidipic acid, diaminopimelic acid and 2-amino-5-phosphonovaleric acid, 2) KA receptors which are blocked, along with NMA receptors, by γ-D-glutamylglycine and 2-amino-4-phosphonobutyric acid and 3) QA receptors which are weakly but selectively antagonized...
TABLE 7. Summary of the Effects of Antagonists on Excitatory Amino Acid-Induced Hypermotility

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>NMA (16.9 nmoles)</th>
<th>KA (0.07 nmoles)</th>
<th>QA (2.6 nmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAA (62 nmoles)</td>
<td>+ (68%)</td>
<td>s1. + (36%)</td>
<td>0</td>
</tr>
<tr>
<td>DAP (100 nmoles)</td>
<td>+ (76%)</td>
<td>+ (90%)</td>
<td>0</td>
</tr>
<tr>
<td>GDEE (500 nmoles)</td>
<td>0</td>
<td>+ (70%)</td>
<td>+ (81%)</td>
</tr>
</tbody>
</table>

D-α-Amino adipic acid (DAA), D,L-α,ε-diaminopimelic acid (DAP) and L-glutamic acid diethyl ester (GDEE) all produced a significant inhibition of the hypermotility response elicited by one or more of the excitatory amino acids shown above. Percent inhibitions are shown in parentheses and were calculated as described in the Methods. + = decrease, 0 = no change, s1. + = slight decrease which was not statistically significant. Doses represent the amount injected on each side of the nucleus accumbens.
by glutamic acid diethyl ester (Watkins, 1981; McLennan and Liu, 1982). When applied to the nucleus accumbens, many of these putative antagonists produced an increase in locomotor activity. However, three of these compounds, diaminopimelic acid, D-amino adipic acid and glutamic acid diethyl ester produced a pattern of inhibition similar to that reported in microiontophoretic studies. Diaminopimelic acid, a relatively specific antagonist for NMA receptor agonists when applied onto spinal neurons (Biscoe et al., 1977a; Evans and Watkins, 1978; Evans et al., 1978; Biscoe et al., 1978), inhibited NMA-induced hypermotility when injected directly into the nucleus accumbens. The intra-accumbens administration of diaminopimelic acid also inhibited KA-induced hypermotility but did not significantly alter the hypermotility induced by QA. This suggests that in the nucleus accumbens, the action of KA and NMA may either be mediated through the same receptor mechanism or through different receptors, which are both blocked by diaminopimelic acid.

In microiontophoretic studies, glutamic acid diethyl ester is a weak antagonist of QA-induced responses (McLennan and Lodge 1979; Davies and Watkins, 1979). The present study also shows that this compound is a weak antagonist of QA-induced responses when injected into the nucleus accumbens. This weak antagonist effect may explain why the relatively low dose of glutamic acid diethyl ester used by Arnt (1981b) did not inhibit the hypermotility response produced by AMPA, a proposed QA receptor agonist (Evans, 1981). In the present study, however, a much higher dose of glutamic acid diethyl ester significantly decreased QA-induced hypermotility while having no effect on NMA-induced responses. In contrast to microiontophoretic studies, glutamic acid diethyl ester
also decreased KA-induced effects. These data support the results with
diaminopimelic acid suggesting that at least two different excitatory
amino acid receptors are present in the nucleus accumbens, one
activated by QA and the other by NMA. Since the effects of KA were
also blocked by glutamic acid diethyl ester, no conclusion can be drawn
at the present time as to whether this agonist stimulates QA receptors
or is activating a third excitatory amino acid receptor which is also
susceptible to glutamic acid diethyl ester antagonism.

The selective antagonist effects produced by D-amino adipic
acid in the nucleus accumbens are identical to those reported in micro-
iontophoretic studies (Biscoe et al., 1977a,b; Evans and Watkins, 1978;
Evans et al., 1978; Lodge et al., 1978; Hicks et al., 1978; Biscoe
et al., 1978; Davies and Watkins, 1979; Collingridge and Davies,
1979; McLennan and Lodge, 1979; McLennan and Liu, 1982). This compound
markedly inhibited NMA-induced hypermotility while having no inhibitory
effect on QA-induced responses. This finding further emphasizes a
distinction between NMA- and QA-receptor mediated effects. Also as
reported in microiontophoretic studies (Biscoe et al., 1977a; Lodge
et al., 1978; Collingridge and Davies, 1979), KA-induced effects are
inhibited to a small extent by D-amino adipic acid, suggesting that the
response to KA may be partially mediated through the stimulation of NMA
receptors. In summary, the above results suggest that in the nucleus
accumbens, at least two receptor types exist for excitatory amino
acids (NMA and QA receptors) which, when stimulated, produce a marked
increase in locomotor activity. In addition, the hypermotility produced
by KA may be caused by the activation of both NMA and QA receptors. Conceivably, this activation could be mediated indirectly through the release of ASP and GLU (Perkany and Coyle, 1983), two endogenous neuroexcitants which preferentially stimulated NMA and QA receptors, respectively (Davies et al., 1980).

Although diaminopimelic acid, D-aminoadipic acid and glutamic acid diethyl ester appear to act as specific excitatory amino acid antagonists in the nucleus accumbens, other explanations could account for their inhibitory effects. These compounds could be acting as dopamine receptor antagonists which have been shown to decrease excitatory amino acid-induced hypermotility after bilateral injection into the nucleus accumbens (see Chapter II). However, this possibility seems unlikely since dopaminergic antagonists, in contrast to the excitatory amino acid antagonists, also produce a significant decrease in spontaneous locomotor activity after intra-accumbens injection (see Chapter II). It is also conceivable that these antagonists act like γ-aminobutyric acid which has been shown to decrease dopamine-induced hypermotility after bilateral injection into the nucleus accumbens (Pycock and Horton, 1979; Kuruvilla and Uretsky, 1981). This mechanism of action also appears unlikely since γ-aminobutyric acid, but not the excitatory amino acid antagonists, decreased spontaneous locomotor activity at doses which are believed to stimulated post-synaptic γ-aminobutyric acid receptors in the nucleus accumbens (Wachtel and Anden, 1978; Jones et al., 1981). Finally, it has been
shown that glutamic acid diethyl ester possesses anticholinergic properties when applied microiontophoretically onto thalamic and spinal neurons (Hicks et al., 1978; Davies and Watkins, 1979; McLennan and Lodge, 1979; Lekic and Padjen, 1981). At the present time this mechanism cannot be ruled out.

A variety of other putative excitatory amino acid antagonists were tested in this behavioral model. γ-D-Glutamylglycine, 2-amino-4-phosphonobutyric acid, 2-amino-5-phosphonovaleric acid and cis-2,3-piperidine dicarboxylic acid did not decrease excitatory amino acid-induced hypermotility. In contrast, γ-D-glutamylglycine unexpectedly produced an additive effect on KA-induced hypermotility and appeared to produce a synergistic effect on NMA-induced hypermotility.

The excitatory amino acid antagonists, diaminopimelic acid, 2-amino-4-phosphonobutyric acid, cis-2,3-piperidine dicarboxylic acid and particularly 2-amino-5-phosphonovaleric acid and γ-D-glutamylglycine increased spontaneous locomotor activity. These results are similar to those reported by Dawbarn and Pycock (1981) who showed that γ-D-glutamylglycine and 2-amino-5-phosphonovaleric acid produced a dose-dependent increase in locomotor activity after bilateral injections into the ventral tegmental area and substantia nigra, pars compacta. The mechanisms(s) responsible for this behavioral response is not clear although it is possible that this effect is mediated through dopaminergic mechanisms since fluphenazine, a dopamine receptor antagonists,
inhibited the increase in locomotor activity produced by \( \gamma \)-D-glutamylglycine and 2-amino-5-phosphonovaleric acid (Dawbarn and Pycock, 1981). It is also possible that diaminopimelic acid, which was shown to possess antagonistic activity in the present study, may be acting as a weak partial agonist on excitatory amino acid receptors to produce an increase in locomotor activity. In contrast to effects produced by nucleus accumbens injections, the intrastriatal injection of \( \gamma \)-D-glutamylglycine and 2-amino-5-phosphonovaleric acid, as well as a variety of other putative antagonists which were ineffective in inhibiting the rotational response induced by KA, elicited no behavioral effects when administered alone (Jenner et al., 1980).

The nucleus accumbens has been described as a functional interface between limbic and motor systems (Mogenson and Yim, 1981). It has been implicated in the initiation and regulation of normal motor function (Mogenson and Yim, 1981) and in pathological states such as schizophrenia (Stevens, 1979; Mackay et al., 1980; Matthysse, 1981; Chronister and DeFrance, 1982), Parkinson's disease (Price et al., 1978) and Huntington's chorea (Hayden, 1981; Bots and Bruyn, 1981). This nucleus receives a dopaminergic input from the ventral tegmental area (Lindvall and Bjorklund, 1978) and a glutamatergic input from both frontal neocortex (Carter, 1980; Walaas, 1981) and allocortex (Walaas and Fonnum, 1979; Walaas, 1981) and may also contain excitatory amino acid interneurons (Walaas and Fonnum, 1979). Evidence suggests that the dopaminergic input into this brain region is involved in the control of both spontaneous locomotor activity (Kelly and Moore, 1976;
Iversen and Koob, 1977; Koob et al., 1978; Makanjuola and Ashcroft, 1982) and drug-induced motor changes (Kelly, 1977; Costall and Naylor, 1979). However, the role of excitatory amino acids in motor function has not been as thoroughly investigated. Recent behavioral studies have shown that several of these neuroexcitants, when injected bilaterally into the nucleus accumbens, produce a marked increase in locomotor activity which appears to be mediated through the release of dopamine and subsequent stimulation of dopamine receptors (Arnt, 1981b; see Chapter II). These findings are supported by in vitro studies which show that L-GLU can stimulate the release of dopamine from accumbal slices (Roberts and Anderson, 1979; Marien et al., 1983). This effect of GLU was inhibited by glutamic acid diethyl ester (Marien et al., 1983). However, for reasons still unknown, L-GLU does not affect spontaneous motor function when injected directly into the nucleus accumbens even though in the same system, L-ASP did produce a weak hypermotility response (see Chapter II). It may be that a larger molecule which contains GLU (e.g. folic acid) or a dipeptide (e.g. N-acetyl-aspartylglutamic acid) is the actual endogenous excitatory neurotransmitter and not GLU itself (Ruck et al., 1980; Zaczek et al., 1983). Since none of the proposed excitatory amino acid antagonists decreased spontaneous locomotor activity, the excitatory amino acid input into the nucleus accumbens does not appear to be of a tonic nature. Thus, the excitatory amino acid-containing neurons in the nucleus accumbens may function as regulators of dopaminergic neurotransmission in this brain region.
CHAPTER IV

MAGNESIUM SELECTIVELY INHIBITS N-METHYL-ASPARTIC ACID-INDUCED
HYPERMOTILITY AFTER INTRA-ACCUMBENS INJECTION

IV.1 INTRODUCTION

The nucleus accumbens is a forebrain structure which has
been postulated to be involved in both psychotic disorders (Chronister
and DeFrance, 1982; Matthysse, 1980) and in the control of motor func-
tion (Mogenson and Yim, 1980; Palmer and Chronister, 1980). This region
is innervated by an ascending dopaminergic neuronal pathway originating
in the ventral tegmental area (Fonnum and Walaas, 1980). The bilateral
injection of dopamine directly into the nucleus accumbens produced an
increase in motility which is blocked by dopamine receptor antagonists
(Palmer and Chronister, 1980). The nucleus accumbens is also believed
to be innervated by glutamatergic neuronal pathways derived from both
allocortex and frontal neocortex and may also contain glutamatergic/
aspartatergic interneurons (Fonnum and Walaas, 1980). Recent studies
have shown that the bilateral injection of GLU analogs into the
nucleus accumbens produces, like dopamine, a hypermotility response
which can be blocked by dopamine receptor antagonists (Arnt, 1981; see
Chapter II). This suggests that excitatory amino acid-induced hyper-
motility is mediated through the release of dopamine and subsequent
stimulation of dopamine receptors within the nucleus accumbens.

NMA, KA and QA have been previously shown to cause the excitation of single neurons in the vertebrate CNS (McLennan et al., 1981; Watkins et al., 1981). The electrophysiological responses to these compounds appear to be mediated by different mechanisms, since they are preferentially inhibited by different antagonists (Davies et al., 1979; McLennan et al., 1981; Watkins et al., 1981). NMA, KA and QA can produce a marked stimulation of locomotor activity after their injection into the nucleus accumbens (see Chapter II). However, it is not clear whether these neuroexcitants act by the same or different mechanisms at this brain site. Numerous organic antagonists of excitatory amino acids, which are effective in microiontophoretic studies, have not been found to be useful in defining the effects of excitatory amino acids on motor function since these antagonists have been shown to be either ineffective in inhibiting the behavioral responses to excitatory amino acids (Arnt, 1981b; Jenner et al., 1980) or to produce motor effects by themselves which are similar to those elicited by excitatory amino acids (Dawbarn and Pycock, 1981; Pycock and Dawbarn, 1980).

In isolated spinal cord preparations, magnesium has been shown to selectively depress the excitatory responses of frog motor-neurons to NMA while having little or not effect on responses to KA or QA (Ault et al., 1980; Evans et al., 1977; Evans and Watkins, 1978). Similar responses were also obtained in vivo in the cat spinal cord
(Davies and Watkins, 1977). This observation provided the first evidence
to suggest that more than one type of excitatory amino acid receptor
may exist in the vertebrate CNS. In the present study, we report
that the intra-accumbens administration of magnesium inhibits the
hypermotility response induced by NMA but had no effect on control
locomotor activity or on the hypermotility responses induced by KA
and QA.

IV.2 METHODS

A. Surgical Procedure

Male Sprague-Dawley rats weighing 175-200 g were used
throughout this study. Injections into the nucleus accumbens were
performed as described in Chapter II.

B. Monitoring Locomotor Activity

Locomotor activity was monitored as described in Chapter
II.

C. Drugs

MgCl₂, MgSO₄ and CaCl₂ were dissolved in normal saline.

D. Statistics

Data were expressed as the mean and standard error of
the mean (SEM). Significant differences were evaluated using the
two-tailed Mann-Whitney U-test, with a level of $P < 0.05$ being considered significant. Percent inhibitions were calculated as described in Chapter III.

E. Histology

Histological verification of the injection sites were performed as described in Chapter III.

IV.3 RESULTS

The intra-accumbens administration of NMA (16.9 moles), KA (0.07 nmoles) and QA (2.6 nmoles) produced an intense increase in locomotor activity when compared to saline-treated controls (Table 8; Fig. 17 and 18). The addition of MgSO$_4$ (7.5 and 15 nmoles) or MgCl$_2$ (15 nmoles) to the injection solutions did not produce a significant change in the locomotor activity of saline-treated rats (Table 8; Fig. 17) or in the hypermotility responses induced by KA (Table 8; Fig. 18) and QA (Fig. 18). In contrast, MgSO$_4$ (7.5 and 15 nmoles) produced a significant dose-dependent inhibition of NMA-induced hypermotility (48 and 75% inhibition, respectively; Table 8). The hypermotility response induced by NMA was also inhibited (90%) by MgCl$_2$ (15 nmoles), suggesting that the inhibitory effect was due to the positive magnesium ion and not the associated anion (e.g., sulfate ion; Fig. 17).

In order to determine whether calcium, a divalent cation like magnesium, possesses similar antagonistic activity, CaCl$_2$
TABLE 8. Effects of MgSO₄ on NMA- and KA-Induced Hypermotility

<table>
<thead>
<tr>
<th>Treatment</th>
<th>None</th>
<th>+ MgSO₄ 7.5 (nmoles)</th>
<th>+ MgSO₄ 15 (nmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0.5 µl)</td>
<td>180 ± 23(6)</td>
<td>256 ± 51(3)</td>
<td>154 ± 55(3)</td>
</tr>
<tr>
<td>NMA (16.9 nmoles)</td>
<td>4172 ± 570(4)*</td>
<td>2335 ± 502(8)**</td>
<td>1240 ± 389(5)**</td>
</tr>
<tr>
<td>KA (0.07 nmoles)</td>
<td>6369 ± 1197(4)*</td>
<td>5361 ± 1409(4)</td>
<td>7397 ± 1754(4)</td>
</tr>
</tbody>
</table>

Rats were injected bilaterally into the nucleus accumbens with NMA or KA alone or in combination with magnesium sulfate. Doses represent the amount injected on each side. Motility was recorded for 1 hour, starting 10 minutes after injection. Values represent the mean ± SEM for the number of observations shown in parentheses. *P < 0.05 with respect to control. **P < 0.05 with respect to NMA or KA alone.
Fig. 17 Effect of MgCl₂ and CaCl₂ on NMA-induced hypermotility. Rats were injected into the nucleus accumbens on each side with MgCl₂ (15 nmoles/0.5 μl) or CaCl₂ (15 nmoles/0.5 μl) alone or in combination with NMA (16.9 nmoles/0.5 μl). Motility was recorded for 1 hour, starting at 10 minutes after injection. Each bar represents the mean and SEM for the number of observations indicated in the parentheses. *P < 0.05 with respect to control. **P < 0.05 with respect to NMA alone.
Fig. 17

Motility/Hour

CONTROL  MgCl₂  CaCl₂  NMA  NMA + MgCl₂  NMA + CaCl₂

(5)  (3)  (3)  (5)  (4)  (5)
Fig. 18 Effect of MgCl₂ on KA- and QA-induced hypermotility. Rats were injected into the nucleus accumbens on each side with KA (0.07 nmoles/0.5 μl) or QA (2.6 nmoles/0.5 μl) alone or in combination with MgCl₂ (15 nmoles/0.5 μl). Motility was recorded for 1 hour, starting at 10 minutes after injection. Each bar represents the mean and SEM for the number of observations indicated in the parentheses. *P < 0.05 with respect to control (140 ± 25 counts/hour; N = 5).
Fig. 18

MOTILITY / HOUR

<table>
<thead>
<tr>
<th></th>
<th>KA</th>
<th>KA + MgCl₂</th>
<th>QA</th>
<th>QA + MgCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>3000</td>
<td>5000</td>
<td>1000</td>
<td>5000</td>
</tr>
</tbody>
</table>

Legend:
- * indicates significant difference
- (4) and (6) represent sample sizes
(15 nmoles) was injected into the nucleus accumbens alone or in combination with NMA. Fig. 17 shows that CaCl₂ produced a much weaker inhibitory effect (37%) than magnesium on NMA-induced hypermotility. Calcium, when added alone, did not change the response of saline-treated controls (Fig. 17).

IV. DISCUSSION

In studies on the isolated frog or rat spinal cord, low concentrations of magnesium (0.5 - 1 mM) were found to selectively inhibit motoneuron depolarization produced by NMA but not that produced by KA or QA (Ault et al., 1980; Evans et al., 1977; Evans and Watkins, 1978). Similar results were also obtained when magnesium was applied by microiontophoresis onto cat spinal neurons in vivo (Davies and Watkins, 1977). These findings suggested that at least two receptor types, a NMA magnesium-sensitive receptor and a non-NMA/magnesium-insensitive receptor, exist for excitatory amino acids in the vertebrate CNS.

In the present study, the intra-accumbens injection of magnesium inhibited NMA-induced hypermotility but not that produced by either QA or KA. In addition, the intra-accumbens injection of calcium was much less effective than magnesium in inhibiting the effects of NMA. These results are consistent with the spinal cord studies (Ault et al., 1980; Davies and Watkins, 1977; Evans et al., 1977; Evans and Watkins, 1978) and suggest that there are at least two receptor types for
excitatory amino acids in the nucleus accumbens, one of which is specifically sensitive to magnesium.

The mechanism by which magnesium produces its inhibitory action is unknown. The effect of magnesium on NMA-induced hypermotility does not appear to be caused by a direct chemical interaction between divalent cations and amino acids because: 1) the amount of interaction between magnesium and excitatory amino acids is small at physiological pH (Puil, 1981), 2) the inhibitory effect of magnesium on NMA-induced hypermotility is relatively selective since calcium produced a much weaker inhibition (Fig. 17), 3) the inhibitory effect of magnesium was selective for NMA, since it did not inhibit the effects of the other amino acids, KA and QA (Fig. 18). Based on previous studies on isolated frog spinal neurons (Davies et al., 1979), magnesium may act in a non-competitive manner to lower the affinity of the receptor for NMA or may act at the receptor-ionophore coupling process to reduce the effectiveness of the receptor-agonist interaction.

Recent behavioral data suggest that excitatory amino acids may produce their hypermotility response through the release of dopamine and subsequent stimulation of dopamine receptors within the nucleus accumbens (Arnt, 1981b; see Chapter II). These findings are supported by in vitro studies which show L-GLU, an endogenous neuroexcitant, to release dopamine from accumbal slices (Marien, 1983; Roberts and Anderson, 1979). This effect of GLU was inhibited by magnesium (Marien, 1983), suggesting a possible interaction at NMA receptors within the
nucleus accumbens. In the present studies, magnesium selectively inhibited the response produced by NMA but not that produced by QA and KA. Thus, in the nucleus accumbens, it appears that at least two excitatory amino acid receptor types (NMA/magnesium-sensitive and non-NMA/magnesium-insensitive receptors) are present which are capable of regulating dopaminergic neurotransmission. The finding that magnesium alone does not affect spontaneous locomotor activity suggests that NMA receptor stimulation in the nucleus accumbens does not function tonically to control dopamine-induced changes in motor function.
CHAPTER V

BIOCHEMICAL STUDIES ON THE ROLE OF EXCITATORY AMINO ACIDS IN THE NUCLEUS ACCUMBENS

V.1 INTRODUCTION

The nucleus accumbens receives a dense dopaminergic input from the ventral tegmental area (Lindvall and Bjorklund, 1978). These neurons are considered to be associated with locomotion (Mogenson and Yim, 1981) and higher mental functions (Stevens, 1979).

Bilateral injections of dopamine (Costall and Naylor, 1979), as well as several excitatory amino acids (Arnt, 1981b; see Chapter II), into the nucleus accumbens has been shown to produce a marked increase in locomotor activity. It has been suggested that excitatory amino acid-induced hypermotility is mediated through the release of dopamine and subsequent stimulation of dopamine receptors within the nucleus accumbens (Arnt, 1981b; see Chapter II). This hypothesis is consistent with recent biochemical studies which have shown that high concentrations of L-GLU produce an increase in the release of (H) dopamine from accumbal slices (Roberts and Anderson, 1979; Marien et al., 1983). The present study was undertaken to test this hypothesis in vivo by examining whether excitatory amino acids, at doses which produce hypermotility,
elicit an increase in dopamine turnover after bilateral administration into the nucleus accumbens. In addition, we have studied the effect of excitatory amino acids on the in vitro release of exogenously accumulated (3H)dopamine from slices of nucleus accumbens.

V.2 METHODS

A. Surgical Procedure

Male Sprague-Dawley rats weighing 175-200 g were used throughout this study. Bilateral injections into the nucleus accumbens were performed as described in Chapter II.

B. Measurement of Dopamine Turnover

The effect of excitatory amino acids on dopamine turnover in the nucleus accumbens in vivo was studied by measuring the extent of dopamine decline after the inhibition of its synthesis by a-methyl-p-tyrosine, a tyrosine hydroxylase inhibitor (see Anden et al., 1970). Rats were administered a-methyl-p-tyrosine (300 mg/kg, i.p.) 45 minutes prior to the intra-accumbens injection of KA (62 ng/0.5 μl), NMA (2.5 μg/0.5 μl) or QA (2.5 μg/0.5 μl). Seventy-five minutes later, the animals were decapitated, the nucleus accumbens removed and endogenous dopamine levels were determined spectrofluorimetrically.

The nucleus accumbens was dissected as described by Horn et al., (1974) and placed on dry ice until analyzed. Dopamine was extracted and isolated using Sephadex G-10 columns according to
the method of Earley and Leonard (1978). Briefly, the nucleus accumbens was weighed and homogenized in 1.0 ml of 0.4 N perchloric acid. Excess perchlorate was precipitated with 50 μl of KOH/HCOOH buffer. Each sample was centrifuged at 4800 x g for 20 minutes at 4°C and the supernatant was decanted onto Sephadex G-10 columns (7 x 40 mm). Dopamine was eluted from the column with 0.01 N HCl and 0.005 M Na2HPO4 and assayed spectrofluorimetrically according to the method of Chang (1964).

C. Measurement of (3H)Dopamine Release

The release of (3H)dopamine from preloaded accumbal slices was determined as described previously for striatal tissue (Liang et al., 1982; Ziance et al., 1972; Uretsky et al., 1975). In this procedure, the slices were incubated with (3H)dopamine (0.05 μM) for 20 minutes, washed extensively and then incubated with KA (1 mM), NMA (1 mM) and/or L-GLU (1 mM) for 15 minutes. The effect of the excitatory amino acids on the amount of (3H)dopamine in the incubation medium and tissue was determined. (3H)Dopamine in tissue and medium was separated from deaminated metabolites by Dowex 50 chromatography and the radioactivity in the appropriate eluates from the Dowex columns determined by liquid scintillation counting (Ziance et al., 1972). Release is expressed as (3H)dopamine in the medium as a percentage of the (3H)dopamine in tissue + medium (Liang et al., 1982; Ziance et al., 1972; Uretsky et al., 1975).

D. Statistics

Statistical comparisons in the turnover studies were performed using the two-tailed Students t-test while statistical analysis
of the release studies were performed using the one-tailed Mann-
Whitney U-test. A level of p < 0.05 was considered statistically
significant.

E. Drugs

α-Methyl-p-tyrosine methyl ester and amphetamine hydro-
chloride were purchased from Sigma Chemical Co., (St. Louis, MO).
(3H)Dopamine (30.4 Ci/m mole) was purchased from New England Nuclear
Co. (Boston, MA).

V.3 RESULTS

A. Effects of Haloperidol and Excitatory Amino Acids on
Dopamine Turnover in the Nucleus Accumbens

As shown in Fig. 19, a 2 hour pretreatment with α-methyl-
-p-tyrosine (300 mg/kg, i.p.) produced a significant decline (45%) in
endogenous dopamine levels in the nucleus accumbens. Haloperidol
(0.25 mg/kg, i.p.) alone did not produce a statistically significant
decrease in dopamine levels but did produce a significant enhancement
of α-methyl-p-tyrosine-induced dopamine decline when given 30 minutes
after the tyrosine hydroxylase inhibitor (Fig. 19). These findings are
similar to those reported previously (Anden et al., 1970; Palfreyman
et al., 1979; Hallman and Jonsson, 1979) and are indicative of an
increase in dopamine turnover (see Anden et al., 1970).
Fig. 19 Effect of Haloperidol (HAL) on endogenous dopamine levels in the nucleus accumbens after pretreatment with α-methyl-p-tyrosine (αMPT). Rats were pretreated with αMPT (300 mg/kg, i.p.) or saline (SAL) 30 minutes prior to the administration of HAL (0.25 mg/kg, i.p.) or SAL. Ninety minutes later, the rats were decapitated, the nucleus accumbens removed and dopamine levels were determined spectrofluorimetrically. Each bar represents the mean and SEM for the number of observations shown in the parentheses. *P < 0.05 with respect to SAL + SAL. **P < 0.05 with respect to αMPT + SAL.
Fig. 19
It is shown in Figs. 20-22 that the intra-accumbens injection of KA (62 ng/0.5 µl), NMA (2.5 µg/0.5 µl) or QA (2.5 µg/0.5 µl) do not affect control dopamine levels. Furthermore, none of these excitatory amino acids significantly enhanced the decline in dopamine levels produced by α-methyl-p-tyrosine. Thus, these compounds, at doses which enhance locomotion, do not appear to increase dopamine turnover in the nucleus accumbens as measured by this procedure.

B. Effects of Potassium, Amphetamine and Excitatory Amino Acids on [³H]Dopamine Release From Accumbal Slices

As shown in Fig. 23, high extracellular potassium levels (12.5 and 26 mM), as well as amphetamine (10⁻⁵M), produce a marked stimulation of release in [³H]dopamine from accumbal slices. Of the excitatory amino acids tested, L-GLU (1 mM) and NMA (1 mM) produced small but statistically significant increases in [³H]dopamine release (Figs. 24 and 25) while KA (1 mM) was ineffective (Fig. 25). The combination of L-GLU with KA did not significantly enhance the release of [³H]dopamine over that seen with L-GLU alone (Fig. 25).

Removal of magnesium, a potential endogenous excitatory amino acid antagonists, from the medium did not influence the above results obtained with the excitatory amino acids except for a very small but significant increase in [³H]dopamine release produced by KA (Figs. 26 and 27).
Fig. 20  Effect of NMA on endogenous dopamine levels in the nucleus accumbens after pretreatment with α-methyl-p-tyrosine (αMPT). Rats were pretreated with αMPT (300 mg/kg, i.p.) or saline (SAL) 45 minutes prior to the bilateral intra-accumbens injection of NMA (2.5 μg/0.5 μl) or SAL (0.5 μl). Seventy-five minutes later, the rats were decapitated, the nucleus accumbens removed and dopamine levels were determined spectrophotometrically. Each bar represents the mean and SEM for the number of observations shown in the parentheses. *P < 0.05 with respect to SAL + SAL.
DOPAMINE (µg/g tissue)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAL + SAL</td>
<td>(3)</td>
</tr>
<tr>
<td>SAL + NMA</td>
<td>(3)</td>
</tr>
<tr>
<td>αMPT + SAL</td>
<td>(4) *</td>
</tr>
<tr>
<td>αMPT + NMA</td>
<td>(3)</td>
</tr>
</tbody>
</table>

Fig. 20
Fig. 21 Effect of KA on endogenous dopamine levels in the nucleus accumbens after pretreatment with a-methyl-p-tyrosine (aMPT).
Rats were pretreated with aMPT (300 mg/kg, i.p.) or saline (SAL) 45 minutes prior to the bilateral intra-accumbens injection of KA (62 ng/0.5 µl) or SAL (0.5 µl). Seventy-five minutes later, the rats were decapitated, the nucleus accumbens removed and dopamine levels were determined spectrofluorimetrically. Each bar represents the mean and SEM for the number of observations shown in the parentheses. *P < 0.05 with respect to SAL + SAL.
Fig. 21

DOPAMINE (μg/g tissue)

- SAL + SAL
- SAL + KA
- αMPT + SAL
- αMPT + KA

(4) (3) (*) (4) (4)
Fig. 22 Effects of QA on endogenous dopamine levels in the nucleus accumbens after pretreatment with α-methyl-p-tyrosine (aMPT). Rats were pretreated with aMPT (300 mg/kg, i.p.) or saline (SAL) 45 minutes prior to the bilateral intra-accumbens injection of QA (2.5 μg/0.5 μl) or SAL (0.5 μl). Seventy-five minutes later, the rats were decapitated, the nucleus accumbens removed and dopamine levels were determined spectrofluorimetrically. Each bar represents the mean and SEM for the number of observations shown in the parentheses. *P < 0.05 with respect to SAL + SAL.
Fig. 22

DOPAMINE (μg/g tissue)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAL + SAL</td>
<td>5.0</td>
</tr>
<tr>
<td>SAL + QA</td>
<td>4.5</td>
</tr>
<tr>
<td>αMPT + SAL</td>
<td>2.0 *</td>
</tr>
<tr>
<td>αMPT + QA</td>
<td>1.5</td>
</tr>
</tbody>
</table>

(4) indicates the number of observations.
Fig. 23 Effect of potassium (K⁺) and amphetamine (AMP) on the release of ³H-dopamine (DA) from accumbal slices. Tissue slices of nucleus accumbens were incubated with ³H-DA (0.05 μM) for 20 minutes, washed extensively and then incubated in control medium or medium containing K⁺ (12.5 or 26 mM) or AMP (10⁻⁵M). The amount of ³H-DA released into the medium was determined as described in the Methods. Each bar represents the mean and SEM for the number of observations shown in the parentheses. *P < 0.05 with respect to control.
Fig. 23

% RELEASE

$^{3}$H-DA \(
\frac{\text{MEDIUM}}{\text{TISSUE} + \text{MEDIUM}}
\)

- Control
- K+ (12.5 mM)
- K+ (26.0 mM)
- AMP (10^-3 mM)

Values: 6, 4*, 3*
Fig. 24 Effect of NMA and L-GLU on the release of $^3$H-dopamine (DA) from accumbal slices. Tissue slices of the nucleus accumbens were incubated with $^3$H-DA (0.05 μM) for 20 minutes, washed extensively and then incubated in control medium or medium containing NMA (1 mM) or L-GLU (1 mM). The amount of $^3$H-DA released into the medium was determined as described in the Methods. Each bar represents the mean and SEM for the number of observations shown in the parentheses. *P < 0.05 with respect to control.
% RELEASE
\[ \frac{[^3]H-DA}{Tissue + Medium} \]

- L-GLU
- NMA
- Control

*(3)*

(3)
Fig. 25 Effect of KA and L-GLU on the release of $^3$H-dopamine (DA) from accumbal slices. Tissue slices of the nucleus accumbens were incubated with $^3$H-DA (0.05 μM) for 20 minutes, washed extensively and then incubated in control medium or medium containing KA (1 mM), L-GLU (1 mM) or KA + L-GLU (1 mM each). The amount of $^3$H-DA released into the medium was determined as described in the Methods. Each bar represents the mean and SEM for the number of observations shown in the parentheses. *P < 0.05 with respect to control.
% RELEASE

\[
\frac{3H-DA}{(MEDIUM + MEDIUM)}
\]

Control (5)

KA (1mM) (3)

L-GLU (1mM) (3)

KA + L-GLU (1mM each)

Fig. 25
Fig. 26 Effect of NMA and L-GLU on the release of $^3$H-dopamine (DA) from accumbal slices in a magnesium ($\text{Mg}^{2+}$) free medium. Tissue slices of the nucleus accumbens were incubated with $^3$H-DA (0.05 $\mu$M) for 20 minutes, washed extensively and then incubated in control medium ($\text{Mg}^{2+}$ free) or in medium containing NMA (1 mM), L-GLU (1 mM) or NMA + L-GLU (1 mM each). The amount of $^3$H-DA released into the medium was determined as described in the Methods. Each bar represents the mean and SEM for the number of observations shown in the parentheses. *P < 0.05 with respect to control.
Figure 26

% RELEASE

\[
\frac{\text{Mg}^{2+} \text{FREE MEDIUM}}{\text{TISSUE + MEDIUM}}
\]

- Control
- NMA (1mM)
- L-GLU (1mM)
- NMA + L-GLU (1mM each)

Fig. 26
Fig. 27 Effect of KA and L-GLU on the release of $^{3}\text{H}$-dopamine (DA) from accumbal slices in a magnesium (Mg$^{+2}$) free medium. Tissue slices of the nucleus accumbens were incubated with $^{3}\text{H}$-DA (0.05 μM) for 20 minutes, washed extensively and then incubated in control medium (Mg$^{+2}$ free) or in medium containing KA (1 mM), L-GLU (1 mM) or KA + L-GLU (1 mM each). The amount of $^{3}\text{H}$-DA released into the medium was determined as described in the Methods. Each bar represents the mean and SEM for the number of observations shown in the parentheses. *P < 0.05 with respect to control.
Fig. 27

% RELEASE

$^{3}H$-DA (MEDIUM) / (TISSUE + MEDIUM)

Mg$^{2+}$ FREE MEDIUM

<table>
<thead>
<tr>
<th></th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KA (1mM)</td>
<td>(5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-GLU (1mM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KA + L-GLU (1mM each)</td>
<td>(3)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* indicates significance.
Biochemical studies have shown that high doses of L-GLU can stimulate the release of \(^{3}\text{H}\)dopamine from accumbal slices (Roberts and Anderson, 1979; Marien et al., 1983; see data in this Chapter). The L-GLU-induced release of \(^{3}\text{H}\)dopamine appears to occur through direct presynaptic activation of excitatory amino acid receptors located on dopaminergic nerve terminals (Marien et al., 1983). The release of \(^{3}\text{H}\)dopamine by L-GLU would be consistent with the hypothesis that excitatory amino acids may exert their behavioral (e.g. hypermotility) effects through the release of accumbal dopamine. This hypothesis would explain why neuroleptics and reserpine inhibit the hypermotility response produced by excitatory amino acids after intra-accumbens injection. However, this mechanism of action is hard to reconcile with the observation that KA, the excitatory amino acid that is most potent in stimulating locomotor activity after intra-accumbens injection (Chapter II), produced only a small release (and only in magnesium free medium) of \(^{3}\text{H}\)dopamine from slices of nucleus accumbens (this Chapter). Furthermore, L-GLU, which did release \(^{3}\text{H}\)dopamine, was ineffective when tested behaviorally (Chapter II). Therefore, based on these in vitro studies, the release of accumbal dopamine does not appear to be a primary mechanism by which excitatory amino acids induce hypermotility.

A more direct way of studying the possible interaction between excitatory amino acids, dopamine and the hypermotility response is to inject the neuroexcitants directly into the nucleus accumbens, at doses
which have been shown to increase locomotor activity, and to observe whether changes in endogenous dopamine content or turnover occur in this nucleus. This type of experiment has been performed in the present study. The intra-accumbens injection of KA, NMA and QA, at doses which produced a marked increase in locomotor activity, did not affect either dopamine levels or turnover in the nucleus accumbens. Thus, these results, in agreement with in vitro studies, would suggest that the behavioral effects of excitatory amino acids on locomotor activity are not due to a primary action on the mesolimbic dopaminergic system. However, the observation that spontaneous motor activity, dopamine-induced and excitatory amino acid-induced hypermotility is inhibited by the intra-accumbens injection of drugs which decrease dopamine neurotransmission would indicate that accumbal dopamine is necessary, in some capacity, for motor function to occur. It may be that only a small portion of the dopamine neurons present in the nucleus accumbens are involved in the stimulation of locomotor activity caused by excitatory amino acids. For example, since a majority of the excitatory inputs to the nucleus accumbens reside predominantly in the medial portion of the nucleus (Yim and Mogenson, 1982) it is conceivable that only those dopamine neurons present in this region are involved in hypermotility. Further studies on the regional effects of excitatory amino acids in the nucleus accumbens may clarify the discrepancies between the biochemical and behavioral results.
CHAPTER VI

THE EFFECTS OF TWO PUTATIVE EXCITATORY AMINO ACID ANTAGONISTS
ON DOPAMINE AND GABA MECHANISMS IN THE NUCLEUS ACCUMBENS

VI.1 INTRODUCTION

The nucleus accumbens is a forebrain region that appears to be involved in the initiation and regulation of motor activity (Morgenson et al., 1980). This nucleus receives a dopaminergic input from the ventral tegmental area (Lindvall and Bjorklund, 1978) and bilateral injections of dopamine into the nucleus accumbens produce a marked stimulation of locomotor activity (Costall and Naylor, 1979). The nucleus accumbens also receives an excitatory amino acid input from the frontal neocortex (Carter, 1980; Walaas, 1981) and allocortex (Walaas and Fonnum, 1979; Walaas, 1981) and may also contain excitatory amino acid interneurons (Walaas and Fonnum, 1979). KA, NMA and QA, three potent excitatory amino acids, can produce a marked increase in locomotor activity after bilateral injection into the nucleus accumbens (see Chapter II). The effects of these excitatory amino acids can be antagonized by the co-administration of certain compounds shown in microiontophoretic studies on vertebrate central neurons to antagonize the electrophysiological responses of excitatory amino acids. After bilateral injection into the nucleus accumbens, magnesium and
D-aminoacidic acid antagonized selectively the hypermotility response produced by NMA while having little or no effect on the hypermotility response produced by KA, QA or spontaneous locomotor activity (see Chapters III and IV). This suggests that magnesium and D-aminoacidic acid may act in the nucleus accumbens by the same mechanism, selectively inhibiting NMA receptors.

Dopamine and picROTOXIN, a γ-aminobutyric acid (GABA) receptor antagonist, like the excitatory amino acids, have been previously shown to stimulate locomotor activity after injection into the nucleus accumbens (Costall and Naylor, 1979; Jones et al., 1981; Wachtel and Anden, 1978; Pycock and Horton, 1979). Excitatory amino acid neurons may be involved in the effects of these drugs on motility.

The present study was undertaken to determine whether NMA receptors play a role in the hypermotility response to dopamine and picROTOXIN. This was done by investigating the effect of magnesium and D-aminoacidic acid on the hypermotility response to these drugs.

VI.2 METHODS

A. Surgical Procedure

Male Sprague-Dawley rats weighing 175–200 g were used throughout this study. Bilateral injections into the nucleus accumbens were performed as described in Chapter II.
B. Monitoring Locomotor Activity

Locomotor activity was monitored as described in Chapter II.

C. Drugs

Dopamine hydrochloride (Sigma Chemical Co., St. Louis, MO) was dissolved in N₂ bubbled saline (pH 5.0) containing 0.1% sodium metabisulfite. PicROTOxin (Sigma Chemical Co., St. Louis, MO) was dissolved in saline (pH 7.4). Nialamide (Sigma Chemical Co., St. Louis, MO) was dissolved in a small amount of 1 N HCl before adding saline to volume.

D. Statistics

Data were expressed as the mean and standard error of the mean (SEM). Significant differences were evaluated using the two-tailed Mann-Whitney U-test, with a level of P < 0.05 being considered significant. Percent inhibitions were calculated as described in Chapter III.

E. Histology

Histological verification of the injection sites were performed as described in Chapter III.

VI.3 RESULTS

A. Effects of D-aminoadipic Acid and Magnesium on DA-induced Hypermotility

Following a 2 hour pretreatment with nialamide (100 mg/kg, i.p.), rats were injected bilaterally into the nucleus accumbens with dopamine (10 μg/0.5 μl) and their locomotor activity recorded. The
administration of dopamine produced a significant increase in locomotor activity as compared to saline-treated controls (Fig. 28).

Of the two excitatory amino acid antagonists tested, only D-amino acid acid (10 μg/0.5 μl) produced a significant inhibition (76%) of dopamine-induced hypermotility. The other antagonist, MgCl₂ (3 μg/0.5 μl), did not produce a significant inhibitory effect.

B. Effects of D-Amino acid Acid, Magnesium and Fluphenazine on Picrotoxin-Induced Hypermotility

Bilateral injection of picrotoxin (0.5 μg/0.5 μl) into the nucleus accumbens markedly enhanced locomotor activity as compared to saline-treated controls (Fig. 29). This response was significantly inhibited (89%) by D-amino acid acid, (10 μg/0.5 μl). In contrast to D-amino acid acid, MgCl₂ produced a slight but statistically insignificant enhancement of picrotoxin-induced hypermotility.

In order to determine whether picrotoxin was producing its hypermotility response through the blockade of GABA neurons which presynaptically inhibit dopaminergic neurotransmission, the dopamine receptor antagonist, fluphenazine, was tested for its ability to inhibit the hypermotility response produced by picrotoxin. It was found that fluphenazine (2.5 μg/μl) did not inhibit picrotoxin-induced hypermotility (Fig. 29), suggesting that GABAergic mechanisms involved in the regulation of motor function are postsynaptic to dopamine receptors in the nucleus accumbens.
Fig. 28 Effect of MgCl$_2$ and D-aminoacidic acid (DAA) on dopamine (DA)-induced hypermotility. Rats were injected into the nucleus accumbens on each side with DA (10 µg/0.5 µl) alone or in combination with MgCl$_2$ (3 µg/0.5 µl) or DAA (10 µg/0.5 µl). Motility was recorded for 1 hour after injection. Each bar represents the mean and SEM for the number of observations indicated in the parentheses. *P < 0.05 with respect to control. **P < 0.05 with respect to DA alone.
Fig. 28
Fig. 29 Effect of MgCl₂, D-amino adipic acid (DAA) and fluphenazine (FLU) on picrotoxin (PIC)-induced hypermotility. Rats were injected into the nucleus accumbens on each side with PIC (0.5 μg/0.5 μl) alone or in combination with MgCl₂ (3 μg/0.5 μl), DAA (10 μg/0.5 μl) or FLU (2.5 μg/μl). Motility was recorded for 1 hour after injection. Each bar represents the mean and SEM for the number of observations indicated in the parentheses. *P < 0.05 with respect to control. **P < 0.05 with respect to PIC alone. ***P = 0.05 with respect to PIC alone.
Fig. 29

MOTILITY / HOUR

CONTROL (0.5 μg)  (5)

PIC + MgCl₂  (4) **

PIC + DAA  (4) *

PIC + FLU  (4)
VI.4 DISCUSSION

Previous studies have shown that magnesium and D-amino adipic acid produce similar effects when injected bilaterally into the nucleus accumbens (see Chapters III and IV). Both compounds inhibit the hypermotility response produced by intra-accumbens injection of NMA, while having little or no effect on the hypermotility response produced by KA or QA. Similarly, neither compound, in doses that inhibit the effects of NMA, produced a significant change in normal locomotor activity.

The present study shows that the effects of magnesium were qualitatively different from D-amino adipic acid on the hypermotility response produced by either dopamine or picrotoxin. Although D-amino adipic acid markedly decreased the hyperactivity induced by both dopamine and picrotoxin, magnesium, at a dose that inhibited the response to NMA, produced no significant change in the hypermotility response to dopamine and slightly enhanced the response to picrotoxin. These studies do not support the hypothesis that magnesium and D-amino adipic acid act in the nucleus accumbens by the same mechanisms.

The interactions between putative neurotransmitters in the nucleus accumbens appear to be quite complex (Jones et al., 1981). However, behavioral and biochemical studies have presented evidence that dopaminergic nerve terminals in the nucleus accumbens may innervate GABAergic interneurons. Thus, (1) dopamine has been shown to inhibit the release of exogenously accumulated (3H)GABA from slices of the nucleus accumbens (Beart et al., 1979); (2) the peripheral
administration of antipsychotic drugs have been shown to produce an increase in GABA turnover in the nucleus accumbens (Moroni et al., 1979); (3) the direct intra-accumbens administration of GABA or picrotoxin can modify the stimulation of locomotor activity produced by dopamine receptor activation (Scheel-Kruger et al., 1977; Pycock and Horton, 1979; Wachtel and Anden, 1978). These findings can best be explained if the GABAergic receptors involved in these effects are located at a site in the neuronal pathway distal to the dopaminergic receptors. The observation that fluphenazine, at a dose which inhibits the hypermotility response produced by dopamine (Costall et al., 1979), did not significantly affect the hypermotility induced by picrotoxin (Fig. 2) is consistent with this hypothesis.

The inability of magnesium to inhibit the hypermotility response to either dopamine or picrotoxin suggests that magnesium may produce its inhibitory effect on NMA-induced hyperactivity by acting at a site (NMA receptors?) in the nucleus accumbens before the post-synaptic dopamine and GABAergic receptors. In contrast, since D-amino adipic acid inhibited the effects of both dopamine and picrotoxin, D-amino adipic acid, may act at a site beyond the dopamine and GABA synapse. The inhibitory effects of D-amino adipic acid are consistent with the hypothesis that the hypermotility response to both dopamine and picrotoxin is mediated through the activation of excitatory amino acid receptors (NMA receptors?) that are blocked by D-amino adipic acid.
CHAPTER VII

SUMMARY

The results from behavioral and electrophysiological studies have suggested that the nucleus accumbens is involved in both limbic and motor processes; however, the neuronal mechanisms which govern these functions are incompletely understood.

The nucleus accumbens receives an inhibitory dopaminergic input from the ventral tegmental area and an excitatory amino acid input from both allocortex and frontal neocortex and may also contain excitatory amino acid interneurons. The endogenous amino acids used as neurotransmitters by these excitatory neurons are, at present, unknown, although there is some evidence that certain of these neurons release GLU. Pharmacological analysis has established that the dopaminergic input is involved in controlling locomotion; however, the functional significance of the excitatory amino acid input has not been fully investigated. Thus, the purpose of this dissertation was to examine the functional role of excitatory amino acids in the nucleus accumbens.

In our initial experiments we examined the animals for any behavioral changes produced by the bilateral injection of excitatory
amino acids into the nucleus accumbens. The most profound behavioral
cchange observed was an immediate and intense increase in locomotor
activity. Unexpectedly, the structural analogs of GLU and ASP were
much more potent than either GLU or ASP themselves. The reason for
this is unknown but may be due to the in vivo inactivation of the
endogenous compounds. It is also possible that L-GLU and L-ASP are not
the endogenous excitatory amino acid transmitters in this brain region.

The mechanism(s) through which excitatory amino acids increase
locomotor activity is not fully understood but our behavioral studies
indicate that dopamine neurotransmission is necessary for their effects
on motility.

We then investigated whether the hypermotility response to
excitatory amino acids was mediated through a receptor mechanism. Electro-
pophysiological-microiontophoretic studies have suggested the presence
of at least three receptor types for excitatory amino acids in the
vertebrate CNS. These receptors are representative by the potent ex-
citatory amino acids, KA, NMA and QA. Since all three of these agonists
produced a potent stimulation of locomotor activity in the nucleus
accumbens, it was initially assumed that all three excitatory amino
acid receptors were present at this site. However, our studies suggest
the presence of two of these receptors (NMA and QA receptors) and that
KA is producing its effects through the activation of both NMA and QA
receptors.
Since it was initially proposed that excitatory amino acids increase locomotor activity through the release of dopamine and subsequent stimulation of dopamine receptors within the nucleus accumbens, our next series of experiments were designed to determine whether these compounds produce any changes in dopamine levels, release or turnover. Through the use of accumbal slices, we found that high concentrations (1 mM) of KA and NMA produced much smaller increases in the release of \(^{3}H\)dopamine than that produced by high potassium (a depolarizing agent) or by amphetamine. In addition, we did not observe any changes in endogenous dopamine levels or turnover after the intra-accumbens injection of KA, NMA and QA, at doses which produced a marked increase in locomotor activity. Thus, the biochemical data do not support the concept that the stimulation of locomotor activity is mediated through the release of dopamine from nerve terminals in the nucleus accumbens. However, our behavioral studies show that drugs which impair dopaminergic neurotransmission, markedly inhibit the hypermotility response produced by the excitatory amino acids. One possible explanation of the biochemical and behavioral results is that normal dopaminergic neurotransmission is necessary, in some capacity, for both spontaneous locomotor activity and excitatory amino acid-induced hypermotility. Alternatively, excitatory amino acids may stimulate the release of dopamine from a small subpopulation of dopamine neurons. This stimulation may be insufficient to influence the measurements of dopamine turnover and release, since these measurements are taken from the total population of accumbal dopamine neurons.
It has been known for sometime that dopamine and picrotoxin increase locomotor activity when injected bilaterally into the nucleus accumbens. It therefore seemed of interest to examine whether excitatory amino acid neurotransmission may be involved in the effects of these drugs on motility. This was done by studying the effects of D-aminoadipic acid and magnesium, two NMA receptor antagonists, on the hypermotility response produced by dopamine and picrotoxin. It was observed that D-aminoadipic acid decreased both dopamine- and picrotoxin-induced hypermotility, suggesting that the hypermotility response produced by both compounds is mediated through NMA receptor activation. However, magnesium did not decrease dopamine- or picrotoxin-induced hypermotility, suggesting that this ion is producing its inhibitory effects at a site (NMA receptor ?) which is presynaptic to both the dopamine and GABA neurons.

In conclusion, these studies suggest that excitatory amino acids, by interacting with specific receptors in the nucleus accumbens, play a role in the regulation of motor function. It is clear that dopamine neurotransmission in the nucleus accumbens is involved in this regulation but the exact nature of the interaction between dopamine neurons and excitatory amino acids is still not known (see Fig. 30).
Fig. 30 Schematic diagram showing possible interactions between excitatory amino acids, dopamine and γ-aminobutyric acid in the nucleus accumbens. (Abbreviations: DA, dopamine; GABA, γ-aminobutyric acid; EAA, excitatory amino acid; KA, kainic acid; QA, quisqualic acid; NMA, N-methyl-aspartic acid; Mg²⁺, magnesium; DAA, D-aminoadipic acid; DAP, diaminopimelic acid; GDEE, L-glutamic acid diethyl ester; FLU, fluphenazine; PIC, picrotobin; SMA, spontaneous motor activity).
Mg\(^{+2}\)
DAA
DAP

Fig. 30
REFERENCES


