CORTICAL REGULATION OF SUBCORTICAL DOPAMINE NEUROTRANSMISSION: CONTRIBUTIONS OF GLUTAMATERGIC MECHANISMS

by

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Abstract

Dysfunction in the cortical regulation of subcortical dopamine (DA) neurotransmission has been proposed to contribute to the pathophysiology of schizophrenia. However, before such a hypothesis can be fully described, it is necessary to establish the normal functional interactions of these systems. To this end, in vivo microdialysis was used to measure extracellular subcortical DA concentrations during electrical stimulation of the prefrontal cortex (PFC) and during feeding in rats. Because excitatory amino acids such as glutamate (Glu) are thought to be the predominant neurotransmitters used by cortical projection neurons, assessing the contributions of Glu receptors to DA release evoked by cortical stimulation and feeding has been the primary focus of these experiments.

Bilateral electrical stimulation of the PFC produced a rapid, current-dependent increase in DA release in the striatum and nucleus accumbens (NAc). This response was not affected by local application of the ionotropic Glu receptor antagonist kynurenic acid (KYN). In contrast, local application of the metabotropic Glu receptor agonist ACPD blocked the effects of cortical stimulation. Application of the ionotropic Glu receptor antagonists, AP5 and CNQX, in the ventral tegmental area (VTA) blocked the effect of stimulation on accumbal DA release. Thus, the PFC modulates subcortical DA release and this effect relies on Glu receptors in the VTA but not the NAc.

Given that electrical stimulation is an artificial stimulus, it was deemed worthwhile to investigate the role of glutamatergic mechanisms in regulating DA release occurring under natural conditions, i.e. during feeding. Thus, in rats deprived of food for 18 hours, feeding resulted in substantial increases in DA release in the NAc. This effect was potentiated by local application of KYN and was blocked by local ACPD application. The effect of feeding

was also markedly attenuated by application of ionotropic Glu receptor antagonists in the VTA. Thus, the neurochemical mechanisms regulating feeding-evoked DA release are consistent with those regulating cortical stimulation-evoked DA release.

These results demonstrate that Glu receptor mechanisms in the VTA mediate increases in DA transmission in the NAc evoked by a variety of stimuli. Secondary to this effect, local Glu receptors inhibit basal and evoked DA release in the NAc.

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I. General Introduction

Schizophrenia is a devastating illness that strikes early in life and often results in the loss of meaningful employment, family relations, and general well-being. The disease affects approximately 1% of the population worldwide (Torrey 1987), exacting tremendous costs both in terms of medical treatment and lost productivity. Substantial gains have been made in the management of this condition with the treatment of choice being the neuroleptic, a class of antipsychotic drug which blocks dopamine (DA) receptors as a primary mechanism of action. Consequently, a major thrust of research has been to establish the role of dopaminergic systems in schizophrenia. However, despite nearly 100 years of research, the biological basis of schizophrenia remains a mystery.

Schizophrenia was first identified as a disease state by Kraepelin (1919) at the end of the nineteenth century. Kraepelin observed a large population of patients in mental institutions who showed a major loss of mental abilities early in life that progressively worsened and culminated in death. Thus, he labeled it "dementia praecox," meaning early deterioration. Describing the primary symptom of the disorder as "...a peculiar destruction of the internal connections of the psychic personality," (Kraepelin 1919, p. 3), he went on to delineate the clinical picture, symptomology, and temporal pattern of the disease in such detail and eloquence that his description remains relevant today.

The clinicians of the day recognized that the symptoms of dementia praecox were not restricted to patients who became demented, but were often transiently observed. This led Bleuler (1950) to conclude that the deterioration occurred secondary to the disease, possibly resulting from the long term isolation in the squalid conditions of the nineteenth century

asylums. Bleuler also observed that not all patients showing symptoms of dementia praecox were hospitalized; many functioned reasonably well outside mental institutions and showed no signs of dementia. Thus the condition was reconceptualized as primarily a disorder of thought, rather than a dementia, and was renamed schizophrenia: a splitting of the mind.

The symptoms of schizophrenia include thought disorganization, hallucinations, delusions, ideas of reference, emotional dullness, loss of volition, and perseveration. These symptoms usually appear without any corresponding loss of orientation in time and space, language, memory, or obvious sensory deficits. The clinical picture is highly varied both in symptomology and time course with many patients showing spontaneous relapse and remission. This observation has resulted in attempts to delineate subtypes of schizophrenia based on groupings of symptoms. The earliest categorization was established by Bleuler and divided the schizophrenic population into subgroups characterized as hebephrenic, paranoid, and catatonic (Bleuler 1950). A system established more recently separates the condition into type I, characterized by *positive* symptoms such as hallucinations and delusions, and type II, characterized by *negative* symptoms such as avolition, emotional withdrawal, and flattened affect (Crow 1980). This model has proven to have heuristic value for describing schizophrenic symptoms. A subsequent model, developed in the last decade, separates the symptoms into three clusters- thought disorganization, psychomotor poverty, and reality distortion- and correlates each with a site of physiological dysfunction (Liddle et al. 1992). The clusters do not serve as categories of schizophrenia, but as syndromes that can coexist.

Between 1910 and 1970 many anatomists examined post-mortem brain tissue from schizophrenics; however no replicable findings were obtained. Unlike Alzheimer's disease or other dementias (Terry and Davies 1980), gross tissue analysis of schizophrenic brains did

not yield any telltale signs of atrophy (Shelton and Weinberger 1987). In fact, Plum (1972), referred to such pursuits as "the graveyard of neuropathologists." The frontal cortex presented a likely candidate for pathology because, in the words of Kraepelin (1919, p. 37), "If it should be confirmed that the disease attacks by preference the frontal areas of the brain... this distribution would in a certain measure agree with our present views about the site of the psychic mechanisms which are principally injured in the disease." This view was based on the observation that lesions of this region caused a syndrome whose symptoms- flattened affect, avolition, and poverty of thought- correspond to some of the negative symptoms of schizophrenia. The suggestion of frontal cortex involvement in schizophrenia led, in part, to the use of frontal leukotomy as a treatment (Greenblatt and Solomon 1953). Although this treatment enabled the medical community to control patients better, no benefit to the patient was demonstrated and the procedure was discredited and abandoned.

The Dopamine Hypothesis

The first evidence for an involvement of DA in schizophrenia actually precedes the confirmation of DA as a neurotransmitter in the central nervous system. Dopamine was first synthesized in 1910, but was not thought to be important because it had only limited actions when injected peripherally (Barger and Dale 1910). It was not until 1958 that DA was localized in the brain by Swedish researchers (Carlsson et al. 1958). As DA is a metabolic precursor to noradrenaline (NA), it was necessary to distinguish between these two compounds by pharmacological means to show that DA itself served as a neurotransmitter. To accomplish this, Carlsson employed reserpine, one of the early antipsychotics. This compound depleted both catecholamines, but after the application of L-DOPA, only DA

levels recovered (Carlsson et al. 1958). This experiment showed that: 1) DA is indeed located in the brain and is regulated in a functionally different manner than NA, confirming that DA is more than just a metabolic intermediary; and 2) reserpine had direct effects on brain DA systems, although the significance of the finding was not recognized for some years. This finding represents the earliest evidence for the DA hypothesis of schizophrenia which states, in its simplest form, that schizophrenia results from the overactivity of central DA systems (Matthysse 1973).

More support for this hypothesis was provided by Carlsson and coworkers who showed that neuroleptics from the other chemical classes, the phenothiazines and butyrophenones, also affected DA metabolism; application of chlorpromazine and haloperidol each increased mouse brain levels of DA when coadministered with monoamine oxidase inhibitors (Carlsson and Lindqvist 1963). Using these data as a starting point, Carlsson made the intuitive leap that the neuroleptics might be blocking a hypothetical DA receptor resulting in the disinhibition of DA metabolism (Carlsson and Lindqvist 1963). The import of this suggestion was bolstered by subsequent reports demonstrating that DA was likely to have an important physiological function. The evidence that DA could be localized in discrete groups of cells (Dahlstrom and Fuxe 1965) and was present in a projection from the substantia nigra to the striatum (Anden et al. 1966) was interpreted as a strong indication that DA could function as a neurotransmitter.

Anatomical studies have revealed a total of three major DA projections originating in the midbrain and projecting to forebrain areas (Lindvall and Bjorklund 1983). In addition to the nigrostriatal projection, there are dopamine projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and to the PFC, called the mesolimbic and

mesocortical DA systems, respectively (Swanson 1982). These two projections originate from distinct but intermingled populations of cells in the VTA (Swanson 1982). These ascending projections are topographically oriented in all three dimensions, medial/lateral, dorsal/ventral, and rostral/caudal, and project to the ipsilateral hemisphere almost exclusively (Fallon and Moore 1978). The mesolimbic projection can be further subdivided into a dense innervation of the ventro-medial NAc and a lighter innervation of the dorso-lateral NAc (Voorn et al. 1989). These two areas of the NAc can also be distinguished on the basis of neuropeptide localization, patterns of afferent and efferent projections, and a number of other neuronal markers and have been labeled a dorsolateral "core" and a ventromedial "shell" (Voorn et al. 1989; Zahm and Brog 1992). Finally, the core but not the shell region also receives a small DA projection from the medial substantia nigra (Fallon and Moore 1979; Lindvall and Bjorklund 1983).

Carlsson's prophetic hypothesis about the mechanism of action of neuroleptics (Carlsson and Lindqvist 1963) was confirmed a decade later. First, Aghajanian and Bunney (1973) showed that acute application of neuroleptics increased the cell firing of midbrain DA neurons. Second, receptor binding studies confirmed that the neuroleptics all competed with DA for binding sites, and the neuroleptic affinity correlated highly with clinical potency (Creese et al. 1976; Seeman et al. 1976). This finding provided compelling evidence that the primary mechanism of action of neuroleptic drugs is blockade of DA receptors; this represents the first line of evidence for the DA hypothesis of schizophrenia.

The second line of evidence for this hypothesis stems from the effects of amphetamine. In the 1950's it was observed that chronic users of this stimulant sometimes developed psychotic symptoms leading to their hospitalization as schizophrenics (Connell

1958; Bell 1965). Amphetamine had been shown to increase extracellular DA as well as serotonin and NA, so this evidence suggested that overactivity of DA systems, among others, could induce psychosis (Snyder 1972). To confirm that the amphetamine-induced psychotic syndrome in the chronic users was a result of the drug and not some confounding factor such as an underlying mental disorder, sleep deprivation, or anxiety, amphetamine was administered to human subjects under controlled laboratory conditions. Amphetamine caused acute psychosis after as few as three days of treatment (Griffith et al. 1972). This evidence, coupled to the clinical efficacy of DA receptor antagonists, seemed to strongly support a role for DA overactivity in the etiology of schizophrenia.

However, it was recognized that the evidence for the DA hypothesis was indirect, so the race was on to find conclusive evidence of DA hyperactivity in schizophrenic patients. In short, such evidence has not been forthcoming. Early studies examined levels of the DA metabolite, homovanillic acid (HVA), in the cerebrospinal fluid and plasma of schizophrenics. These studies did not yield consistent findings, and the findings that were obtained were confounded by the issue of whether differences in blood-serum HVA corresponded to differences in brain levels (Widerlow 1988; Davis et al. 1991). Post-mortem studies revealed that schizophrenics sometimes showed higher brain levels of DA and/or HVA than normals (Davis et al. 1991), but the sites of differences were not consistent between studies. Thus it is possible that the observed effects could be attributed to technical or statistical differences between studies or to differences between the patient groups. Most importantly, neuroleptic medication could affect measures of DA metabolism so patient groups differing in their history of treatment might show artifactual effects.

Studies examining DA receptor levels post-mortem or with PET imaging have yielded some interesting, but inconclusive results. At the time of the early studies of this type, there were only thought to be two DA receptors, D1 and D2, and neuroleptics were thought to act as D2 receptor antagonists. Early binding studies targeted these receptors with the ligand ^{3}H-methylspiperone, and found evidence for small increases in D2 receptor densities in the striatum of schizophrenics, including neuroleptic-naive patients (Wong et al. 1986; Seeman et al. 1987; Davis et al. 1991). However, subsequent PET studies using the more selective agent ^{3}H-raclopride found no evidence for altered D2 binding in schizophrenics (Farde et al. 1987; 1990). Then in the early 1990's additional subtypes of the D2 receptor were identified, labeled D3 and D4, that could be pharmacologically differentiated (Van Tol et al. 1991; Sokoloff et al. 1990). Indeed, it was found that ^{3}H-methylspiperone binds to all the D2 subtypes, whereas ^{3}H-raclopride binds only D2 and D3 receptors (Seeman et al. 1987; Van Tol et al. 1991; Sokoloff et al. 1990). This finding suggested that the difference between results obtained with the two ligands could be attributable to D4 binding (Seeman et al. 1993). As no D4 selective ligand is currently available for research purposes, Seeman subtracted D2 and D3 (^{3}H-raclopride) binding from D2, D3, and D4 (^{11}C-spiperone) binding and found evidence of sixfold increased D4 binding in schizophrenics relative to controls (Seeman et al. 1993). However, this finding remains controversial as other studies using similar approaches have failed to replicate this finding (Seeman et al. 1993; Reynolds and Mason 1994). Debate on this issue continues unabated (Murray et al. 1995; Seeman and Van Tol 1995;1996; Reynolds 1996), and the question must be considered unresolved until a selective D4 ligand becomes available.

Taken together, the data described to this point indicate at most a subtle dysfunction of DA signaling in schizophrenia. However, because the DA system readily compensates in response to insult (Hornykiewicz 1979), it is hard to imagine that such a subtle dysfunction could lead to the full-blown disorder. For example, the symptoms of Parkinson disease do not become apparent until more than 80% of nigrostriatal neurons are lost (Hornykiewicz 1979). For these reasons, the simple form of the DA hypothesis of schizophrenia has been all but abandoned.

Connectionist Models of Schizophrenia

A revised form of the DA hypothesis has persisted in which DA activity may be affected secondary to some other primary pathology. In searching for this primary source of pathophysiology in schizophrenia, the PFC has been implicated. In particular, a reduction in basal metabolic activity in frontal areas of the brains of schizophrenics, referred to as hypofrontality, has been described. This phenomenon was first identified by Ingvar and Franzen who used radioactive xenon gas to measure cerebral blood flow (Ingvar and Franzen 1974a; 1974b). They found that schizophrenics as a whole showed less activity in the dorsolateral PFC than normals and that patients with more negative symptoms showed significantly lower PFC blood flow (Ingvar and Franzen 1974a; 1974b). This finding has been replicated by some researchers but not by others (Weinberger et al. 1986). The inability of some investigators to replicate Ingvar and Franzen's findings may relate to the substantial overlap in basal cortical metabolism between normals and patients.

To address this issue in more detail, Weinberger and coworkers examined blood flow in the PFC of schizophrenics during performance of the Wisconsin Card Sort. Performance of

this task is thought to depend on the integrity of the PFC (Milner 1963), and schizophrenic patients show deficits at this task (Taylor and Abrams 1984). Weinberger found that performance of the Wisconsin Card Sort activated the dorsolateral PFC of schizophrenics to a lesser degree than that of normals (Weinberger et al. 1986). This finding confirmed that, regardless of basal metabolism in the PFC, schizophrenics showed a deficit in the evoked activity of this brain region. Differences were not observed in any other brain region including the temporal, parietal, or occipital lobes. This finding, recently replicated in a group of neuroleptic-naive patients (Andreasen et al. 1992), provides strong evidence of hypofrontality in schizophrenic patients.

The pathophysiological basis for the hypofrontal syndrome is not fully understood. As mentioned above, early anatomical studies did not reveal any major abnormality in schizophrenic brains such as a lesion or evidence of gliosis. Then, in the 1970's major advances were made possible with the advent of the Computerized Tomography (CT) scan. Studies of CT scans of some schizophrenic brains have revealed a number of reasonably consistent abnormalities including larger volume of the lateral ventricles, larger volume of the third ventricle, and shrinking of cortical gyri (Shelton and Weinberger 1987). These measures are indicators of general dysfunction rather than a specific lesion, and are thought to be evidence of a deficit occurring early in development given the lack of gliosis. Studies reporting localized anatomical abnormalities in schizophrenic brains often implicate prefrontal areas, although other brain regions may also be involved (Shelton and Weinberger 1987; Gur and Pearlson 1993; Ross and Pearlson 1996). In fact, a substantial case is building that structural abnormalities are present in frontal areas of schizophrenic brains at the microscopic level. Benes and coworkers have found a preponderance of vertical fibers and a

loss of GABAergic interneurons in the PFC (Benes et al. 1991; 1992). Akbarian and coworkers, on the other hand, report a loss of the synthetic enzyme for GABA, glutamic acid decarboxylase, in the PFC of schizophrenics without a concomitant loss of neurons (Akbarian et al. 1995). Additionally, there is some evidence for a loss of spines in the PFC of schizophrenics (Glantz and Lewis 1995). Taken together, these findings suggest that the PFC may be characterized by a subtle, anatomical disorganization rather than massive cell death.

To summarize, three generalizations can be made about the pathophysiology of schizophrenia. 1) Drugs that block DA receptors have antipsychotic efficacy. 2) Schizophrenia is characterized by abnormally low metabolism in the PFC under a variety of conditions. 3) The schizophrenic brain shows subtle, widespread anatomical abnormalities that are consistent with a "lesion" early in development. These findings have led to the hypothesis that schizophrenia results not from a single, localized abnormality, but by the dysfunction of broadly distributed circuitry.

In addition to the PFC, the NAc may be a component of this circuitry. The NAc is heavily innervated by DA terminals (Lindvall and Bjorklund 1983) and it appears to be a common site of action of neuroleptic drugs based on neurochemical, electrophysiological, and immunohistochemical findings (White and Wang 1983; Moghaddam and Bunney 1990; Robertson and Fibiger 1992). Furthermore, as neuroleptics primarily affect positive symptoms of schizophrenia, it is conceivable that these symptoms result from overactivity of DA neurotransmission in the NAc (Reynolds 1992). This observation, coupled with the possibility that the negative symptoms might result from deficits in the PFC, has provoked a number of investigators to propose that schizophrenia may result from a dysfunction of

cortical regulation of subcortical DA neurotransmission (Weinberger 1987; Robbins 1990; Davis et al. 1991; Grace 1991; Goldstein and Deutch 1992).

In the earliest and most influential of these models Weinberger (1987) proposed that a lesion early in development could result in the loss of DA input into the PFC. Part of the rationale for this hypothesis stemmed from the observation that a 6-hydroxydopamine (6-OHDA) lesion of the primate PFC resulted in a syndrome with components similar to the negative symptoms of schizophrenia (Brozowski et al. 1979). Also, humans with idiopathic Parkinson's disease or MPTP-induced parkinsonism show flattened affect, loss of volition, and other cognitive deficits associated with schizophrenia that could result from a loss of PFC DA function (Langston et al. 1983; Bowen et al. 1975). Weinberger (1987) further suggested that the PFC dysfunction could result in disinhibition of subcortical DA neurotransmission. The primary evidence for the latter assertion came from ex vivo studies by Pycock et al (1980) showing that a 6-OHDA lesion of the rat frontal cortex increased subcortical DA metabolism. Other studies had previously shown that disruption of the PFC caused a hyper-responsiveness to the locomotor and stereotypy producing effects of amphetamine (Iversen et al. 1971), behaviors thought to be DA mediated.

This hypothesis was substantially weakened by the failure of subsequent studies to substantiate the Pycock findings (Rosin et al. 1992; Bubser 1994). Various reports have also indicated that disruption of the corticostriatal projection, excitotoxic lesions of the PFC, and suction ablation of the PFC all fail to influence subcortical DA metabolism or release in the striatum or NAc (Scatton et al. 1982; Jaskiw et al. 1990; Whishaw et al. 1992). An early study, in fact, points to the opposite relationship, showing that electrical stimulation of the cortex increased subcortical DA release (Nieoullon et al. 1978). Thus it is difficult to

conclude that the PFC has a substantial inhibitory influence in regulating DA activity in the NAc. To reconcile these inconsistent and/or contradictory findings, a closer analysis of the anatomy and neurochemistry is necessary.

As early as the 1970's it was recognized that the entire neocortex projects topographically to the striatum (Graybiel and Ragsdale 1979), and further studies indicated that the NAc receives projections specifically from regions of the PFC (Berendse et al. 1992; Sesack et al. 1989; Fuller et al. 1987). Both of these projections are thought to use glutamate (Glu) as a neurotransmitter (McGeer et al. 1977; Christie et al. 1987; Fuller et al. 1987). Ultrastructural analysis of the striatum and NAc showed that the efferents from the PFC do not form axo-axonal contacts on the DA terminals, but the PFC and DA containing projections were found to synapse in close proximity on the same dendritic spines (Bouyer et al. 1984; Sesack and Pickel 1990). Some authors have suggested that this organization leaves open the possibility that Glu or some other excitatory amino acid could escape the synaptic cleft and activate Glu receptors on DA terminals (Moghaddam et al. 1990; Sesack and Pickel 1990; Keefe et al. 1992). In support of this possibility, studies have shown that Glu application in the striatum can increase DA release in vivo (Carter et al. 1988; Imperato et al. 1990a; Moghaddam et al. 1990; Leviel et al. 1990; Keefe et al. 1992; Westerink et al. 1992) and in vitro (Giorguieff et al. 1977; Jones et al. 1993; Desce et al. 1992). These findings suggest that the PFC has an excitatory influence on subcortical DA release.

This line of evidence led Grace (1991) to propose an alternative hypothesis of schizophrenia in which the PFC regulation of subcortical DA release is abnormal. Grace proposed that under normal conditions the glutamatergic projection from the PFC to the NAc maintains tonic, basal DA release in the NAc. This influence is hypothesized to be lost in the

schizophrenic brain, resulting in sensitization of the post-synaptic response to DA. The mesolimbic DA system, thought to be transiently active in response to discrete phasic-type stimuli, would then evoke an abnormally enhanced post-synaptic response.

The Grace hypothesis fits much of the anatomical, electrophysiological, and neurochemical organization of the PFC/NAc circuitry with two notable exceptions. First, if the glutamatergic input into the NAc maintains basal DA concentrations, then Glu receptor antagonists should reduce basal DA when applied in this region. In fact, Glu receptor antagonists consistently increase DA release when applied to this region (Moghaddam et al. 1990; Imperato et al. 1990b; Youngren et al. 1993). Secondly, if the meso-striatal projection is only phasically active, then blockade of impulse flow in the DA cells should not affect basal striatal DA release. In fact, blockade of this projection with tetrodotoxin (TTX) abolishes extracellular DA in the striatum (Keefe et al. 1992). These observations make it difficult to accept the Grace hypothesis as stated.

When examined carefully, neither the Weinberger nor the Grace model adequately integrates the known anatomy and neurochemistry of forebrain DA systems into a fully convincing model to describe the PFC regulation of subcortical DA transmission. In fact, because these two connectionist models of schizophrenia are based on opposing assumptions about the regulation of DA release in the NAc, it is clear that more research into this question is necessary. The experiments described in this thesis were undertaken to describe the specific mechanisms by which the PFC modulates subcortical DA neurotransmission. Three general questions were addressed. 1) Does the PFC regulate subcortical DA release? 2) As the efferents from the PFC use an excitatory amino acid as a neurotransmitter, do glutamatergic mechanisms participate in the PFC regulation of subcortical DA release? 3)

Do other stimuli that activate the mesolimbic DA system do so via similar mechanisms as direct stimulation of the PFC?

II. Electrical Stimulation Of The Medial Prefrontal Cortex Increases Dopamine And Acetylcholine Release In The Striatum

Introduction

Data from a variety of sources indicate that cortical areas can regulate subcortical DA neurotransmission. For example, surgical ablation of the PFC enhances amphetamine's stimulant effects on locomotor behavior (Iversen et al. 1971) and apomorphine's stereotypy inducing properties (Scatton et al. 1982). 6-OHDA lesions of the medial PFC increase DA uptake and binding site density in the striatum (Pycock et al. 1980). Electrical stimulation of motor or visual cortex has been observed to increase striatal [^{3}H]DA release as measured by the push-pull cannula technique (Nieoullon et al. 1978).

Given that the PFC sends glutamatergic afferents to the striatum (Divac et al. 1977; McGeer et al. 1977), the chemical and anatomical substrates for these observations may involve glutamatergic terminals in the vicinity of axons and terminals of nigrostriatal neurons (Grace 1991). Pharmacological studies have found that Glu applied to striatal slices in vitro stimulate [^{3}H]DA release even in the presence of TTX (Giorguieff et al. 1977; Roberts and Anderson 1979; Marien et al. 1983). In vivo studies with push-pull cannulae have also found increased [^{3}H] DA release following local applications of low (10^{-8}-10^{-6} M) concentrations of Glu (Cheramy et al. 1986; Leviel et al. 1990). At higher concentrations (10^{-5}-10^{-3} M) this effect was reversed, with DA release being decreased (Cheramy et al. 1986; Leviel et al. 1990). In vivo microdialysis experiments also support the facilitation of striatal DA release following a local injection of Glu receptor agonists (Moghaddam et al. 1990; Keefe et al. 1992; Westerink et al. 1992). Moreover, in these studies depression of DA release was not

observed even at relatively high concentrations of these agonists (1 mM AMPA, 1 mM NMDA, 0.1 mM kainate). These results show primarily, though not exclusively, facilitatory actions of Glu on DA release in the striatum.

The relative contributions of the corticostriatal and nigrostriatal systems in regulating striatal DA release are not fully understood. Basal striatal DA release is decreased by manipulations that decrease the activity of nigrostriatal neurons, but not by local administration of Glu receptor antagonists at concentrations which block Glu evoked DA release (Keefe et al. 1992). These data point to a tonic role for nigrostriatal neurons in DA release with an additional phasic component being mediated by glutamatergic mechanisms.

The purpose of the present study was to investigate the role of the PFC in regulating endogenous DA release in the striata of awake, freely moving animals. To this end in vivo microdialysis was used to monitor extracellular concentrations of endogenous DA and its metabolites in rats during bilateral electrical stimulation of the PFC. Additionally, a fluorescent retrograde labeling technique was employed to confirm a prefrontal origin of corticostriatal afferents innervating the region of the dialysis probe.

To further examine the role of the PFC in regulating striatal neurotransmission, the effect of PFC stimulation on striatal acetylcholine (ACh) release was also examined. Cholinergic neurons make up about 2% of the total cell population of the striatum (Fibiger 1983), and these cells are thought to be interneurons due primarily to their lack of labeling following injection of retrograde tracers into known targets of the striatum (Parent 1990). Several lines of evidence suggest that the corticostriatal projections may regulate the activity of these neurons. Application of Glu receptor agonists to the cortex has been shown to increase striatal acetylcholine (ACh) release in the anesthetized rat (Herrera-Marschitz 1991).

In vitro studies have also shown that stimulation of Glu receptors increases the release of endogenous or tritiated ACh from striatal slices (Scatton and Lehman 1982; Arenas et al. 1991), and this effect can be blocked by antagonists acting at NMDA Glu receptors (Scatton and Lehman 1982; Lupp et al. 1992). In addition, microdialysis studies demonstrate that cortical ablation decreases baseline ACh release and prevents the increased ACh release that is normally induced by oxiracetam and apomorphine (Consolo et al. 1990). Cortical ablation has also been shown to reduce ACh synthesis in the striatum ex vivo (Wood et al. 1979). Together, these results point to an excitatory influence of the cortex on striatal ACh release. The present experiments examined this question directly by determining if the efferent projections from the PFC can regulate striatal ACh release phasically. To this end, extracellular ACh concentrations were measured in the striata of awake, freely moving rats during electrical stimulation of the PFC.

Materials and Methods

Fifteen male Wistar rats weighing between 300 and 360 g at the time of surgery were used in these experiments. The rats were anesthetized with pentobarbital (50-60 mg/kg) and were stereotaxically implanted with transverse dialysis probes passing through both striata (see Fig. 2.1). Coordinates were measured from bregma (A:+.7 V:-4.75) according to the atlas of Paxinos and Watson (1986). The probe consisted of a hollow dialysis membrane (outer diameter = 0.320 mm, Mol. wt. cutoff = 40,000 Da; AN69 Hospal) with an active surface length of 3.2 mm on each side of midline according to the lateral boundaries of each striatum. Ten rats were additionally implanted bilaterally with bipolar stainless steel electrodes in the PFC (0.2 mm diameter; MS303/2, Plastic Products). Coordinates were

measured from bregma (A:+3.0 V:-3.0 L:+.8/-.8) according to the atlas of Paxinos and Watson (1986). Probe outlets and electrodes were attached to the skull via four anchoring screws and dental acrylic. Following surgery all animals were housed individually in Plexiglas cages (35 X 35 X 40 cm) where they remained for the duration of the experiment. Subjects had free access to food and water at all times.

The ten animals with electrodes and probes were used in the ACh and DA microdialysis experiments. For DA microdialysis experiments, approximately 48 h after surgery dialysis probes were perfused at a rate of 5 µl/min with a solution containing mM $CaCl_2$ 1.3, $MgCl_2$ 1.0, KCl 3.0, NaCl 147, in 1 mM aqueous phosphate buffer to maintain pH at 7.4. The resulting dialysate passed through outlet tubing (PE10, Clay Adams) into the sample loop (50 µl) of a high performance liquid chromatography (HPLC) system where samples were automatically injected every ten minutes. For both DA and ACh dialysis, analysis was performed "on-line," and samples were injected with the use of an electrically controlled valve (EC10W,Valco Inc.). Dopamine was separated with reverse phase liquid chromatography (Nucleosil, 5µm, C18, Chrompack) and detected electrochemically. The mobile phase was delivered at 1.2-1.35 ml/min using an HPLC pump (1350, BIO-RAD) and contained 0.5 mM octane sulfonic acid, 0.01 mM ethylenediamine-tetraacetic acid (EDTA), and 12% methanol in sodium acetate buffer (pH = 4.1). The concentration of DA was determined by sequential oxidation and reduction using a coulometric detector (Coulochem II, ESA) and a flow-through analytical cell (5011, ESA) with the voltage set at +0.4 and -0.35 V, respectively. A pulse dampener (SSI) and guard cell (ESA, potential = 0.45 V) were placed between the HPLC pump and the injector. A DA

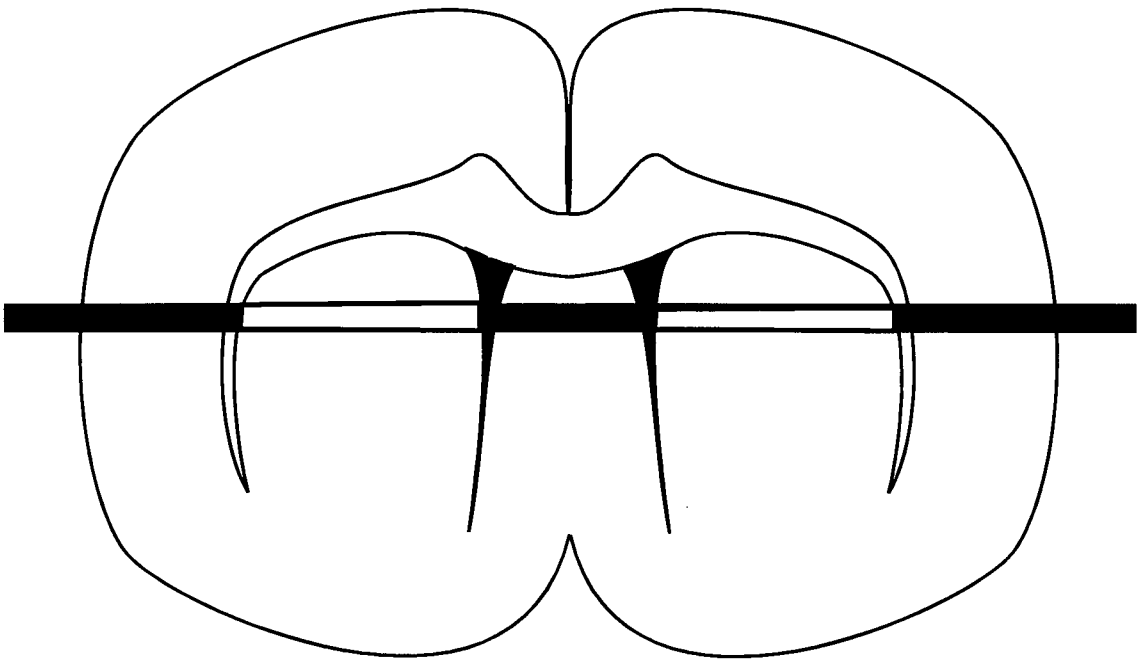


Figure 2.1. Schematic diagram depicting a horizontal microdialysis probe implanted in the dorsal striatum. The clear areas of the probe indicate the areas of active dialysis; the black areas were blocked with glue.

peak on the chromatogram representing a signal to noise ratio of 2:1 was deemed the smallest peak measurable. This corresponded to a detection limit of 5 fmol/sample.

Measurement of ACh using on-line microdialysis and HPLC with electrochemical detection (Damsma et al. 1988) was also initiated approximately 48 hours after surgery. A solution containing (mM) NaCl 125, KCl 3, NaCl 147, $CaCl_2$ 1.3, $MgCl_2$ 1.0, in 1 mM aqueous phosphate buffer , pH 7.4, and neostigmine bromide 0.1 μM, was perfused through the microdialysis probes at 5 μl/min. The resulting dialysate was first separated on a column pretreated with lauryl sulfate and then passed through an enzyme reactor containing acetylcholinesterase and choline oxidase (Sigma) covalently bound to glutaraldehyde-activated Lichrosorb NH_2 (Merck). These reactions gave a stoichiometric yield of peroxide that was electrochemically detected at a platinum electrode set at +0.5 V versus a Ag/AgCl reference electrode (BAS-LC4B).

PFC stimulation began after a stable baseline was established (4 samples with <10% variation). The electrodes of the 5 rats undergoing DA microdialysis were electrically stimulated at 50 μA (sine wave, frequency = 60 Hz, stimulus duration = 0.5 s, interstimulus interval = 5 s) for 20 min, and two hours later the animals received stimulation at a higher current (100 μA, same parameters). The ACh rats received the same currents but in randomized order, and only 1 hour separated the stimulation periods. The currents were chosen on the basis that rats have been found to self administer electrical stimulation to the PFC at these parameters (Phillips and Fibiger 1978). At the end of the experiment rats were killed; brains were cut in 50 μM sections and stained with cresyl violet to confirm electrode and probe placements.

Retrograde labeling of cells innervating the region of the dialysis probe was performed on the remaining five rats using fluorescence histology. Striatal probes were filled with a solution containing 4% Fluorogold in saline (0.9%) and were flushed with saline 18h later. Five days later these animals were given a lethal injection of pentobarbital and were perfused with 4% paraformaldehyde. Following a two hour post-fix, brains were bathed overnight in 0.05 M phosphate buffer, cut in 35 μm coronal slices, and mounted on slides. The sections were observed under a fluorescent microscope using ultraviolet light.

Figure 2.2A shows chromatograms of dialysis samples from the striatum before and after electrical stimulation. To analyze the microdialysis results, peak heights were measured with ruler to the nearest 0.5 mm. The average of the first four samples with <10% variability was considered to make up baseline. To calculate basal DA concentrations sample peak heights were compared to peak heights from a standard solution containing DA (100 nM). To determine treatment effects, data were represented as percentage of baseline and analyzed using one-way and two-way analysis of variance (ANOVA's) with repeated measures.

Results

Dopamine Microdialysis

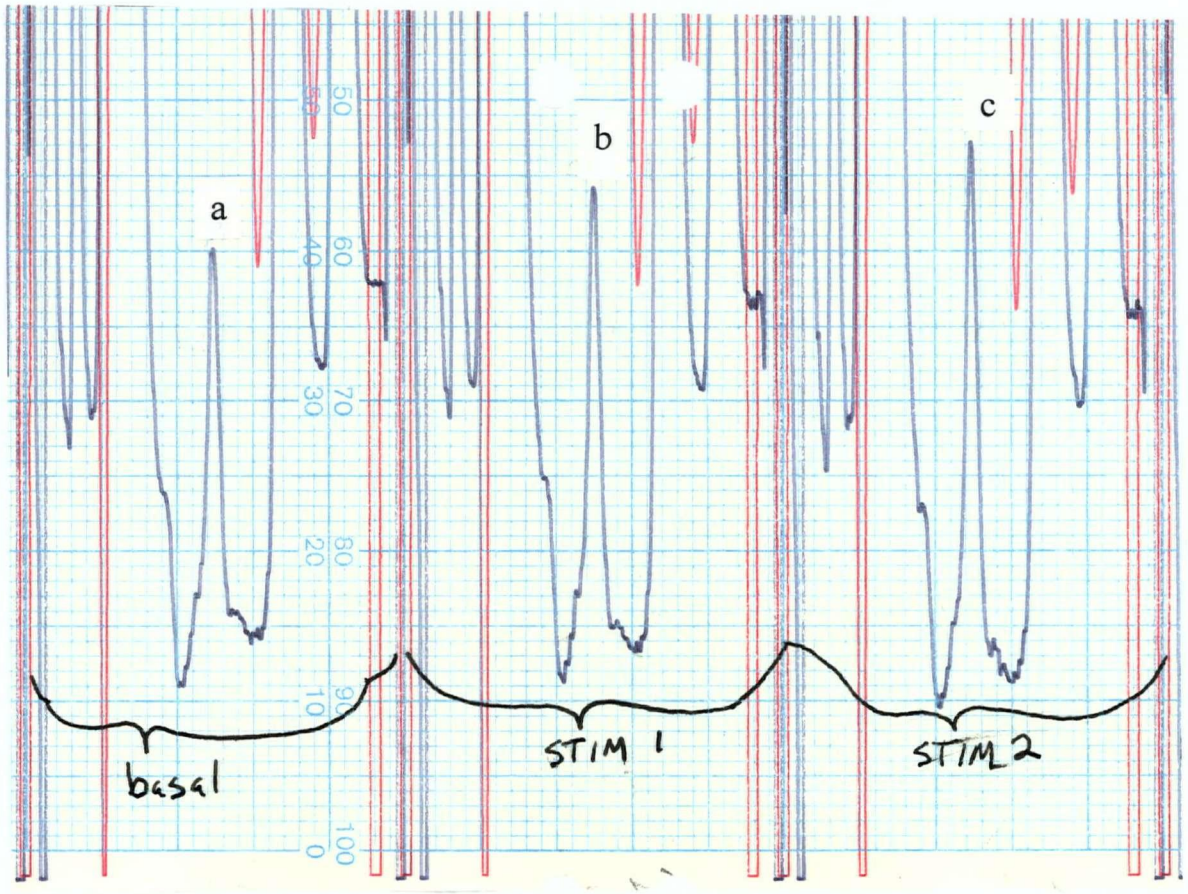
The average basal extracellular DA output was 150 ± 22 fmol/sample (n=5). Electrical stimulation increased DA output in all animals, and the increase was found to rise with current intensity (Fig. 2.2B). Fifty μA increased DA output to a maximum of 118% of baseline values; 100 μA increased DA output to a maximum of 138% of baseline values. The effects of both stimulation currents were statistically significant across time ($p < 0.001$).

A significant Stimulation Intensity X Time interaction was also found across the two levels of stimulation ($p<.02$). The DA metabolites DOPAC and HVA and the serotonin metabolite 5-HIAA were also increased by both levels of stimulation ($p < 0.001$, data not shown), although in all cases the peak percent increase was lower than the corresponding DA values. DOPAC peaked at 117% and 128% for 50μA and 100μA, respectively; HVA peaked at 113% and 121%; and 5-HIAA peaked at 106% and 107%. Significant Stimulation Intensity by Time interactions were found for DOPAC ($p < 0.002$) but not HVA or 5-HIAA.

Dopamine concentrations rose rapidly during each 20 min stimulation and peaked in the second 10 min sample of the stimulation period. DA decreased thereafter, and returned to baseline 50-60 min after PFC stimulation. Formal behavioral measures were not performed, but casual observation indicated that the stimulation did not produce behavioral activation. Indeed, rats appeared more active during the 50 μA stimulation than at 100 μA.

Acetylcholine Microdialysis

The average baseline dialysate ACh output for all five rats was 828 ± 232 fmol/sample. Figure 2.3 A shows sample chromatographs of basal and stimulated ACh. Both stimulation currents produced significant increases in ACh output ($p < 0.001$; Fig. 2.3B), although there was no difference between the effects of the two stimulation currents. Fifty μA caused a maximum increase to 134% of baseline whereas 100 μA led to an increase of 127% of baseline. ACh output returned to baseline within 30 min of termination of the stimulation. During electrical stimulation no behavioral abnormalities such as seizures or high levels of behavioral activation were observed in any of the animals. The rats appeared awake and alert, but locomotion was minimal.



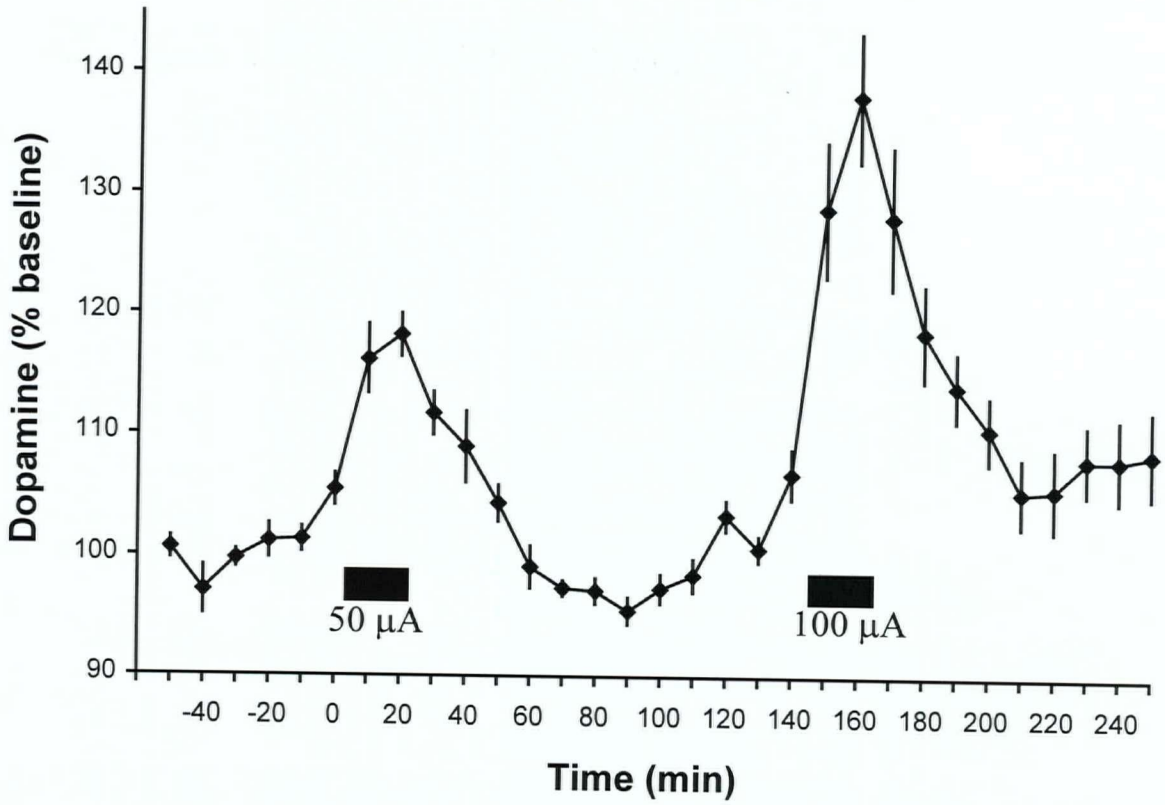
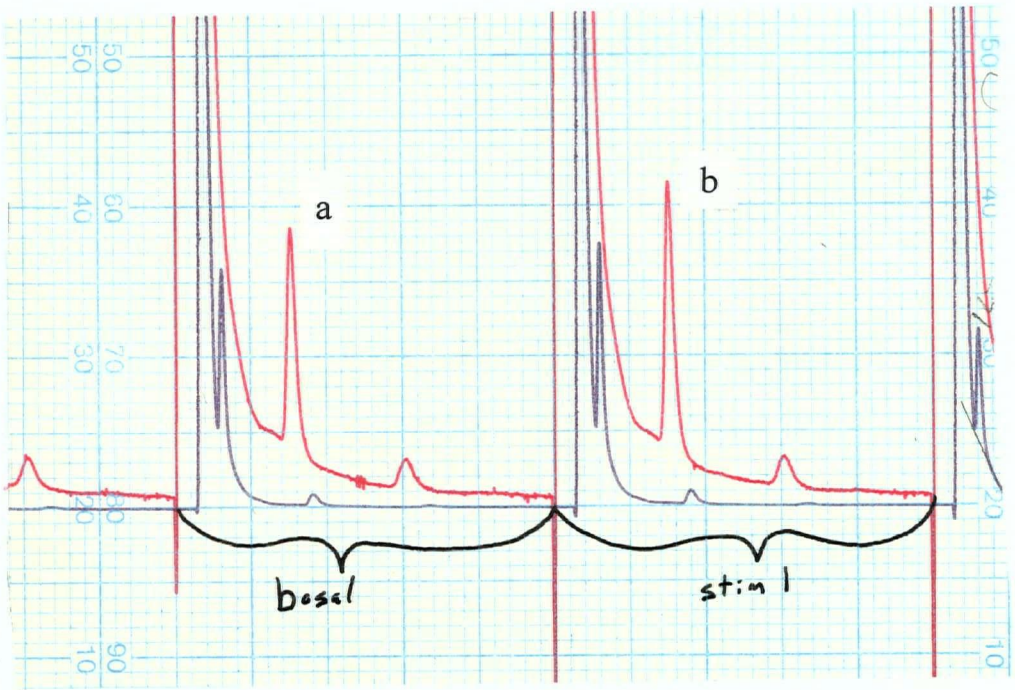


Figure 2.2**A)** Chromatograph showing sample striatal DA peaks taken before (a) and during

(b,c) electrical stimulation of the PFC (100 μA). **B)** Dialysate DA concentrations sampled bilaterally from the striatum during electrical stimulation of the PFC (60 Hz, 0.5 s pulse, 5.0 s interpulse interval). The amplitude of the increase in DA was intensity dependent. In this and all following figures data represent the mean ± SEM. Black bar indicates the onset and duration of electrical stimulation.



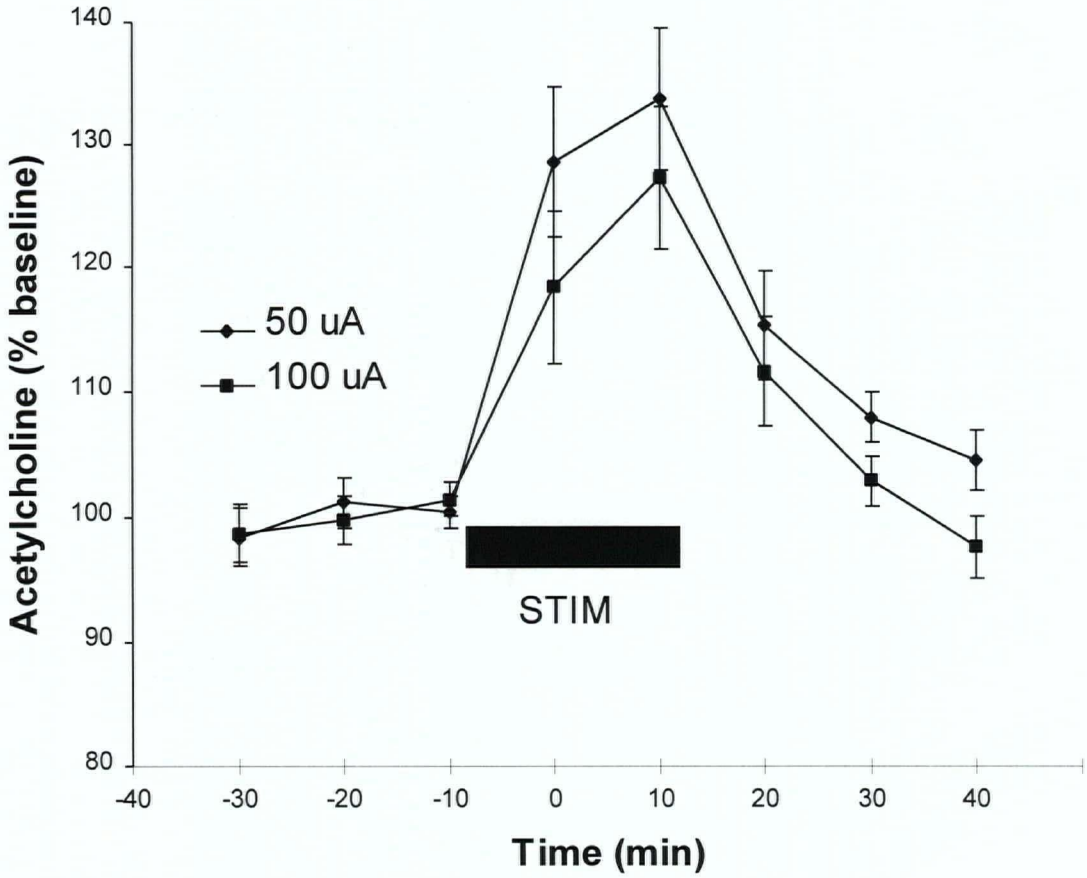


Figure 2.3**A**) Chromatograph showing sample striatal ACh peaks taken before (a) and during

the first sample of (b) electrical stimulation (100 μA). **B**) Dialysate ACh concentrations sampled bilaterally in the striatum during electrical stimulation of the PFC (sine wave, 60 Hz, 0.5s pulse, 5 s interpulse interval). The effects of the two currents were not different. Black bar indicates onset and duration of the electrical stimulation.

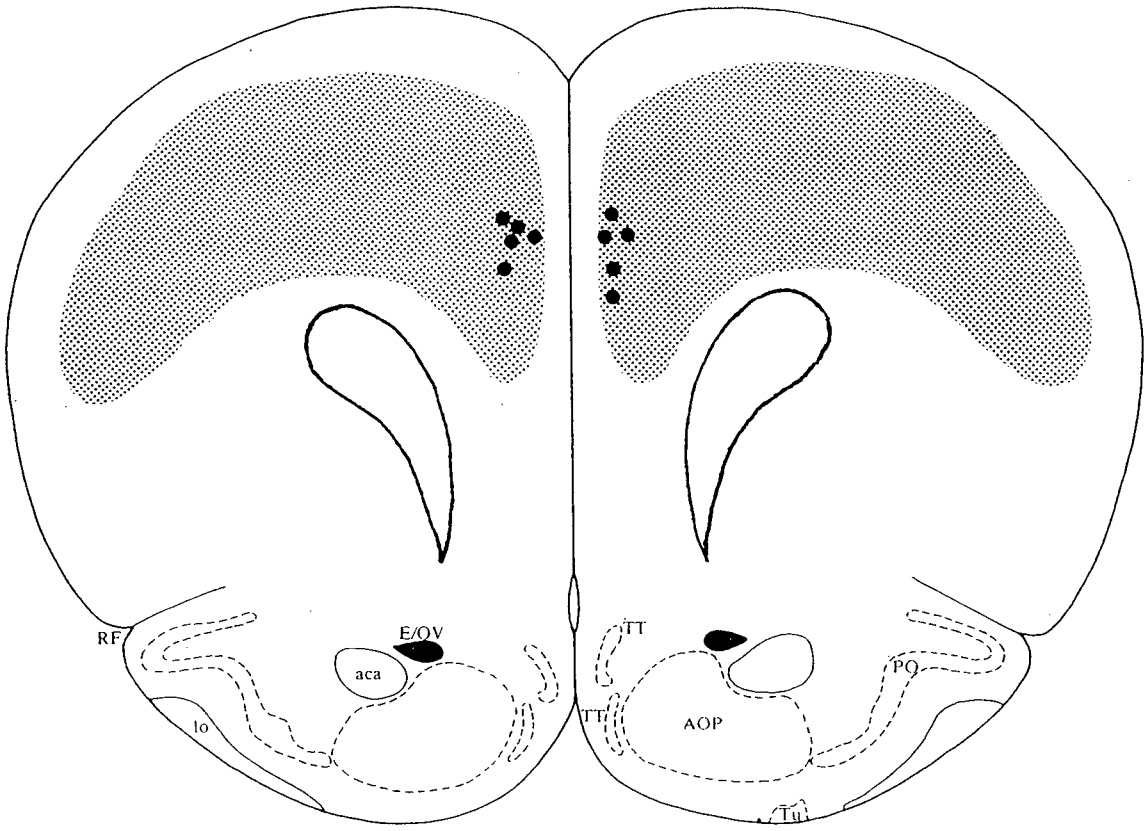


Figure 2.4. Drawing of a coronal section through the PFC showing electrode tip locations. Stippling represents areas of retrograde labeling following infusion of Fluorogold through the dialysis probe in the striatum.

Histology

Histological analysis of the 10 rats with electrodes and dialysis probes implanted confirmed all placements. Electrodes were located in medial PFC at 3.0 to 3.2 mm anterior to bregma, 2.5 to 3.0 mm ventral to skull surface, and within 1 mm of the midline (Fig. 2.4). All dialysis probes were located in dorsal striatum 0.5 to 0.7 mm anterior to bregma.
In rats infused with Fluorogold, fluorescence was examined in coronal slices from the frontal pole to the dialysis probe. The area of densest retrograde labeling was the dorsal cortex in all sections viewed, and the labeling extended across the entire medial/lateral aspect of this region. Dense labeling was found in the area surrounding the electrode sites in all animals viewed (Fig. 2.4). These data indicate that the medial PFC in the vicinity of the stimulation electrodes project directly to the region of the striatum containing the dialysis probe. This agrees with previous retrograde and anterograde labeling studies (McGeorge and Faull 1989; Berendse et al. 1992).

Discussion

The primary result presented here is direct evidence that increased activity in the medial PFC can evoke striatal DA and ACh release. The stimulation parameters that were used (50 and 100 μA) have been found to maintain self-stimulation in rats (Phillips and Fibiger 1978), and can therefore be considered behaviorally relevant. No behavioral abnormalities such as seizures were observed in any animal. The time course of the increased DA output is most consistent with a phasic excitatory regulation of DA release. During stimulation DA concentrations rose rapidly, peaked immediately after cessation of the stimulation, then declined. Additionally, electrical stimulation of the PFC elevated striatal

acetylcholine release. These results demonstrate the ability of the microdialysis technique to detect transient neurotransmitter fluctuations following discrete phasic stimuli.

Nieoullon et al. (1978) have reported that cortical stimulation produces long-lasting increases in extracellular concentrations of [^{3}H]DA in the striatum. These researchers found that extracellular DA concentrations were still rising an hour after termination of the cortical stimulation. This observation led to the hypothesis that the corticostriatal projection has a tonic facilitatory influence on striatal DA release (Nieoullon et al. 1978; Romo et al. 1986; Grace 1991). However, later studies have indicated that Glu in the striatum does not have a tonic influence on DA release (Leviel et al. 1990; Keefe et al. 1992) and suggest that exogenous Glu produces phasic increases in striatal DA release. The present results are consistent with this view in that they demonstrate that PFC stimulation produces transient increases in DA release, an effect that may be mediated by the glutamatergic corticostriatal projection.

It is unclear why the DA increases seen in the Nieoullon et al. (1978) study lasted so much longer than in the current study. Technical differences suggest three possibilities. First, the cats in the Nieoullon study were stimulated at a much higher frequency (300 Hz) and in a different area of cortex (motor) than in the current study. The second involves behavioral state; the rats in the current experiment were awake and not restrained, whereas the cats in the Nieoullon study were halothane-anesthetized. Keefe et al. (1992) suggest that behavioral state may contribute to baseline levels of DA mediated through the corticostriatal projection; perhaps behavioral state also affects stimulation-evoked DA release. The second possibility is that the dialysis and push-pull cannula techniques are measuring different pools of DA. Microdialysis samples total extracellular DA concentrations whereas the push-pull

cannula technique used by Nieoullon et al. measured recently synthesized [^{3}H]DA. It is possible that cortical stimulation influences release of [^{3}H]DA differently than it affects endogenous stores of DA.

The present data stand in contrast with earlier lesion-based studies which have suggested that the PFC has inhibitory actions on subcortical dopaminergic systems (Iversen et al. 1971; Pycock et al. 1980; Jaskiw et al. 1990). The chronic and irreversible nature of PFC lesions used in those studies makes direct comparisons with the present acute manipulations difficult. Clearly, however, the present findings point to an excitatory influence of the PFC on subcortical DA systems in awake, relatively intact, behaving animals. A report from Louilot et al. (1989) is somewhat more difficult to reconcile with the present results. These authors found that infusions of TTX into the PFC increased extracellular concentrations of DOPAC in the NAc, suggesting that the PFC normally inhibits DA metabolism in this structure. In the present experiments electrical stimulation of the PFC increased extracellular concentrations of DOPAC in the dorsal striatum. The basis of these apparent discrepancies is not presently known and requires investigation.

Anatomical systems other than the glutamatergic corticostriatal projection may have contributed to the stimulation-induced increase in DA release observed in the present experiments. Most significantly, efferents from the PFC synapse directly on tyrosine hydroxylase containing neurons of the VTA (Sesack and Pickel 1990). Also, a projection from the PFC terminates in the substantia nigra (Gerfen et al. 1982). Excitation of either of these systems may have activated dopaminergic projections to the striatum. Another channel through which stimulation of the PFC could evoke striatal DA release involves the intralaminar nuclei of the thalamus. For example, the centrolateral nucleus sends axon

collaterals to both the cortex and the striatum (Cesaro et al. 1979). Antidromic stimulation of the projection to the PFC may have activated striatal collaterals to evoke DA release.

These results also provide direct evidence that cortical stimulation enhances striatal ACh release. The lack of difference in ACh release evoked by the two stimulation currents probably reflects a "ceiling" effect whereby even the lower current produced a maximal effect. The most direct mechanism by which the PFC could mediate striatal ACh increases is via stimulation of the corticostriatal glutamatergic projection. Electrical stimulation of the cortex was directed at a site in the PFC shown to project to the region of the dorsal striatum that contained the dialysis probe. In addition, previous in vitro studies have shown that pharmacological stimulation of NMDA receptors evokes ACh release in the striatum (Scatton and Lehman 1982; Arenas et al. 1991; Lupp et al. 1992). However, several recent anatomical studies have indicated that few (Dimova et al. 1993) if any (Lapper and Bolam 1992), terminals of the corticostriatal projection synapse directly with cholinergic neurons in the striatum. In contrast, Lapper and Bolam (1992) have demonstrated that these cholinergic neurons are heavily innervated by the centromedian-parafascicular complex of the thalamus, another putatively glutamatergic projection (Wilson et al. 1983). This raises the possibility that electrical stimulation of the PFC increases striatal ACh release indirectly, perhaps via actions on thalamic nuclei.

The present results provide a potential explanation for the ability of indirect DA receptor agonists and of specific D1 agonists to increase striatal ACh release via actions at D1 receptors that are extrinsic to the striatum (Damsma et al. 1991). D1 receptors have been localized to the medial PFC (Vincent et al. 1993). It is possible that actions of DA at these receptors alters cortical activity in a manner that stimulates corticostriatal efferents which in

turn results in increased ACh release in the striatum. These events could occur concomitantly with actions at D1 receptors located in the substantia nigra pars reticulata which also appear to regulate ACh release in the striatum (DeBoer and Abercrombie 1993).

III. Electrical Stimulation Of The Prefrontal Cortex Increases Dopamine Release In The Nucleus Accumbens: Involvement Of Local Glutamate Receptors

Introduction

Electrical and chemical stimulation of the sensorimotor or PFC have been shown to enhance DA release in the striatum and NAc (Chapter I; Nieoullon et al. 1978; Murase et al. 1993a). The neurotransmitter Glu may be implicated in the mechanism underlying DA release evoked by PFC stimulation. The PFC projects heavily to the dorsal and ventral striatum in a topographically organized manner (Sesack et al. 1989; Berendse et al. 1992). This projection is thought to use Glu or aspartate (ASP) as a neurotransmitter since lesions of the PFC have been shown to reduce Glu uptake in the dorsal striatum (Divac et al. 1977; McGeer et al. 1977), and [^{3}H] -ASP injected into the NAc is retrogradely transported to the prelimbic areas of the PFC (Christie et al. 1987; Fuller et al. 1987). In vivo studies have demonstrated that electrical or chemical stimulation of the frontal cortex increases the release of both ASP and Glu in the dorsal striatum (Godukhin et al. 1980; Young and Bradford 1986; Palmer et al. 1989; Perschak and Cuenod 1990). Although these findings have not been extended to the NAc, the general parallel nature of the cortical projections to these areas of the striatal complex are suggestive of a similar organization. Taken together these findings suggest that stimulation of the PFC may activate the corticostriatal projection and increase Glu release in the NAc which may in turn enhance DA release from meso-accumbens nerve terminals.

The extent to which Glu may modulate DA release via direct presynaptic actions within the NAc remains a critical question to this hypothesis. Axo-axonic synapses between cortical and midbrain afferents in the NAc or striatum rarely, if ever, occur (Bouyer et al. 1984; Sesack and Pickel 1990). Recent in vivo microdialysis studies have examined this issue by applying Glu or Glu receptor agonists locally to the NAc via a dialysis probe while concurrently monitoring DA concentrations in the same area (Imperato et al. 1990a,b; Youngren et al. 1993). One group of researchers found that the specific Glu receptor agonist NMDA (1 mM) failed to affect DA release (Imperato et al., 1990b), while the agonists quisqualate and kainate at similar concentrations increased DA release (Imperato et al., 1990a). Others have reported that high concentrations of either Glu or ASP (1-10 mM) evokes DA release with ASP causing the larger increase (Youngren et al., 1993). Additionally, the NMDA receptor antagonist AP5 was found to be more effective than the non-NMDA antagonist CNQX at reducing the effect of ASP, whereas the reverse held true for the effects of Glu (Youngren et al., 1993). Utilizing electrochemistry to measure DA concentrations, Svensson et al. (1994) found that local administration of either NMDA or AMPA could increase DA release in the NAc, but only at high concentrations that also evoked physiological events consistent with spreading depression. These reports indicate that DA release can be evoked by Glu acting at either NMDA or non-NMDA ionotropic receptors, but that the effect may not be physiological. A survey of the literature addressing the same question in the dorsal striatum points to a similar, inconclusive picture (Moghaddam and Bunney 1990; Keefe et al. 1992; Westerink et al. 1992; Morari et al. 1993).

The current study was performed to establish whether electrical stimulation of the PFC can increase DA release in the NAc and if so, the extent to which this effect is mediated by Glu receptors in the NAc. To this end, the noncompetitive NMDA antagonist dizocilpine maleate (MK-801) and the broad spectrum ionotropic Glu receptor antagonist kynurenic acid (KYN) were applied locally to the NAc alone or in combination with electrical stimulation of the PFC. Additionally, because the NAc contains relatively high concentrations of metabotropic Glu receptors (Albin et al. 1991; Shigemoto et al. 1992; Testa et al. 1994), we sought to investigate the possible role of these receptors in modulating DA release. Activation of metabotropic Glu receptors mediates a variety of physiological effects in striatal slice preparations (Calabresi et al. 1993; Lovinger et al. 1993), and produces behavioral effects in the intact rodent (Sacaan et al. 1991; Sacaan et al. 1992; Klitgaard and Laudrup 1993; Laudrup and Klitgaard 1993). Furthermore, a recent study has implicated interactions between Glu metabotropic and dopaminergic receptors in regulating locomotor activity (Sacaan et al. 1992). To address whether Glu metabotropic receptors may play a role in regulation of DA release, the effects of the specific metabotropic agonist trans (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) on basal and stimulation-evoked DA release were examined.

Materials and Methods

Surgery

Male Wistar rats weighing 280-350 g were anesthetized with 50-60 mg/kg Pentobarbital (i.p.) and placed in a stereotaxic apparatus. A vertical, concentric type

dialysis probe with 2.0 mm of active membrane (AN69, Hospal, m.w. cutoff = 40,000 Da) was implanted in the NAc. Coordinates relative to bregma were A: +3.6 mm, L: +1.5 mm, and V: = -7.8 mm (at the ventral extent of the active membrane) according to the atlas of Pellegrino et al. (1979). Bilateral, bipolar stimulating electrodes (MS303/2, Plastic products, Roanoke VA) separated at the tip (< 100 μm) were implanted in the prelimbic area of the PFC. This represents a more ventral site in the PFC than the site stimulated to evoke DA release in the neostriatum (previous chapter) due to the topography of the PFC-striatal projections (McGeorge and Faull 1989). Electrodes were implanted bilaterally because the NAc receives afferents from the PFC of both hemispheres (Sesack et al., 1989; current results). Electrode coordinates were A: +5.0 mm, L: ± 2.4 mm, and V: -4.2 mm from bregma. The electrodes were implanted at an angle 20° lateral to the dorsal/ventral axis for ease of implantation. Another group of rats had electrodes implanted in the VTA in combination with the NAc dialysis probes. VTA electrodes were implanted unilaterally at A: +3.5 mm, L +2.4 mm, and V: +1.8 mm relative to interaural zero according to the atlas of Paxinos and Watson (1986). Probe outlets and electrodes were anchored to the skull with two or three stainless steel screws and dental acrylic. Following surgery, animals were housed individually in Plexiglas cages and were given 48 h to recover prior to initiation of dialysis. Rats were maintained on a 12:12 h light:dark schedule and had ad libitum access to food and water. See Chapter II for details of microdialysis and the DA assay.

Treatment was initiated after establishment of a baseline, defined as 3 consecutive samples with <10% variation in DA levels, and involved either local drug administration or electrical stimulation. The stimulation period started 20 min after initiation of local administration of drug, except in animals receiving TTX which had a 40 min interval before the start of stimulation. Stimulation consisted of a 60 Hz sine wave applied for 20 min in all stimulation treatments, although parameters varied for PFC and VTA stimulation. PFC electrodes were stimulated at 50 and 100 μA with pulse trains of .5 s duration and 5.1 s inter-train interval. VTA electrodes were stimulated at 20 μA with 0.2 s pulse train duration and 1.1 s inter-train interval. The PFC stimulation parameters were chosen on the basis of a previous study which found that rats will self-stimulate at these currents (Phillips and Fibiger 1978). The VTA stimulation parameters were chosen not only because they support self-stimulation in rats, but also because the DA release evoked by this amount of VTA stimulation approximates that found with PFC stimulation in the present study (Fiorino et al. 1993). The concentrations of DA were measured after stimulation to determine the time course of return to baseline.

In some animals locomotor activity was measured during the experiment using an automated locomotion detector (Digiscan, Omnitech, Columbus, OH). This apparatus gave a count of horizontal activity measured as the number of interruptions of an infrared light beam during each 10 min dialysis sample.

A within-subjects, counterbalanced design was used in the experimental groups that received both 50 and 100 μA currents to the PFC and in the group that

received stimulation of the VTA alone and in combination with ACPD. Rats in these groups had an interval of at least 90 min between treatments for recovery of baseline. All other analyses employed a between-groups design in which rats received only one treatment.

Drugs

All drugs were administered locally in the NAc via reverse dialysis through the same probe used to measure DA. Compounds delivered in this way were dissolved in the perfusion medium and pH was adjusted to 7.1-7.4 with dilute acetic acid or NaOH. MK-801, KYN, and ACPD were obtained from Research biochemical Inc. (Natick, MA). TTX was purchased from Sigma Chemical Co. (St. Louis, MO). All drugs were diluted from frozen aliquots immediately prior to use.

Histology

To confirm that the area of the NAc perfused by the dialysis probe received a projection from the area of the PFC containing the stimulating electrodes, the retrograde label Fluorogold was again employed. A solution of 3% Fluorogold in distilled water was infused through the dialysis probes of four rats at the end of the experiment and again 12 h later. After an additional 24 h, the dialysis probe was rinsed with distilled water to wash out excess Fluorogold. Seven days later animals were given an overdose of chloral hydrate and were perfused with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were removed, postfixed for 2 h, and transferred to 0.05 M phosphate buffer for 12 h. The following day brains were cut in 30 μm coronal slices, mounted on slides, coverslipped with a mounting

solution containing 5% n-propyl-gallate (as an antifading agent) in a mixture of 80% glycerol and 20% Tris-Cl, and examined under fluorescent light.

The remaining rats were overdosed with chloral hydrate and the brains were removed and fixed in 4% paraformaldehyde. At least three days later brains were cut in 40 μm sections, mounted on slides, and stained with cresyl violet to confirm electrode and probe locations. All rats used in the present study were confirmed to have dialysis probes located in the NAc and electrodes in the PFC and VTA as appropriate.

Data Analysis

For statistical analysis all data except DA in the presence of TTX were represented as percent baseline, calculated as the three samples preceding experimental manipulation. A one-way ANOVA with repeated measures across time was employed to test each individual treatment. Two-way ANOVAs were used to compare the stimulation effects with and without drug. In all cases Huynh-Feldt corrections for degrees of freedom were used to account for time as a repeated measure. Post hoc analysis was performed with Tukey's test. To test the effects of TTX on DA release elicited by PFC stimulation, absolute values of DA across time were tested with a one-way ANOVA.

Results

The mean dialysate DA concentration for baseline NAc samples in the group of rats receiving 50 or 100 μA was 51.0 ± 3.9 fmol/sample. No differences in the

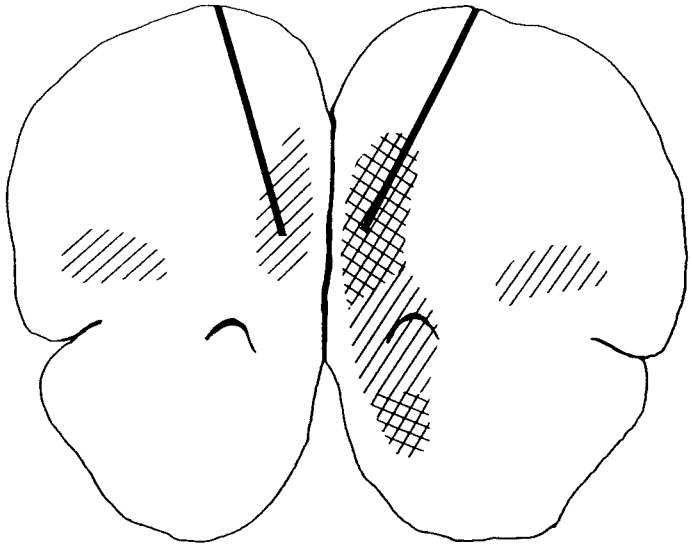


Figure 3.1. *Camera lucida* drawing of a coronal section through the PFC showing electrode location. Hatching represents areas of retrograde labeling following infusion of Fluorogold through the dialysis probe in the NAc. Cross hatching represents dense labeling and single hatching represents sparse labeling.

absolute baseline values were observed between experimental groups ($p > 0.05$).

Histology

Figure 3.1 shows a camera lucida drawing of a coronal section through the PFC showing a typical electrode placement; hatching has been added to show the approximate area of fluorescent labeling following infusion of Fluorogold through the dialysis probe. Cross hatching represents higher levels of retrograde labeling and single hatching represents lower levels. Bilateral labeling of the PFC was obtained following unilateral perfusion in the NAc although ipsilateral labeling was stronger; this is in agreement with previous work which shows substantial crossing of corticostriatal fibers (Sesack and Pickel, 1990). Fluorescent labeling was found in the prelimbic, infralimbic, insular, and piriform cortices as well as the claustrum, amygdala, and olfactory bulbs. All electrode tips were located in the ventral half of the prelimbic area of the PFC.

Effects of PFC Stimulation on DA in the NAc

During the 20 min periods of 50 or 100 μA stimulation, DA concentrations rose significantly above baseline ($p < 0.01$; Fig. 3.2A). The higher current caused a significantly larger increase than the lower current ($p < 0.05$) with 100 μA causing a peak increase of 69% above baseline, and 50 μA causing a peak increase of 37%. Extracellular DA concentrations peaked in the second stimulation sample and returned to baseline 20 min following termination of the stimulation. A group of rats

receiving only the 100 μA (Fig. 3.4) stimulation current showed an increase in DA from stimulation (71 % above baseline) almost identical to that seen in the first experiment (Fig. 3.2A).

Stimulation had an activating effect on locomotor activity at both currents in all rats although the variation was substantial. Measured as the difference between the sample preceding stimulation and the sample with peak locomotor activity, locomotor counts ranged from 432 to 3043. Rats displayed normal behaviors such as sniffing, rearing, grooming, eating, and drinking during stimulation. No signs of seizure or stereotypy were observed in any rat.

Effects of TTX on DA Release Evoked By PFC Stimulation

TTX (1 μM) applied locally in the NAc caused a substantial drop in extracellular DA concentrations (Fig. 3.2B). Within 30 min DA was no longer detectable in 3 out of the 4 rats, and the fourth rat's DA levels were undetectable 20 min thereafter. When the PFC was stimulated (100 μA) under these conditions, no increase in absolute amounts of extracellular DA was observed ($p > 0.05$). In a group of rats that received PFC stimulation without TTX treatment (Fig. 3.2A) extracellular DA rose an average of 35 fmol/sample.

Effects of Locally Applied Glu Receptor Antagonists on DA Release

Figure 3.3 shows the effects of MK-801 and KYN at various concentrations on DA release from the NAc. Both drugs caused dose dependent increases in DA release. MK-801 (Fig. 3.3A) had a significant effect at a moderate concentration (10 μM: $p < 0.01$) whereas a lower concentration (1 μM) had no effect ($p > 0.05$). A high

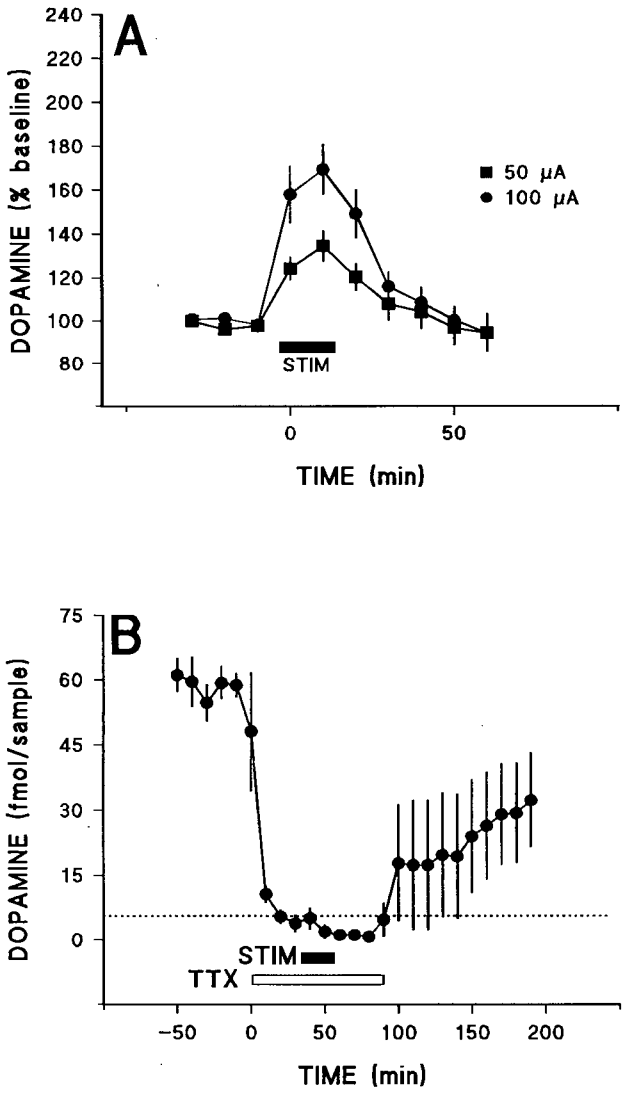


Figure 3.2.**A**: Dialysate concentrations of DA in the NAc (n = 6) during stimulation of the PFC at 50 µA (squares) and 100 µA (circles; 60 Hz, pulse duration = .5 s, interstimulus interval = 5 s). **B**: Absolute values of dialysate DA in the NAc during PFC stimulation (100 µA) and concurrent administration of TTX (1 µM) locally via reverse dialysis (n = 4). In this and all subsequent figures data represent the mean ± SEM. Black bars indicate the onset and duration of electrical stimulation; clear bars indicate the onset and duration of local drug application.

concentration of KYN (5 mM; Fig. 3.3B) caused a significant increase in extracellular DA ($p < 0.05$), while 1 mM had no effect ($p > 0.05$). Both drugs required administration for at least 30 min before changes in DA concentrations were clearly seen. For both MK-801 and KYN the lower concentration was deemed subthreshold on DA release, and was subsequently used in combination with stimulation of the PFC.

Effects of MK-801 and KYN Applied Locally to the NAc on DA Release Evoked by Electrical Stimulation of the PFC

When MK-801 (1 μM) or KYN (1 mM) was applied locally in the NAc for 2 h beginning 20 min before the initiation of PFC stimulation at 100 μA, the time course of DA release was not different from that observed during stimulation alone (Fig. 3.4). Stimulation still evoked a significant increase in extracellular DA during treatment with either MK-801 ($p < 0.01$) or KYN ($p < 0.01$). When the effect of stimulation co-administered with KYN or MK-801 was compared to DA release induced by stimulation alone, there was no significant effect of drug treatment ($p > 0.05$). Not only did the antagonists fail to block the stimulation-induced increase in DA release, but the tendency was for release to be slightly potentiated. This finding is consistent with the ability of each drug to increase DA release on its own. Higher concentrations of the antagonists were not used because they had significant effects on their own, thereby confounding interpretation.

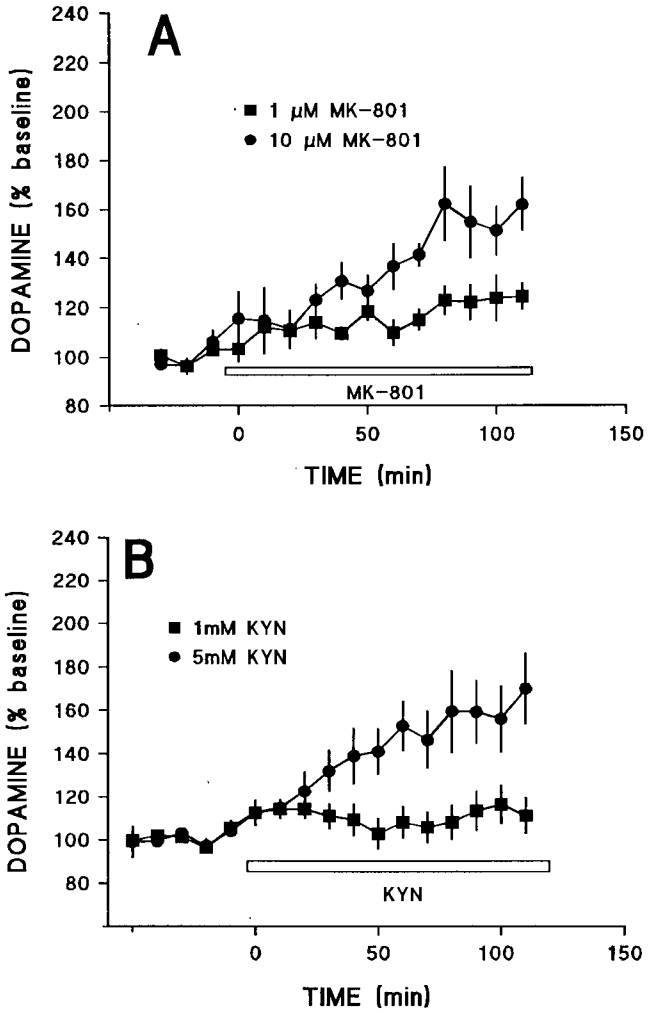


Figure 3.3. Dialysate concentrations of DA in the NAc during local administration of **A**: MK-801 (1 μM; squares, 10 μM; circles; n = 5, each group) and **B**: KYN (1 mM; squares, 5 mM; circles) through the NAc dialysis probe (n = 5, each group). Clear bars indicate the onset and duration of local drug application.

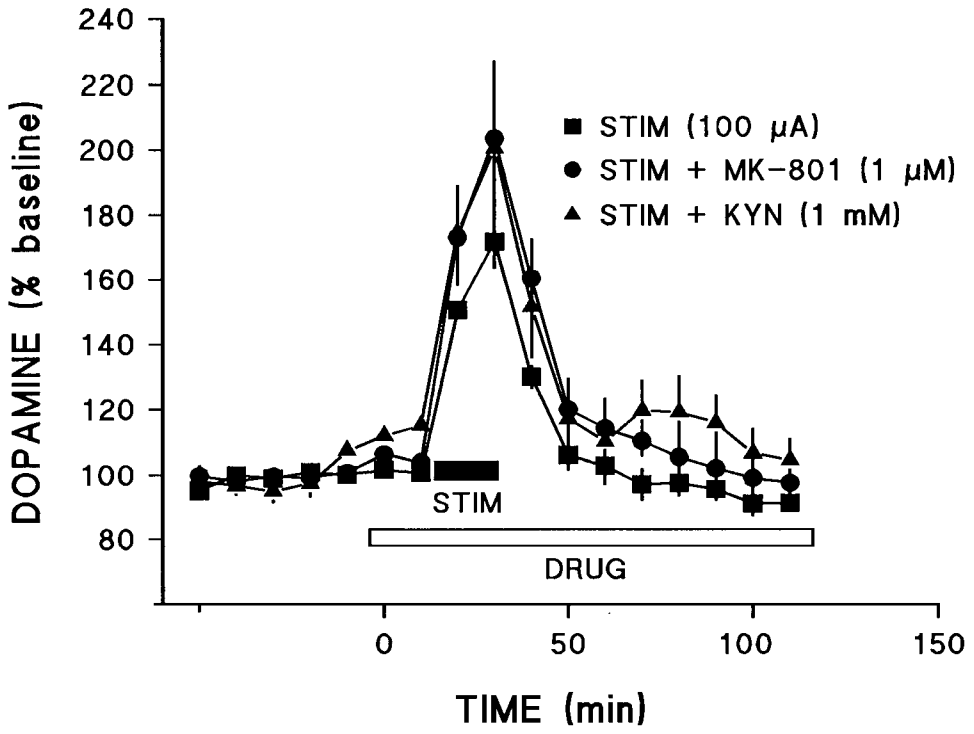


Figure 3.4. Dialysate concentrations of DA during PFC stimulation (100 μA) in combination with local perfusion of MK-801 (1 μM; circles; n = 6), KYN (1mM; triangles; n = 6), or vehicle solution (squares; n = 5). Neither MK-801 nor KYN significantly affected stimulated DA release. Black bar indicates the onset and duration of electrical stimulation; clear bars indicate the onset and duration of local drug application.

Effects of ACPD on DA Release

Figure 3.5A shows the effects on DA release of two concentrations of ACPD. The higher concentration of ACPD (1 mM) caused a biphasic increase in DA release peaking at 52%, falling back to baseline, then rising gradually over a much longer period ($p < 0.05$). A lower dose of ACPD (100 μM) caused a small decrease in DA levels compared to baseline ($p < 0.01$) or compared to a control group that only received the standard perfusion solution ($p < 0.01$). This effect was quite small and delayed, however, as the decrease was not statistically significant until an hour after the beginning of local drug application. On the basis of these results, the middle concentration (100 μM) was judged to be subthreshold with respect to DA release and was therefore used in the subsequent experiments.

Effects of ACPD on DA Release Evoked by PFC Stimulation

Figure 3.4B shows the effect of two concentrations of ACPD administered locally to the NAc on DA release evoked by PFC stimulation (100 μA). At the higher concentration ACPD completely blocked the effect of PFC stimulation on DA release. Accordingly, the stimulation did not cause a significant deviation from baseline across time ($p > 0.05$). The lower concentration of ACPD (10 μM) failed to attenuate the stimulation induced effect; following administration of this concentration; DA release was not different from that of stimulation alone ($p > 0.05$).

Effects of ACPD on DA Release Elicited by Stimulation of the VTA

To establish whether the effect of ACPD on stimulated DA release was specific to cortical stimulation or was representative of a more general phenomenon,

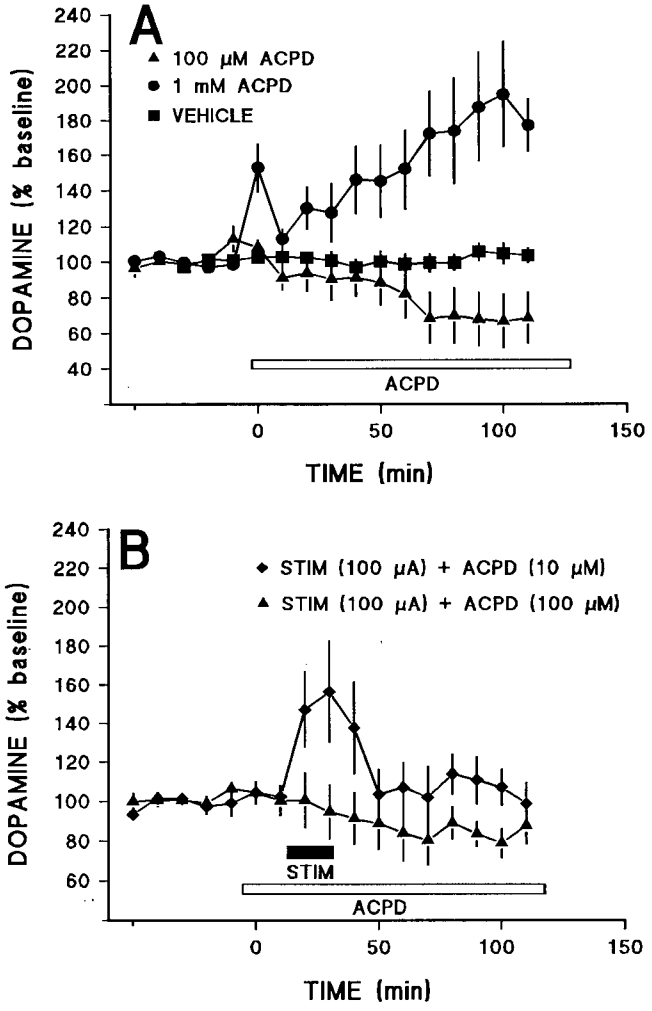


Figure 3.5. Dialysis concentrations of DA in the NAc during **A**) administration of ACPD (100 μM; triangles, 1 mM; circles) or vehicle solution (squares; n = 5, all groups) **B**) PFC stimulation (100 μA) and concurrent local administration of ACPD (10 μM; diamonds, 100 μM; triangles; n=5 per group). Black bar indicates the onset and duration of electrical stimulation; clear bars indicate the onset and duration of local drug application.

extracellular DA was monitored during electrical stimulation of the VTA with and without concurrent local administration of ACPD (100 μM). VTA stimulation (20 μA) caused a significant increase in DA in the NAc to 55% above baseline ($p < 0.01$; see Fig. 3.5). During local application of ACPD, stimulation of the VTA failed to significantly raise extracellular DA concentrations in the NAc ($p > 0.05$).

Discussion

These results demonstrate that phasic activation of the medial PFC increases DA release in the NAc. The stimulation-evoked increase was TTX sensitive and varied directly as a function of current intensity. The peak increase in extracellular DA obtained during the 100 μA stimulation period was 69% above baseline. As shown in Chapter I, identical stimulation currents applied to the dorsal PFC caused an increase of 38% in the dorsal striatum. This finding is consistent with previous studies showing that a variety of stimuli increase extracellular DA to a greater extent in the NAc than in the dorsal striatum (Abercrombie et al. 1989; Damsma et al. 1992). When applied locally via reverse dialysis, the ionotropic Glu receptor antagonists MK-801 and KYN failed to attenuate the increase in DA release produced by PFC stimulation. This suggests that ionotropic Glu receptors intrinsic to the NAc do not mediate this effect of PFC stimulation. The metabotropic Glu receptor agonist ACPD, also applied locally, blocked the increase in DA release. ACPD also effectively blocked the increase in DA release produced by electrical stimulation of

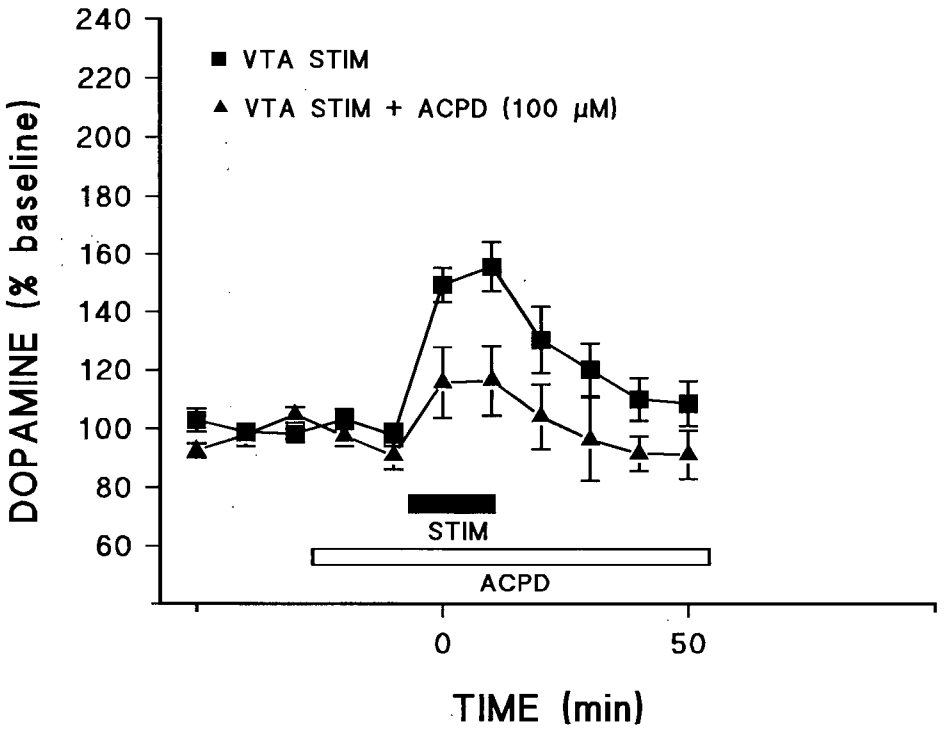


Figure 3.6. Dialysis concentrations of DA in the NAc (n = 5) during VTA stimulation (20 μA, pulse duration = 0.2 s, interstimulus interval = 1 s) and concurrent local administration of ACPD (100 μM; triangles) or vehicle (squares). Black bar indicates the onset and duration of electrical stimulation; clear bar indicates the onset and duration of local drug application.

the VTA. These findings indicate that activation of metabotropic Glu receptors has an inhibitory effect on stimulated DA release in the NAc.

Stimulation currents were chosen on the basis of previous studies which showed that rats will self-stimulate the PFC and VTA at approximately the same intensities and train frequencies as those used in the present study (Phillips and Fibiger 1978; Fiorino et al. 1993). Unlike in the previous chapter, during PFC stimulation the animals became active, probably reflecting the different site of stimulation. The animals demonstrated non-stereotyped sniffing, locomotion, and some rearing, although the variation between animals was substantial; they showed no behavioral abnormalities such as seizures or specific motor responses. These observations suggest that the stimulation currents used in the present study can be considered behaviorally relevant. Circling behavior was not apparent during bilateral PFC stimulation. In contrast, during unilateral VTA stimulation, animals often rotated contralateral to the stimulation, consistent with a unilateral increase in DA release (Ungerstedt 1971).

Due to the fact that the stimulating electrodes in the ventral PFC were 2-3 mm from the NAc dialysis probe, it was important to establish that the effect of stimulation was not due to direct depolarization of the DA terminals as a result of current spread. When the Na^+ channel blocker TTX was applied locally to the NAc, DA was no longer detectable in the dialysate, and PFC stimulation failed to increase DA release to detectable levels. Given that the detection limit of our assay was 5 fmol/sample, the stimulation could not have increased DA release by this amount. In contrast, an increase of 35 fmol/sample was observed in rats stimulated without

concurrent TTX treatment. These results indicate that the effect of PFC stimulation was impulse-dependent and not due to current spread.

Local administration of the specific Glu antagonists MK-801 and KYN through the dialysis probe produced dose-dependent increases in extracellular DA in the NAc in agreement with the findings of previous studies (Imperato et al. 1990b; Keefe et al. 1992). While the increase in DA release was small and required considerable time to appear, it was consistent across animals. The increases were produced by high concentrations of the antagonists and may have involved non-specific (i.e. non-Glu receptor) actions of the compounds. It is noteworthy in this regard that MK-801 potently activates DA neurons in the VTA (French et al. 1993; Murase et al. 1993b), and French et al. (1993) concluded that this effect was due to effects of MK-801 not involving NMDA receptors because competitive NMDA antagonists failed to mimic the response. Kynurenic acid was employed in the present study because it is one of the few Glu analogues with antagonist properties at all of the ionotropic receptor subtypes (Perkins and Stone 1982). When concentrations of MK-801 or KYN that did not by themselves affect DA release were applied to the NAc in combination with electrical stimulation to the PFC, the increase in DA release did not differ from that evoked by stimulation alone. Indeed there was a tendency for DA release to be slightly potentiated in this circumstance, although this effect did not reach statistical significance. Following the stimulation period, extracellular DA returned to baseline levels within 30 min in both treated and control rats. This provides further evidence that neither of these drug treatments interacted with the stimulation-induced increases in DA release. It is unlikely that the

lack of effect could be due to insufficient drug concentrations being applied through the dialysis probe. Previous studies of dialysis probe recoveries suggest that approximately 5-10% of a solute crosses the dialysis membrane (Kendrick 1988). This indicates that in the present experiments during PFC stimulation extracellular concentrations at the probe surface would reach at least 50-100 nM and 50-100 μM for MK-801 and KYN, respectively. MK-801 at these concentrations should effectively block NMDA receptors in the vicinity of the dialysis probe since the K_d for this compound in the striatum is 9.0 nM (Wong et al. 1988). This concentration of MK-801 has also been shown to attenuate the effect of local NMDA (1 mM) on DA release in the striatum (Morari et al. 1993). Electrophysiological studies have found that KYN in this dose range antagonizes the postsynaptic effects of Glu at both NMDA and non-NMDA Glu receptors in the striatum (Herrling 1985; Mereu et al. 1991). The concentration of KYN used in the present study was found to block the effects of locally applied Glu and NMDA on DA release (see Chapter VI). Kynurenic acid (100 μM) has also been found to attenuate NMDA (50 μM) induced DA release in a slice superfusion preparation (Krebs et al. 1991). We conclude that the increase in DA release produced by stimulation of the PFC occurs independently of ionotropic Glu receptors in the NAc.

Studies showing that Glu receptor antagonists applied to the striatum fail to decrease DA release preclude a tonic role for Glu in maintaining basal DA release (Imperato et al. 1990b; Moghaddam and Gruen 1991; Keefe et al. 1992). The present results confirm and extend these findings and suggest that ionotropic Glu receptors in

the NAc do not contribute to the increased DA release that is evoked by a *phasic* stimulus that is presumed to increase Glu release in the NAc. This finding agrees with the results of a recent study showing that ionotropic Glu receptor antagonists applied to the dorsal striatum fail to block the increase in DA release induced by pharmacological stimulation of the PFC (Karreman and Moghaddam 1996). Similarly, Keefe et al. (1993) have demonstrated that stress-induced DA release in the dorsal striatum also occurs independently of local Glu receptors. Taken together, these results suggest that neither basal nor stimulated DA release is regulated by ionotropic Glu receptors in the striatum.

The present results indicate that Glu may have inhibitory effects on basal and stimulated DA release via activation of metabotropic receptors within the NAc. Thus, when PFC stimulation was applied in the presence of ACPD in the NAc, no increase in DA release was observed. This appeared to be a general phenomenon as ACPD also blocked the increases in DA release produced by electrical stimulation of the VTA. The percent increase in DA release produced by stimulation in the present study (55%) is similar to that found previously in rats self-stimulating or receiving experimenter delivered stimulation at the same currents (Fiorino et al. 1993). The effects of ACPD on stimulation-induced increases in DA release could be either direct or indirect. With respect to direct effects it is noteworthy that in mesencephalic brain slices ACPD has been observed to depolarize and increase the firing rates of presumed DA neurons (Mercuri et al. 1993). These neurons have recently been demonstrated to express mRNA for one of the metabotropic Glu receptor subtypes (mGlur1: Martin et al. 1992; Shigemoto et al. 1992; Testa et al. 1994). In addition,

the VTA and the NAc demonstrate metabotropic Glu receptor binding sites (Albin et al., 1991). These findings, together with the present results, raise the possibility that DA neurons synthesize and then axonally transport mGlur1 receptors to their terminal regions where they serve to regulate DA release. With respect to possible indirect effects, Lovinger and coworkers (1991; 1993) have provided electrophysiological evidence in striatal slice preparations that activation of metabotropic Glu receptors inhibits Glu release at corticostriatal synapses. It remains possible that the effects of ACPD observed in the present experiments were mediated indirectly via actions on metabotropic Glu receptors located on non-dopaminergic elements.

Application of ACPD produced biphasic, dose-related effects on DA release with the lower concentration (100 μM) causing a delayed decrease and the higher concentration (1 mM) causing a gradual increase (Fig. 4A). Inasmuch as subtypes of metabotropic Glu receptor have different affinities for ACPD (Schoepp and Conn 1993), the biphasic response raises the possibility that metabotropic Glu receptor subtypes may have opposing actions on DA release in the NAc. The increase in DA release produced by the higher concentration of ACPD is consistent with two behavioral studies which have reported that locally applied ACPD increases contralateral turning behavior in rats (Sacaan et al. 1991; 1992). In the later report it was demonstrated that this behavioral response to ACPD could be blocked with α-methyl-p-tyrosine induced depletions of DA or by the DA receptor antagonist haloperidol. In addition, ACPD increased tissue concentrations of DOPAC and HVA in the striatum (Sacaan et al. 1992).

The anatomical substrate through which stimulation of the PFC enhances DA release remains to be determined. The present results suggest that the corticostriatal Glu projection is not directly involved. Antidromic stimulation of meso-cortical DA axons leading to activation of meso-accumbens DA collaterals cannot account for the present findings as few if any DA-containing neurons project to both PFC and NAc (Lindvall and Bjorklund 1983). A more plausible mechanism involves the projection from the PFC to the VTA (Sesack and Pickel 1990). Gariano and Groves (1988) have shown that electrical stimulation of the PFC produces bursts of action potentials in mesencephalic dopaminergic neurons of anesthetized rats. This pattern of electrophysiological activity is associated with increases in DA release from the terminals of these neurons (Nissbrandt et al., 1994). In a study that combined electrophysiological and voltammetric techniques in anesthetized rats, Murase et al. (1993a) demonstrated that activation of the PFC by intracerebral injections of Glu increased burst firing of single dopaminergic neurons in the VTA and enhanced DA release in the NAc. Finally, Glu receptor antagonists applied to the VTA have been shown to block the increase in striatal DA release produced by pharmacological stimulation of the PFC (Karreman and Moghaddam 1996). The present results obtained in awake, freely moving animals are entirely consistent with these observations and suggest that the PFC stimulation-induced increases in accumbal DA release may have been due to the activation of the cortico-VTA projection, which in turn increased burst firing of the dopaminergic neurons in the VTA.

On the basis of behavioral studies, the PFC has long been thought to inhibit subcortical DA mechanisms. For example, DA-mediated behaviors such as

amphetamine-induced stereotypy and locomotor activity are enhanced by PFC lesions (Adler 1961; Iversen et al. 1971; Jaskiw et al. 1990). On the other hand, while lesions of the PFC have been reported to produce transient (2 week) increases in measures of subcortical DA turnover (Jaskiw et al 1990), most studies have failed to observe such effects (Scatton et al. 1982; Wilkinson et al. 1993). Notably, a recent microdialysis study found that PFC lesions did not affect amphetamine-induced DA release 5 or 15 days after a PFC lesion, times at which the same study showed amphetamine-induced locomotor activity to be enhanced (Whishaw et al. 1992). The present findings point to an excitatory influence of the PFC on subcortical DA and are consistent with the results of other stimulation studies that have examined this issue (Nieoullon et al, 1978; Murase et al., 1993a; Taber and Fibiger, 1993). It is unlikely therefore, that PFC lesions enhance DA-mediated behaviors by "disinhibiting" subcortical DA systems.

In conclusion, the increase in DA release in the NAc evoked by activation of the PFC does not appear to be mediated by ionotropic Glu receptors intrinsic to the NAc. On the other hand, local application of the metabotropic Glu receptor agonist ACPD attenuates the effect of PFC stimulation on DA release in the NAc. A general inhibitory effect of ACPD on evoked DA release is indicated as this compound also antagonized increases in DA release produced by stimulation of the VTA. These data implicate metabotropic Glu receptors in the NAc in mediating Glu/DA interactions in limbic striatum.

IV. The Prefrontal Cortex Regulates Dopamine Release In The Nucleus Accumbens Via Projections To The Ventral Tegmental Area

Introduction

Results presented in the previous chapter indicate that electrical stimulation of the PFC can evoke DA release in the NAc, and that this effect is not mediated via Glu receptors in the NAc. Thus the cortico-accumbens projection is not involved in PFC stimulation induced DA release. Further experiments were performed to investigate the role of another pathway, the PFC-VTA projection, in modulating accumbens DA release. Efferents from the PFC project to nuclei containing the perikarya and the terminals of the meso-accumbens DA projection, and these projections are thought to utilize an excitatory amino acid such as Glu as a neurotransmitter (Christie et al. 1985; Sesack et al. 1989; Sesack and Pickel 1990). Thus, the PFC may regulate DA release in the NAc via an indirect, cortico-midbrain-accumbens pathway.

This hypothesis is supported by a variety of neurochemical and physiological data. For example, DA release in the dorsal striatum can be influenced by the PFC, and the anatomical substrate for this effect appears to include glutamatergic afferents to the midbrain (Karreman and Moghaddam 1996). This finding is consistent with reports demonstrating that stress-induced increases in DA release are not attenuated by application of Glu receptor antagonists to the NAc or dorsal striatum (Keefe et al. 1993; Gratton and Doherty 1994). Electrophysiological studies in slice preparations have shown that Glu receptors mediate excitatory responses in the VTA (Johnson and North 1992; Wang and French 1993).

Furthermore, Glu receptor activation in the VTA increases extracellular concentrations of DA in the NAc (Kalivas et al. 1989).

The present experiments sought to determine whether DA release in the NAc evoked by stimulation of the PFC can be attenuated by application of Glu receptor antagonists to the VTA. To this end, dialysis probes were implanted unilaterally in the VTA and the ipsilateral NAc in conjunction with bilateral stimulating electrodes in the PFC. Extracellular DA concentrations were monitored in the NAc during application of Glu receptor antagonists to the VTA before and during electrical stimulation of the PFC. In some rats a solution containing the fluorescent retrograde tracer Fluorogold was subsequently infused through the dialysis probe in the VTA to determine the sources of cortical afferents to this area. This technique enabled confirmation that the area of the PFC being stimulated projects directly to the area of the VTA containing the dialysis probe.

Materials and Methods

Male Wistar rats weighing 300-325 g were anesthetized with 50-60 mg/kg pentobarbital (i.p.) and placed in a stereotaxic apparatus. Microdialysis probes were implanted in the NAc and electrodes were implanted in the PFC as described in the previous chapter. A similar dialysis probe with 1.0 mm of active membrane was implanted into the VTA; coordinates were A: -5.3 mm, L: +0.7 mm, V: -8.6 relative to bregma according to the atlas of Paxinos and Watson (1986). Chapter II contains the details of the microdialysis procedure and Chapter III describes the details of the stimulation protocol.

All drugs were administered locally in the VTA via reverse dialysis. Compounds were dissolved in the perfusion medium and pH was adjusted to 7.2-7.4 with dilute NaOH. 2-Amino-5-phosphonopentanoic acid (AP5) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were obtained from Research Biochemicals Inc. (Natick, MA). Tetrodotoxin was purchased from Sigma Chemical Co. (St. Louis, MO). All drugs were diluted from frozen aliquots immediately prior to use.

To confirm that the area of the VTA containing the dialysis probe received a projection from the area of the PFC containing the stimulating electrodes, Fluorogold was perfused through the VTA probe in four rats using the same procedure described in the previous chapter. All rats used in the study were confirmed to have dialysis probes located in the NAc and VTA and electrodes in the PFC.

For statistical analysis all data were represented as percent baseline, calculated as the three samples preceding experimental manipulation. A one-way ANOVA with repeated measures across time was employed to test each individual treatment. In all cases Huynh-Feldt corrections for degrees of freedom were used to account for time as a repeated measure. Post hoc analysis was performed with Dunnett's test.

Results

The mean basal dialysate DA concentration of all animals was 41.6 ± 6.1 fmol/sample ($n = 15$). Basal DA concentrations did not differ significantly between experimental groups ($p > 0.05$).

As shown in Fig. 4.1A, application of AP5 (200 μM) and CNQX (50 μM) to the VTA decreased dialysate DA concentrations obtained from the NAc ($p < 0.01$). Post hoc analysis showed that the third sample following initiation of drug application was significantly lower than baseline. The maximal decrease observed during the 70 min drug administration period was 67% of baseline. When the solution perfusing the VTA probe was switched back to vehicle, DA concentrations returned to baseline values within three samples. To assess whether perfusion alone had any effect on accumbal DA release, DA concentrations in three rats were measured prior to the onset of vehicle perfusion through the VTA probe. None of these rats showed a change in dialysate DA as a result of vehicle perfusion (data not shown).

Stimulation of the PFC increased extracellular DA in the NAc (Fig. 4.1B) to a peak of 157% of baseline ($p < 0.01$). Dialysate concentrations of DA returned to baseline during the sample taken immediately after the stimulation period.

When CNQX and AP5 were applied to the VTA beginning 20 min prior to the 20 min PFC stimulation period (Fig. 4.1B), electrical stimulation failed to affect accumbal DA release ($p > 0.05$). Thus, administration of the antagonists to the VTA blocked the effect of PFC stimulation on DA release. This was not simply an additive effect since stimulation alone caused an increase of 57% whereas the antagonists decreased DA by only 20% in the period during which stimulation was applied. To confirm that the VTA dialysis probe was in a position to affect meso-accumbens DA neurons, a solution containing TTX (1 μM) was perfused through the VTA probe at the end of the experiment in four of the rats that received the combination of stimulation and drug treatment (Fig. 4.1B). This treatment caused a

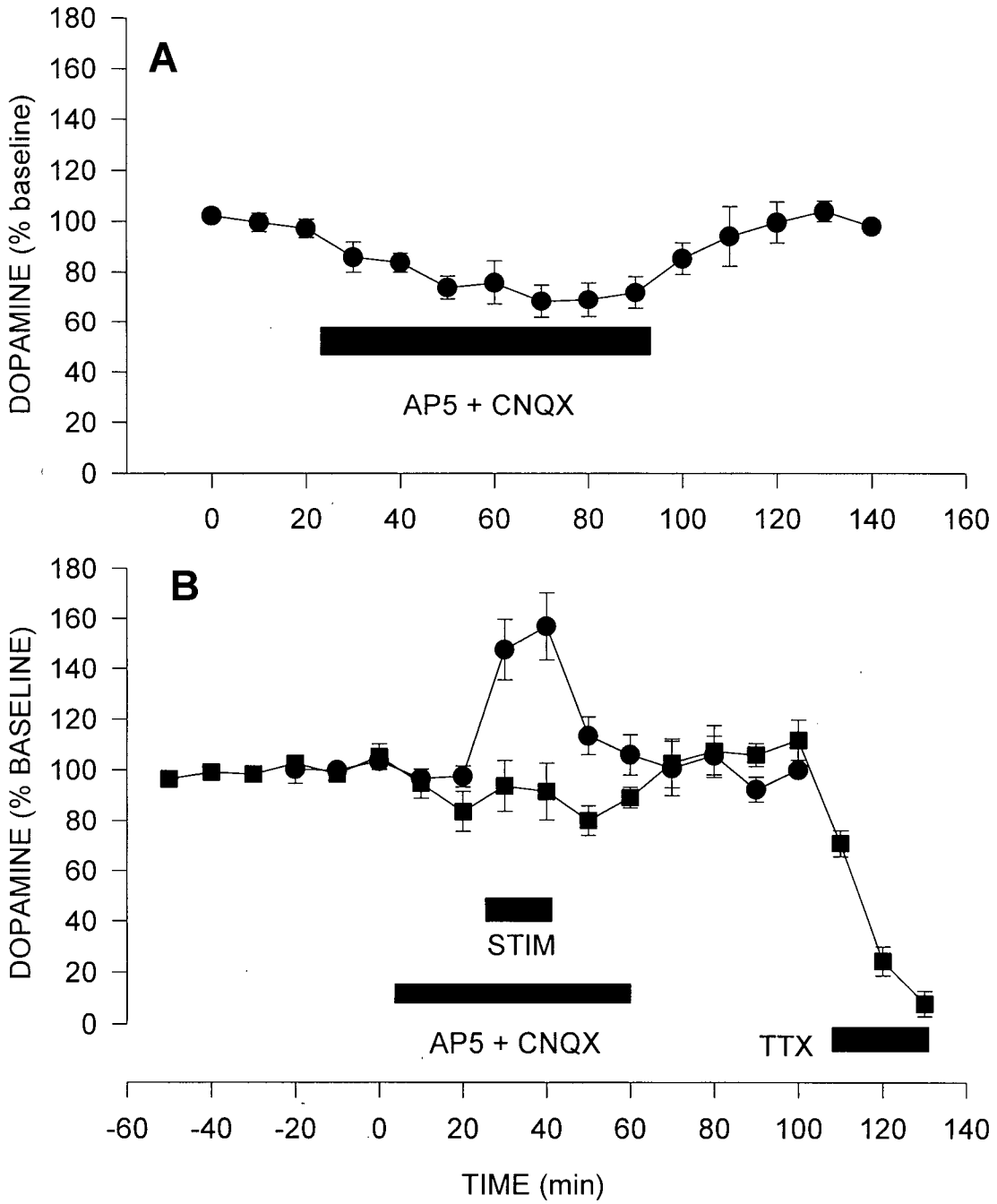


Figure 4.1. Dialysate DA concentrations sampled from the NAc. **A)** A combination of AP5 (200 μM) and CNQX (50 μM) applied locally via reverse dialysis in the VTA decreased extracellular DA. **B)** Electrical stimulation of the PFC (100 μA) during vehicle infusion into the VTA increased DA release (circles). Application of AP5+CNQX in the VTA blocked the effect of stimulation on DA release (squares). Data represent the mean ± SEM.

rapid, massive decrease in extracellular DA ($p < 0.01$). Dopamine was no longer detectable by the fourth sample of TTX application in any of the four rats. In one rat the midbrain dialysis probe was placed 1 mm above the VTA due to an error in stereotaxic surgery. In this animal application of the Glu receptor antagonists failed to reduce the effect of stimulation, there being a 75% increase in DA release during stimulation.

Figure 4.2 shows a schematic diagram of a coronal section of the PFC, corresponding to the location of the stimulating electrodes (A: +5.0: Pellegrino et al. 1979). Retrogradely labeled perikarya were observed in the prelimbic, infralimbic, anterior cingulate, and insular cortices. Labeling was predominantly limited to the side of the PFC ipsilateral to the site of Fluorogold perfusion, although some contralateral labeling was also observed.

Discussion

The present experiments demonstrate that ionotropic Glu receptor activation in the VTA regulates basal and stimulation-evoked DA release in the NAc. Application of a combination of AP5 and CNQX to the VTA reduced and TTX application eliminated accumbal DA release. This finding indicates that basal DA concentrations in the NAc are critically dependent on midbrain DA cell activity and that glutamatergic inputs to the VTA influence this basal activity. Application of the same antagonists to the VTA during electrical stimulation of the PFC blocked the effect of stimulation on DA release. This finding confirms that the PFC regulates accumbal DA neurotransmission and indicates that this effect is dependent upon ionotropic Glu receptors in the VTA.

This conclusion is consistent with other studies examining the effects of phasic excitation of the PFC on DA neurons in the VTA. Gariano and Groves (1988) showed that

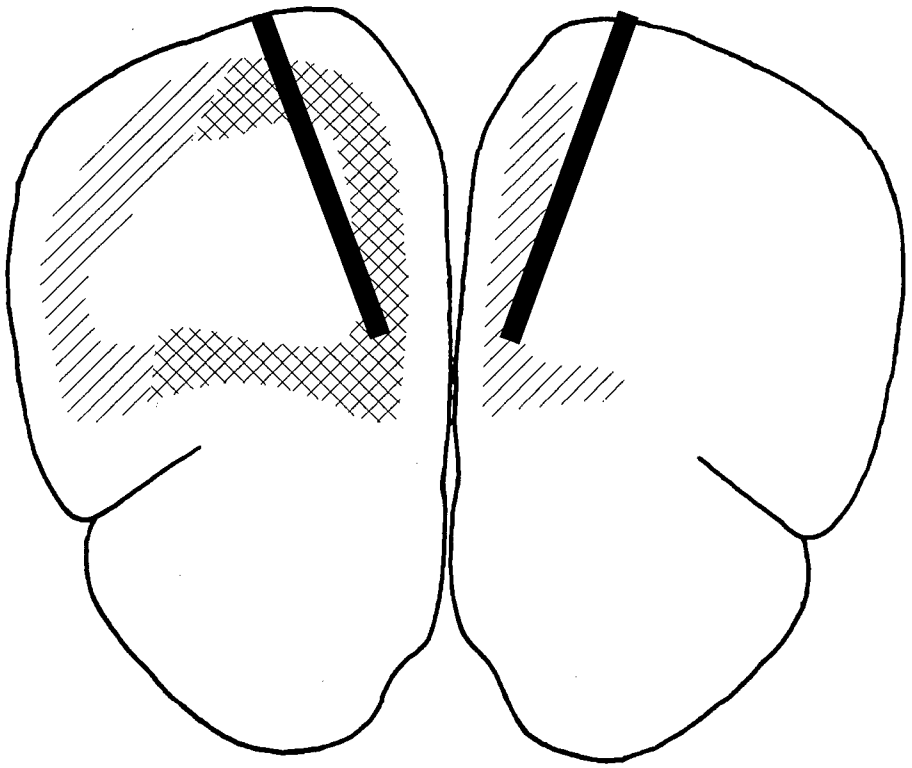


Figure 4.2. Schematic diagram of a coronal section through the PFC indicating typical electrode placements and fluorescent labeling following infusion of Fluorogold through the VTA dialysis probe. Cross hatching represents greater labeling.

electrical stimulation of the PFC increased burst firing in identified DA neurons of the VTA. Another study used a combination of single cell recording and voltammetric measurement of DA to show that pharmacological stimulation of the PFC increased burst firing in the VTA concomitant with increased DA release in the NAc (Murase et al. 1993a). Studies showing that Glu receptor antagonists applied to the dorsal or ventral striatum have no effect on DA release induced by PFC stimulation or by environmental stressors indicate that ionotropic Glu receptors in the NAc are not involved in the regulation of DA release under a wide variety of conditions (previous chapter; Keefe et al., 1993; Gratton and Doherty, 1994). Another report, however, found that an NMDA antagonist applied to the dorsal striatum was able to block stress induced DA release in this area (Wheeler et al. 1995). However, the data of Wheeler et al. (1995) are suspect due to the lack of Mg^{++} in the perfusion medium and the high concentration of MK-801 that was employed (100 μM).

The combination of drugs used in the present study, AP5 and CNQX, was intended to block all ionotropic Glu receptors in the VTA. This was deemed necessary because excitatory responses in the VTA are mediated by both NMDA and non-NMDA ionotropic Glu receptors (Wang and French 1993). Indeed, excitatory responses can be mediated by both types of receptors on the same VTA neuron (Johnson and North 1992). For this reason, no attempt was made to separate the NMDA and non-NMDA components of the response.

The present results suggest that basal extracellular DA in the NAc is dependent on the activity of DA perikarya inasmuch as application of TTX to the VTA reduced extracellular DA in the NAc to undetectable levels. This finding agrees with reports showing that TTX applied to the midbrain eliminates extracellular DA in the dorsal striatum (Robertson et al.

1991; Keefe et al. 1993). The present study also shows that the activity of midbrain dopaminergic neurons is specifically dependent on glutamatergic afferents as evidenced by the decrease in basal DA resulting from application of CNQX and AP5 to the VTA. An electrophysiological study has specifically implicated the cortico-midbrain-accumbens projection in mediating basal DA release by showing that cooling of the PFC transiently reduces cell firing in VTA neurons (Svensson and Tung 1989). These results provide further evidence that cortical regulation of DA neurotransmission in the NAc occurs via midbrain mechanisms.

In the present study it was demonstrated that neurons in the vicinity of the PFC electrodes project directly to the area containing the VTA dialysis probe. This finding is consistent with the possibility that the projection from the PFC to the VTA mediated the increase in accumbal DA release. Also consistent with this hypothesis are anatomical and biochemical studies which suggest that this projection uses Glu as a neurotransmitter (Christie et al. 1985), and that it has direct synaptic connections with dopaminergic neurons (Sesack and Pickel 1990). However, at least three other pathways could mediate this effect. 1) The PFC projects to the habenula which in turn projects to the VTA (Beckstead 1979; Herkenham and Nauta 1979). In fact, in a slice preparation stimulation of the fasciculus retroflexus, which connects the habenula to the VTA, evokes excitatory postsynaptic potentials in VTA neurons (Matsuda and Fujimara 1992). 2) The PFC projects to the subthalamic nucleus which also innervates the VTA (Kita and Kitai 1987). 3) The PFC projects to pontine nuclei which project to VTA (Semba and Fibiger 1992). These and other pathways involving limbic structures such as the amygdala and hippocampus may also have contributed to the observed phenomena.

V. Feeding-Evoked Dopamine Release in the Nucleus Accumbens: Regulation by Glutamatergic Mechanisms

Introduction

The previous two chapters show that Glu receptors in the VTA but not the NAc mediate increases in DA release in the NAc evoked by electrical stimulation of the PFC. A similar observation has been made for DA release evoked in the limbic striatum by chemical stimulation of the PFC (Karreman and Moghaddam 1996). Other studies have examined the role of Glu receptors in the NAc and VTA in modulating stress induced DA release. Gratton and Doherty (Gratton and Doherty 1994) found that an NMDA receptor antagonist applied in the NAc potentiated the DA response to stress. In contrast, another study found that neither NMDA nor non-NMDA receptor antagonists applied in the NAc affected the amplitude of the increase in DA release evoked by conditioned stress (Saulskaya and Marsden 1995). In other investigations NMDA antagonists given locally in the VTA have been reported to attenuate stress-induced DA turnover in the PFC (Kalivas et al. 1989; Morrow et al. 1993), but not in the NAc (Morrow et al. 1993).

The present study was designed to compare directly the role of Glu receptors in the NAc and VTA in modulating the enhanced DA release in the NAc that occurs during some types of motivated behavior. The behavior selected for the present study was feeding in food-deprived rats. Dopaminergic mechanisms are known to be critically involved in the regulation of food intake (Ungerstedt 1971; Zhou and Palmiter 1995), and robust increases in DA release during feeding behavior occur in a variety of situations including scheduled feeding, lever pressing for food reward, and consumption of a palatable meal (Church et al.

1987; Radhakishun et al. 1988; Hernandez and Hoebel 1988; Yoshida et al. 1992; Wilson et al. 1995).

Materials and Methods

Training

Male Wistar rats weighing 280-320 g at the beginning of the experiment were housed individually with a 12:12 h light-dark cycle (lights on at 7:00 AM), and food (Ratdiet, PMI Feeds) and water were available ad libitum except where indicated. The initial training was performed in the rat's home cage. At 5:00 PM on the evening prior to the first day of training food was removed from the animal's cage although water was still available. Eighteen hours later, food was placed in the cage and the rat had the opportunity to eat until 5:00 PM when the food was removed again. Following a second 18 h interval, rats again had the opportunity to eat. After at least two hours of food availability, rats underwent stereotaxic surgery for implantation of microdialysis probes (described below). Following surgery, the rats were housed in Plexiglas cages with a plastic mesh floor suspended 1" above the animal's bedding (Crown Inc.). During the first 24 recovery period the animals again had free access to food and water. The following day food was again removed at 5:00 PM although some food remained in the bottom of the cage, unavailable to the rat, to retain habituation to the smell of the food. Experiments were initiated the following day shortly after the onset of the light cycle. Food was placed in the cage between 10:00 and 11:00 AM, after approximately 18 h of deprivation, at which time the rat had access to food for two hours. The latency to begin eating, and the volume of food consumed over the two hour period were recorded.

Surgery

Rats (n = 52) were anesthetized with equithesin and implanted in the NAc with a vertical, concentric type dialysis probe at coordinates A: +3.5, L: +1.5, and V: -7.8 relative to bregma according to the atlas of Pellegrino et al. (1979). In 21 rats a second dialysis probe was implanted in the VTA at coordinates A: -5.3, L: +0.7, V: -8.6 relative to bregma according to the atlas of Paxinos and Watson (1986). Microdialysis procedures were identical to those used in the previous chapter.

Histology

In four rats with dual probes Fluorogold was perfused through the microdialysis probe in the NAc at the end of the experiment. Fluorescent labeling was examined in the VTA to confirm that the VTA probe was appropriately situated to perfuse cells that terminated in the region of the NAc perfused by the dialysis probe.

Results

The amount of food consumed and the latency to eat on the experimental day for all groups are shown in Table 5.1. The mean food consumption for all rats during the 2 h test period was 7.2 ± 0.43 g (mean ± SEM: n=36), and there was no difference in food consumption between the groups ($p > 0.05$). Training also did not significantly affect the volume of food consumed as there was no difference in food consumption across the two days of training and the experimental day ($p > 0.05$: data not shown). Rats did not lose weight during the course of the experiment; they typically lost less than 10% of body weight during each overnight deprivation period and recovered this weight during the feeding

Table 5.1 Amount of food consumed and latency to eat on the experimental day

Group	**Food (g) ± SEM**	**Latency (sec) ± SEM**
No VTA Probe		
Vehicle	6.97 ± 0.7	25.2 ± 6.0
KYN	6.5 ± 1.9	22.8 ± 5.4
ACPD	7.6 ± 0.9	52.8 ± 12
With VTA Probe		
Vehicle	8.3 ± 1.0	25.2 ± 8.4
AP5+CNQX	6.9 ± 0.9	24.0 ± 4.8

In no case did food consumption or latency to eat differ from control.

period. Because rats typically gained 25 g after eating less than 10 g of food, the primary factor affecting weight appeared to be water consumption. The mean latency to eat for all rats was 24 ± 3.6 s (mean ± SEM: n = 36) and there was no difference between groups ($p > 0.05$). Typically the rats fed continuously for about 20 min with pauses for drinking and grooming, and then went to sleep. Often later during the feeding session rats fed again sporadically.

Microdialysis

Basal DA concentration in the NAc for all rats (n = 52) was 34.7 ± 4.81 (mean ± SEM) fmol/sample, and no difference in basal values was observed between groups. Feeding significantly elevated extracellular DA concentrations to a peak of 51% above baseline ($p < 0.03$: Fig. 5.1). Dopamine concentrations declined toward baseline 90 min after food presentation, while food was still available. The greater variability observed in the DA signal after 80 min reflects sporadic increases in DA release evoked during later periods of eating (after the initial bout). Removal of food from the cage after 2 h (Fig. 5.1) had no further effect on DA concentrations ($p > 0.05$). Because the increase in DA release peaked during the first four samples following the introduction of food, the effects of drug treatments were examined over this period.

Local application of KYN (1 mM) in the NAc did not attenuate the effect of feeding on DA release; indeed the increase in DA release during feeding was enhanced ($p < 0.05$: Fig. 5.2). Treatment with KYN increased the peak effect of feeding from 51% above baseline to 94% above baseline when applied at a concentration that did not itself affect basal DA concentrations (Fig. 5.2).

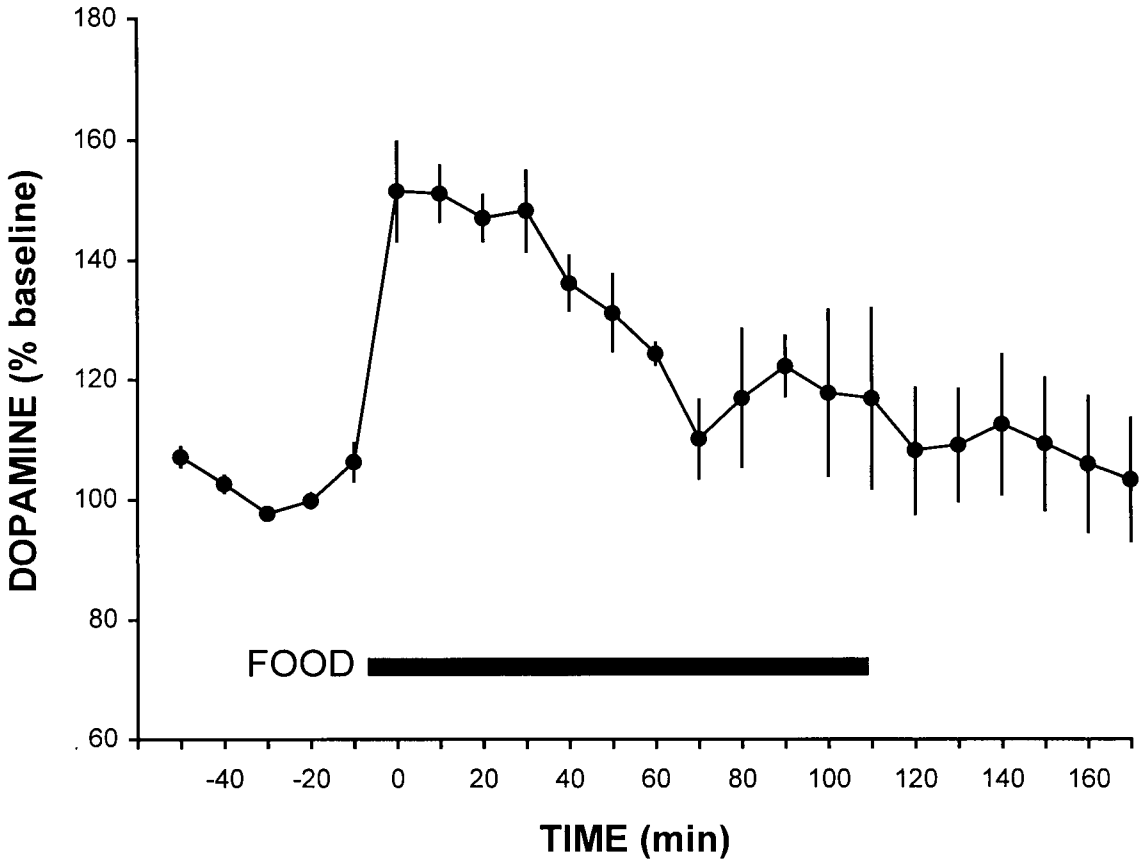


Figure 5.1. Effects of feeding on dialysate DA concentrations obtained in the NAc. In this and all following figures data are represented as the mean ± SEM of percent baseline, calculated as the mean of the first three samples shown. Black bar indicates the duration of food availability.

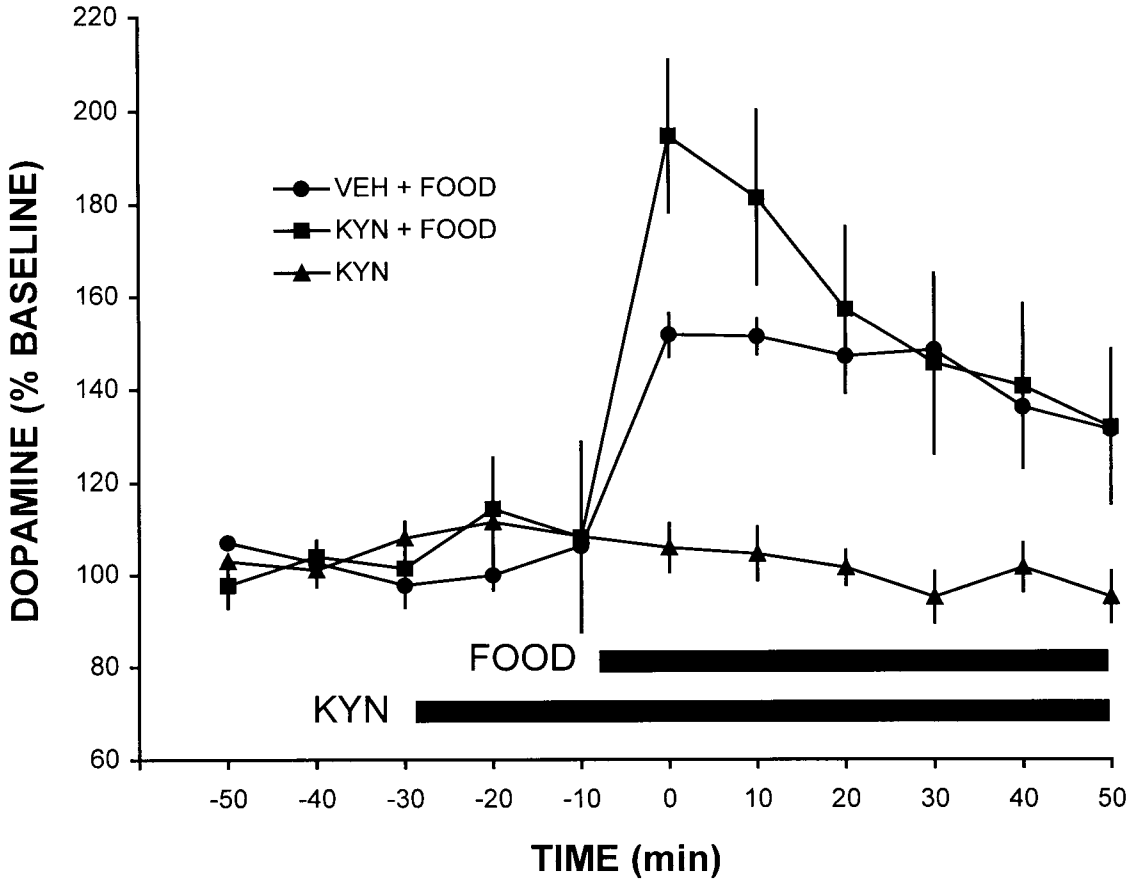


Figure 5.2. Effects of local application of kynurenic acid (1 mM) through the NAc dialysis probe during feeding on dialysate DA concentrations obtained in the nucleus accumbens. The feeding group shown in this figure is the same one shown in figure 5.1. Black bars indicate the duration of food availability and local drug application.

Local application of ACPD (100 μM) in the NAc blocked the effect of feeding on DA release ($p < 0.05$: Fig. 5.3). However, ACPD did not affect basal DA concentrations over the time course employed in these experiments ($p > 0.05$). Results presented in Chapter III showed that ACPD at the same concentration can reduce extracellular DA when applied over a longer period of time.

In rats with VTA probes, feeding significantly increased DA release to 66% above baseline ($p < 0.001$: Fig. 5.4). The amplitude of the DA increase in rats with VTA probes was not significantly different than that observed in rats without VTA probes ($p > 0.05$), although the time course varied significantly ($p < 0.025$). The difference in time course reflected the fact that DA concentrations did not return to baseline after feeding in rats with VTA probes. Application of the combination of CNQX (50 μM) + AP5 (200 μM) in the VTA did not significantly affect DA release in the NAc when the data were analyzed over the 2 h application period ($p > 0.05$). However, the treatment did reduce extracellular DA concentrations in the first four samples ($p < 0.01$). The difference between the two analyses arises from the fact that in some rats DA concentrations returned to baseline after an initial decrease while in others it did not.

Treatment with AP5+CNQX reduced the mean DA concentration to 86% of baseline and feeding caused a subsequent increase to 105% of baseline (Fig. 5.4), a 19% increase that was statistically significant ($p < 0.01$). Analyzed as change in percentage of baseline, AP5+CNQX in the VTA greatly attenuated the effect of feeding on DA release in the NAc (Fig. 5.4: $p < 0.01$). However, because the drug treatment affected the baseline, the data were also analyzed in terms of the absolute the change in DA concentrations (fmol). Feeding

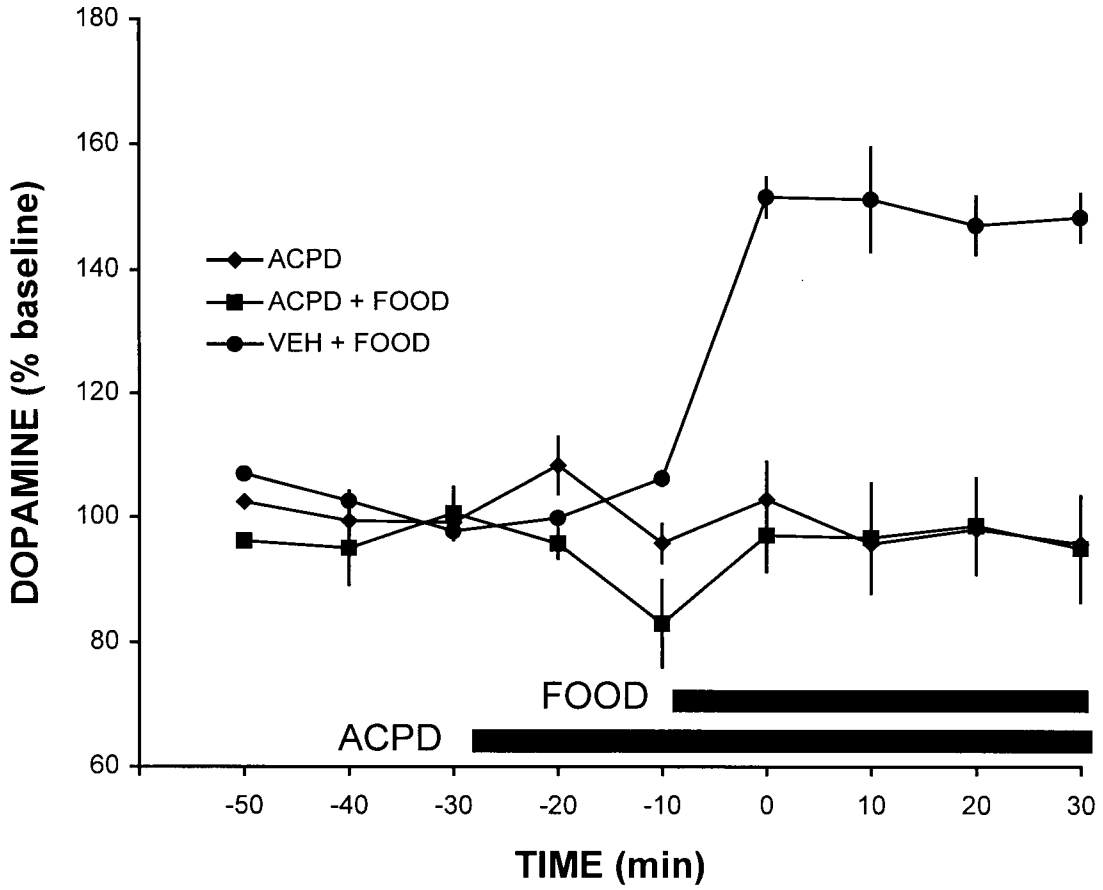


Figure 5.3. Effects of local application of ACPD (100 μM) alone and in combination with feeding on dialysate DA concentrations obtained from the NAc. The feeding group represented in this figure is the same one shown in figure 5.1. VEH refers to saline vehicle. Black bars indicate the duration of food availability and local drug application.

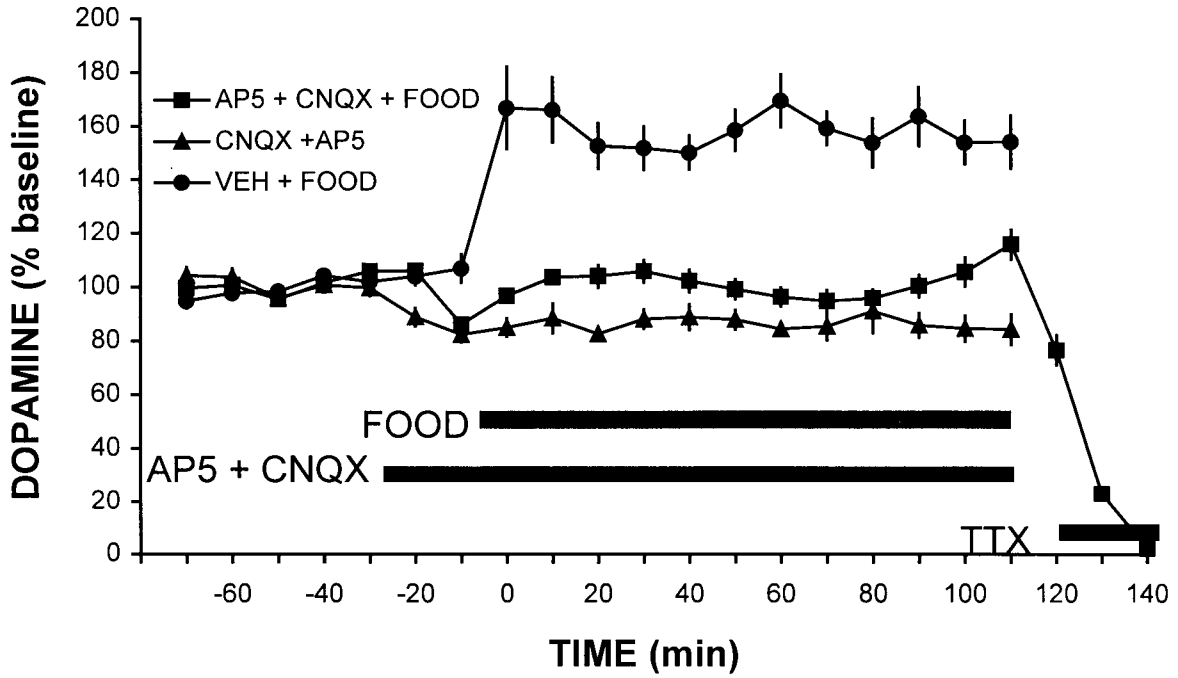


Figure 5.4. Effect of local application of AP5 (200 μM) +CNQX (50 μM) in the VTA alone and in combination with feeding on dialysate DA concentrations obtained in the NAc. Tetrodotoxin (1 μM) was applied through the VTA probe following the feeding period. Black bars indicate the duration of food availability and local drug application.

resulted in a peak increase of 12.4 ± 1.8 fmol/sample whereas AP5+CNQX treatment reduced this increase to 5.6 ± 2.2 fmol/sample. This represents a 55% reduction in the concentration of DA evoked by feeding ($p < 0.05$).

To confirm that the VTA dialysis probe was appropriately situated to affect DA cell bodies with projections to the region of the NAc containing the dialysis probe, two control experiments were performed. First, TTX (1 μM) was applied in the VTA following the end of the feeding period in rats that received AP5+CNQX during feeding. As shown in Figure 5.4, TTX reduced DA to undetectable amounts within four samples of application. Also, the pattern of fluorescent labeling in the VTA was examined after application of Fluorogold to the NAc via the dialysis probe. Two examples are shown in Figure 5.5. In all four rats examined, the VTA dialysis probe was situated amidst a group of fluorescently labeled, putatively dopaminergic, cells. Figure 5 also shows sample locations for the NAc and VTA dialysis probes in cresyl violet labeled sections.

Discussion

The primary finding of the present study is that feeding-evoked increases in DA release in the NAc are mediated by glutamatergic mechanisms in the VTA and not the NAc. Furthermore, Glu receptors of the metabotropic and the ionotropic subtypes located in the NAc appear to inhibit evoked DA release. These findings are highly consistent with the previous results addressing the effects of electrical stimulation of the PFC on DA release in the NAc presented in Chapters III and IV.

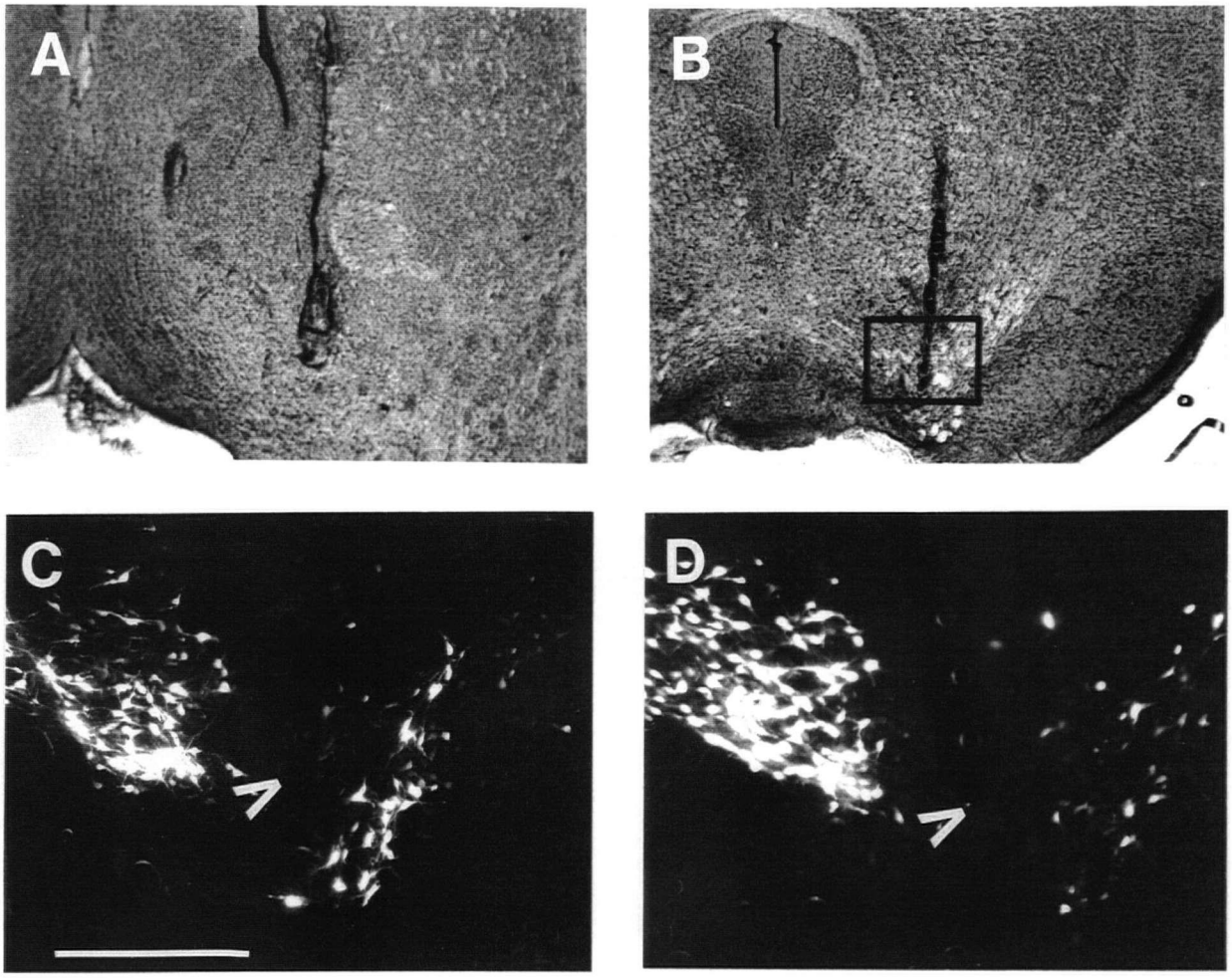


Figure 5.5. **A, B** Photomicrographs of coronal sections of the NAc (**A**) and VTA (**B**) stained with cresyl violet. The box in **B** indicates the approximate area shown in **C** and **D**. **C, D** Representative photomicrographs of the VTA from two rats showing meso-accumbens neurons labeled with fluorogold. Arrowheads indicate the location of the ventral tip of the microdialysis probes. Scale bar in **C** is 500 μm and also pertains to **D**.

Application of the broad spectrum ionotropic Glu receptor antagonist KYN, at a concentration (1 mM) that did not itself affect DA release, potentiated the increase in DA release associated with feeding. This is consistent with the finding that high concentrations of KYN can increase basal DA release (Chapter III; Keefe et al. 1992). These results suggest that under normal physiological conditions Glu in the NAc has inhibitory actions on DA release. Studies on stress-induced DA release offer some support for this hypothesis. Gratton and Doherty (1994) observed that Glu receptor antagonists applied locally in the NAc potentiated what they interpreted to be stress-induced DA release as estimated by voltammetry. Similarly, Keefe et al. (1993) found that local application of AP5 or CNQX enhanced the duration of stress-induced DA release in the striatum. While Chapter III indicates that the KYN did not significantly affect the increase in DA release evoked by PFC stimulation, there was a strong trend toward a potentiation of DA release. These observations are in marked contrast with results of a number of studies that have reported increased DA release in the NAc after local application of high concentrations of Glu receptor agonists (Imperato et al. 1990a; Svensson et al. 1994; Youngren et al. 1993), results which suggest that activation of Glu inputs to the NAc should increase DA release. The possible bases of these inconsistencies will be discussed in Chapter VI. Regardless, the present data suggest that under physiological conditions Glu concentrations do not rise sufficiently in the NAc to stimulate DA release and that Glu normally serves to inhibit DA release in this structure.

The mechanisms by which activation of ionotropic Glu receptors in the NAc inhibits DA release are uncertain. The most direct mechanism would be the presence of such receptors on DA terminals; however, as ionotropic receptors mediate depolarizations, this mechanism is not tenable. Furthermore, studies of synaptosomal preparations do not support

an inhibitory role for Glu receptors on DA terminals (Desce et al. 1992). Another possibility is that Glu acts at receptors located on interneurons in the NAc, activation of which could, in turn, inhibit DA release. The primary candidate here would be GABAergic neurons which make up the majority of accumbal neurons (Meredith et al. 1993). However, as GABA receptor agonists and antagonists applied locally to the striatum generally fail to affect DA release, such a mechanism is not likely involved (Tanganelli et al. 1994; Westerink et al. 1994). Another possibility is that activation of efferents from the NAc could inhibit DA release via actions on DA perikarya in the VTA. A GABA-containing projection from the NAc to the VTA has been identified (Kalivas et al. 1993), and activation of GABA receptors in the VTA decreases accumbal DA release (Westerink et al. 1996). In support of such an organization, electrical stimulation of the NAc decreases the activity of dopaminergic neurons in the VTA via a GABAergic mechanism (Yim and Mogenson 1980). At present, therefore, activation of GABAergic efferents from the NAc to the VTA seems to be the most likely mechanism by which Glu in the NAc inhibits DA release in this structure.

To examine the contribution of glutamatergic afferents to the VTA on feeding evoked DA release, a combination of an NMDA receptor antagonist (AP5) and a non-NMDA receptor antagonist (CNQX) was applied to the VTA. This combination of antagonists was used to block all ionotropic Glu receptors in the VTA, because both subtypes of receptor contribute to Glu-evoked EPSPs in DA neurons (Mereu et al. 1991). This treatment has been shown previously to block the effect of PFC stimulation on DA release in the NAc (Chapter IV) and the limbic striatum (Karreman and Moghaddam 1996). These antagonists could not be applied together in the NAc as CNQX interfered with the electrochemical detection of DA in the present assay system. Instead, KYN was chosen for application to the NAc as it is

not electrochemically active and it blocks the effects of locally applied Glu in the NAc (see Chapter VI).

Consistent with previous findings, AP5+CNQX in the VTA reduced extracellular DA concentrations in the NAc by about 20% (Karreman and Moghaddam 1996). This treatment also greatly reduced the effect of feeding on DA release. These results contribute to a growing body of evidence implicating glutamatergic inputs to the VTA in the regulation of basal and evoked DA release in the NAc. Neurochemical studies have shown that application of Glu receptor agonists in the VTA increases DA release in the NAc (Kalivas et al. 1989; Suaud-Chagny et al. 1992). Cooling the PFC, which sends glutamatergic projections to the VTA (Christie et al. 1987), reduces burst firing in the VTA (Svensson and Tung 1989), whereas PFC stimulation increases cell firing in the VTA and enhances DA metabolism and release in the NAc (Murase et al. 1993a). The latter effect is blocked by Glu receptor antagonists applied to the VTA. In the only previous study examining the effect of Glu receptor antagonists in the VTA on DA responses to a physiological stimulus, Morrow et al. (1993) have reported that a glycine/NMDA receptor antagonist failed to affect stress-induced DA metabolism in the NAc. Although measurements of DA turnover and release may not be directly comparable, this finding raises the possibility that different types of stimuli regulate DA release via different mechanisms.

To investigate the contribution of metabotropic Glu receptors in the NAc to the regulation of feeding evoked DA release, ACPD was applied in the NAc. This treatment blocked the effect of feeding on DA release. This is consistent with a previous study showing that application of ACPD blocks the increase in DA release evoked by PFC and VTA stimulation (Chapter III). These results suggest that metabotropic Glu receptors have a

local inhibitory influence on *evoked* DA release. On the other hand, the effect of locally applied of Glu in the NAc can be fully blocked with ionotropic receptor antagonists, suggesting that metabotropic receptors do not modulate *basal* DA release (Imperato et al. 1990a; Youngren et al. 1993). Another possibility is that ACPD acts via a non-glutamatergic mechanism to impair DA release. Unfortunately, all other compounds with metabotropic activity that were tested in the present analytical system were electrochemically active and could not therefore be used.

The results of this research indicate that glutamatergic mechanisms in the VTA mediate the response of the mesolimbic DA system to feeding in food deprived rats. This parallels the glutamatergic contribution to DA release evoked by PFC stimulation. Thus, these data are consistent with a potential role for the PFC in triggering DA release evoked by feeding. However, in the absence of additional data, such a role for the PFC remains speculative as there are other inputs to the VTA which may use Glu as a neurotransmitter (Herkenham and Nauta 1979; Kita and Kitai 1987; Semba and Fibiger 1992).

VI. Glutamate Receptor Agonists Applied Locally Decrease Dopamine Release In The Nucleus Accumbens

Introduction

The experiments described in Chapter III and V indicate that local application of KYN in the NAc increased DA release alone and potentiated the DA increase evoked by feeding. This suggests that Glu acts at ionotropic receptors in the NAc to inhibit basal and evoked DA release. The experiments presented in this chapter further examined this issue.

Anatomical studies do not support a direct glutamatergic regulation of DA release in the NAc as axo-axonic synapses are not commonly observed in this structure (Sesack and Pickel 1990; Johnson et al. 1994). However, tyrosine hydroxylase-containing terminals have been observed to synapse on the same dendrites as putatively glutamatergic terminals originating in the PFC and the amygdala (Sesack and Pickel 1990; Johnson et al. 1994). Neurochemical studies have shown that application of ionotropic Glu receptor agonists in the NAc increases extracellular DA via AMPA/kainate or NMDA subtypes of Glu receptors (Jones et al. 1987; Imperato et al. 1990; Youngren et al. 1993; Svensson et al. 1994). Studies of the striatum have also reported increases in DA release produced by local application of Glu receptor agonists (Moghaddam et al. 1990; Keefe et al. 1992; Westerink et al. 1994). However, there is also substantial evidence that weakens the case for Glu-mediated stimulation of subcortical DA release via presynaptic actions on DA terminals. For example, Glu-evoked DA release co-occurs with electrophysiological events indicative of spreading depression (Moghaddam et al. 1990; Svensson et al. 1994). Also, Glu receptor antagonists do not decrease basal extracellular DA in the NAc; indeed, when applied to the NAc these

compounds invariably increase DA release, often more potently than the agonists (Imperato et al. 1990; Chapter III). It is interesting, however, that Glu receptor antagonists may attenuate the increase in extracellular DA produced by local application of DA uptake inhibitors (Moghaddam and Bolinao 1994).

To evaluate further the role of Glu in mediating DA release in the NAc, the present experiments utilized in vivo microdialysis to examine the effect of locally applied Glu receptor agonists on DA release. In addition to the endogenous agonist Glu, and the subtype-specific agonist N-methyl-D-aspartate (NMDA), a potent and specific Glu uptake blocker, l-trans-pyrrolidine-2,4- dicarboxylic acid (PDC) was also employed (Bridges et al. 1991; Griffiths et al. 1994). The latter compound provides novel data on the physiological role of Glu in mediating DA release as PDC selectively enhances endogenous Glu, thus avoiding artifacts that may result from exogenous application of direct agonists.

Materials and Methods

Male Wistar rats (300-350 g) were anesthetized with xylazine (6 mg/kg) and pentobarbital (50-60 mg/kg) and placed in a stereotaxic apparatus. A vertical dialysis probe with 2.0 mm of permeable membrane (Hospal, AN69) was implanted into the left NAc. Stereotaxic coordinates relative to bregma were A: +3.5, L: +1.4, and V: -7.8 at the ventral extent of the open area of the dialysis probe according to the atlas of Pellegrino et al. (1979). Following surgery rats were singly housed in Plexiglas cages and had ad libitum access to food and water at all times.

Dopamine Microdialysis

Dialysis procedures were used as described in the preceding chapters. Experimental treatment was initiated after the establishment of a baseline that was defined as three consecutive samples with less than 10% variation. Treatment involved the application of Glu, NMDA, PDC, or KYN alone or a combination of one of the agonists with KYN. The agonists were each applied for 20 min whereas the antagonist, KYN, was applied for 40 min. In the treatments involving co-application, KYN was applied 20 min before the agonist and both compounds were removed after an additional 20 min. All drugs were applied locally through the dialysis probe in the NAc. The compounds were dissolved in perfusion medium and pH was adjusted to 7.2-7.4 with dilute NaOH. L-Glutamate and monosodium Glu were obtained from Sigma Chemical Co. (St. Louis, MO); NMDA, PDC, and KYN were obtained from Research Biochemicals Inc. (Natick, MA). At the end of the experiment rats were overdosed with chloral hydrate, brains were removed, and cresyl violet staining was used to assess for neurotoxic effects and verify probe placement. Only rats with probes located within the NAc were included in the analysis.

Glutamate Microdialysis

Animals used for Glu microdialysis were prepared in a manner identical to that described above until the point that microdialysis was initiated. The dialysis samples from the first hour were discarded after which samples were collected off-line every ten minutes and immediately frozen at -80°C to await subsequent analysis. Six baseline samples were collected, then two samples during PDC (1 mM) application, then six additional samples.

o-Phthalaldehyde derivatives were prepared by reacting a 10 μl aliquot of the dialysate with Fluoraldehyde reagent (Pierce Chemicals); homoserine was used as the internal standard (Sloley et al. 1992). Separation of amino acid derivatives was achieved using isocratic reverse-phase liquid chromatography. The mobile phase consisted of 0.04 M NaH_2PO_4/methanol/acetonitrile/tetrahydrofuran (135:40:5:3 by volume). The pH was adjusted to 6.3 with 10N NaOH, and the solution was filtered and degassed and pumped at a flow rate of 1.0 ml/min. The apparatus for HPLC detection consisted of a Waters Model 510 pump coupled to an automatic injector (WISP, Waters model 712B). Separations were achieved using a 250 x 4.6 mm analytical column packed with spherical 5 μm particles (Spherisorb ODS2; Phenomenex) and protected by a C18 μbondapak guard column. The derivatives of the amino acids were detected by fluorometric detection using a Waters Model 420-AC fluorescence detector with an excitation wavelength of 254 nm (Mercury lamp) and an emission wavelength of 455 nm. Peak heights of the derivatives of the amino acids and the internal standard were integrated using a Hewlett-Packard 3392A integrator.

Data Analysis

For statistical analysis all data were converted to percent of baseline, calculated for DA as the mean of three samples preceding the experimental treatment and for Glu as the first five samples obtained. One Glu dialysis rat was excluded from analysis because the baseline was judged too variable (over 100% variance around the mean of the baseline samples). One-way and two-way ANOVA with Time as a repeated measure were used to test experimental treatments, and Huynh-Feldt corrections for degrees of freedom were used to account for Time as a repeated measure.

Results

Dopamine Microdialysis

The mean basal dialysate concentration of DA was 54.4 ± 3.0 (mean ± SEM) fmol/sample (n = 68). Basal values of DA did not differ significantly between groups ($p > .05$).

Effects of Glu Application

Local application of a high concentration of Glu (10 mM; Fig. 6.1B) decreased extracellular DA in the NAc ($p < 0.01$), whereas a lower concentration (1 mM) had no effect ($p > 0.05$). Dopamine concentrations returned to baseline rapidly following removal of Glu. The Glu receptor antagonist KYN (1 mM) had no effect on dialysate DA concentrations (Fig. 6.1A, $p > 0.05$). This concentration of KYN was chosen on the basis of previous results demonstrating that it is below the threshold required to increase DA release in the NAc (Chapter III). As shown in Figure 6.1A, application of KYN in the NAc beginning 20 min prior to Glu application blocked the Glu-evoked decrease in DA ($p < 0.05$). Following removal of the drug combination, extracellular DA increased for 30 min and then returned to baseline. Post-mortem examination of Nissl-stained brain sections, following application of Glu, revealed no evidence of toxicity in the area surrounding the microdialysis probe or any other area that was examined.

To address whether this effect of Glu could be explained as a loss of DA recovery by the microdialysis probe, in vitro experiments were performed (data not shown). The mean in vitro recovery was 9.3 ± 1.4 % (±SEM; n=4) during perfusion of the normal medium. The

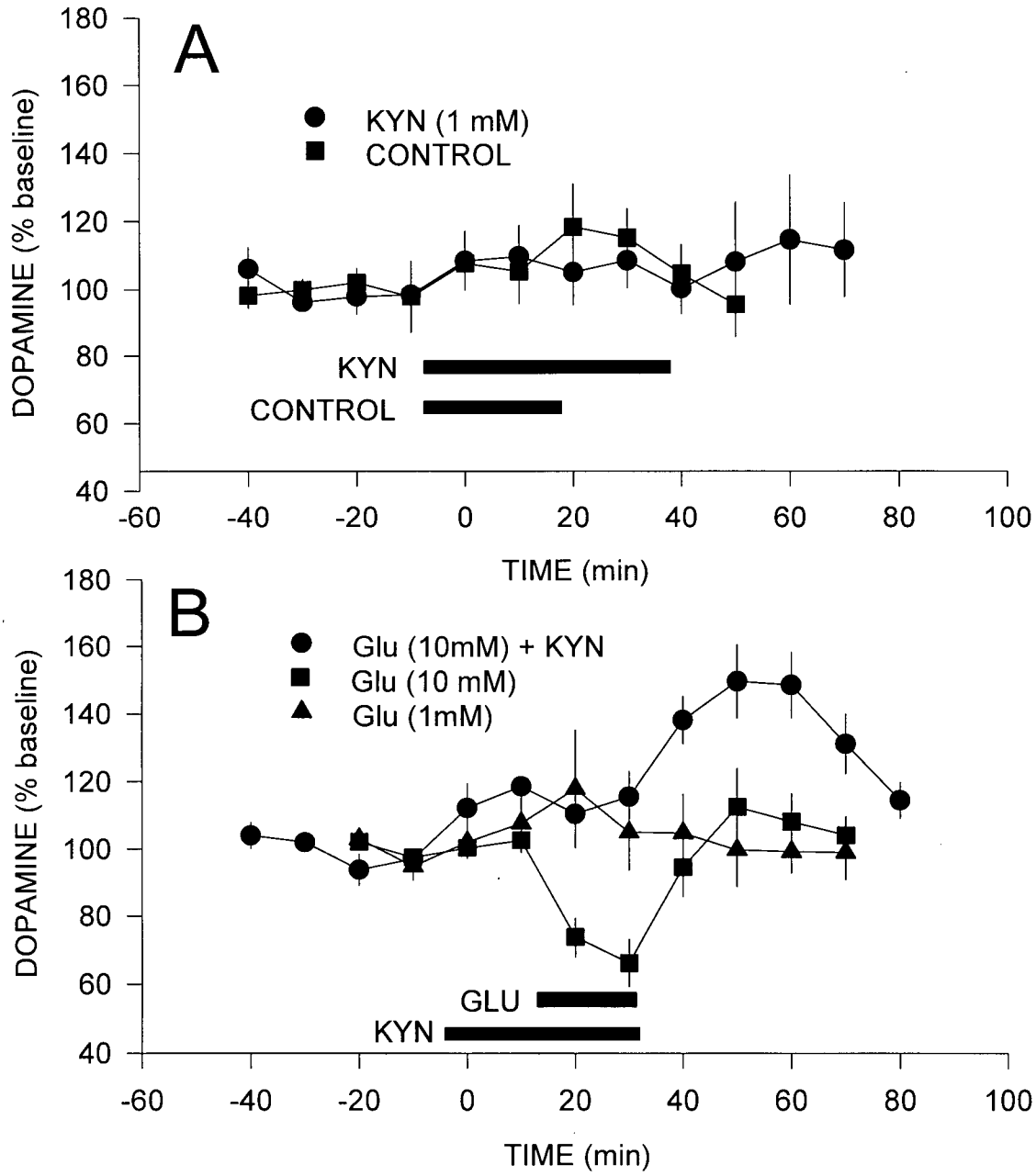


Figure 6.1. Dialysate DA concentrations sampled from the NAc during local application of **A)** KYN (1 mM) and **B)** Glu alone and in combination with KYN (1 mM). In this and all following figures data represent the mean ± SEM. Black bars indicate onset and duration of local drug application.

DA recovery of these dialysis probes was not altered by the addition of 10 mM Glu to the perfusion medium ($p > 0.05$).

Another possible explanation for Glu's effects could be that some aspect of the pH balancing of the Glu solution may have caused the decrease in DA. Although concentrations of all ions were balanced in all conditions, we were concerned that an unknown contaminant in the NaOH solution may have influenced the results. Therefore, in two rats the monosodium salt of Glu (10 mM) was applied instead of the free acid since the former compound does not require pH balancing. As the effect of monosodium Glu on extracellular DA matched that of the free acid (10 mM), these groups were pooled for the sake of data analysis.

Effects of NMDA Application

Figure 6.2 shows the effects of three concentrations of NMDA on dialysate DA concentrations. The effect of NMDA was concentration-dependent; the lowest concentration (10 μM: Fig. 6.2A) did not have a statistically significant effect ($p > 0.05$), the middle concentration (100 μM: Fig. 6.2A) decreased DA ($p < 0.05$), and the highest concentration (1 mM: Fig. 6.2B) increased DA ($p < 0.01$). Application of KYN (1 mM) blocked both the increase in DA produced by the higher concentration of NMDA ($p < 0.05$), and the decrease in DA seen with the lower concentration ($p < 0.05$). After the removal of the combination of NMDA (1 mM) and KYN (1 mM), dialysate DA increased briefly (Fig 6.2B); in all other cases DA returned to baseline rapidly. Post-mortem analysis did not reveal any evidence neurotoxicity following NMDA application.

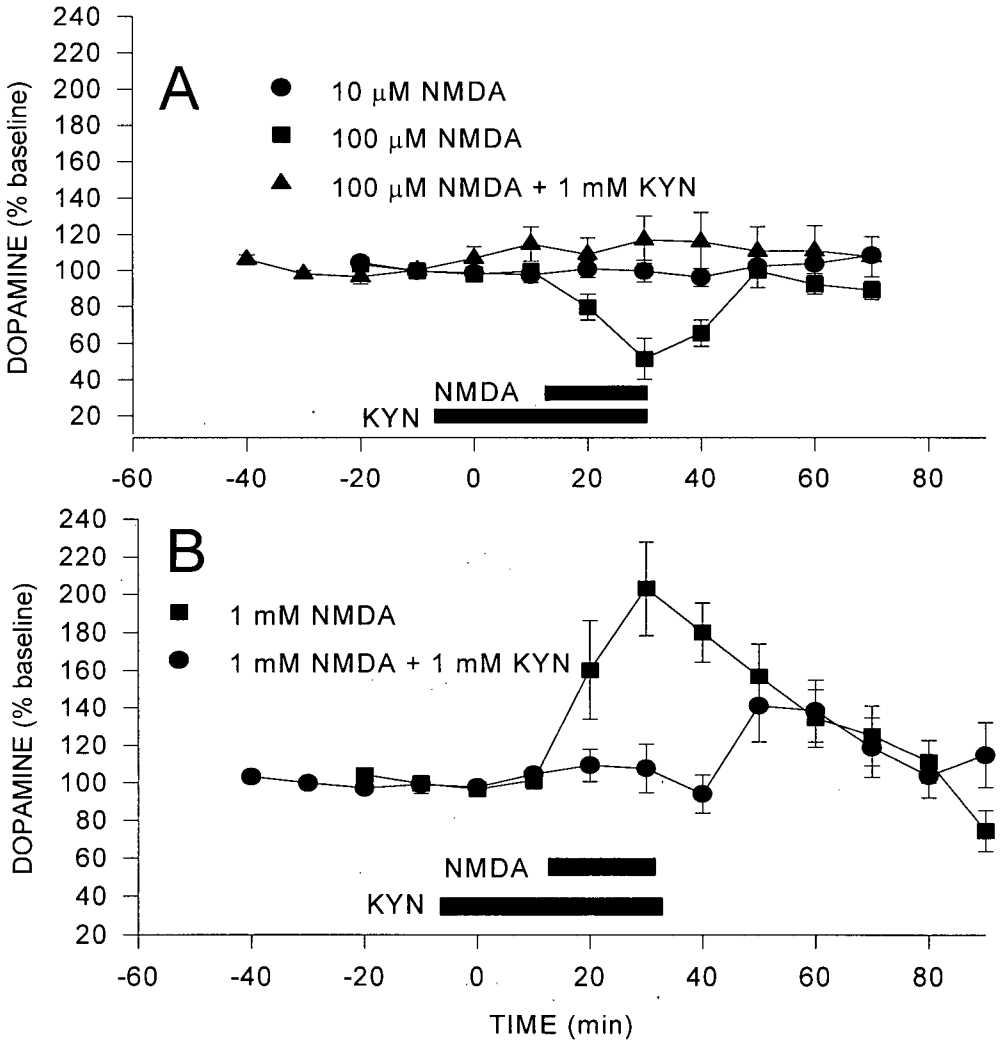


Figure 6.2. Dialysate DA concentrations sampled from the nucleus accumbens during local application of NMDA alone or in combination with KYN (1 mM). Black bars indicate onset and duration of local drug application.

Effects of PDC Application

The Glu uptake inhibitor PDC was used to assess the effect of increasing extracellular concentrations of endogenous Glu on extracellular DA (Fig. 6.3). Application of PDC (1 mM) to the NAc caused a statistically significant decrease in extracellular DA ($p < 0.01$); the maximal effect was a decrease to 70% of baseline. A lower concentration of PDC (100 μM) did not affect extracellular DA ($p > 0.05$; data not shown). Kynurenic acid significantly reduced the effect of PDC on extracellular DA ($p < 0.05$), although a complete blockade was not obtained.

Glutamate Microdialysis

Mean basal dialysate concentrations of Glu were 24.2 ± 0.6 pmol/sample (mean ± SEM: n=6). Mean basal dialysate concentrations of glutamine were 415.2 ± 46.5 pmol sample (same group). Application of PDC (1 mM) increased extracellular Glu in the NAc by 102% during the second sample of PDC ($p < 0.05$: Fig. 6.4). Application of PDC did not affect glutamine concentrations ($p > 0.05$). Post-mortem assessment of brain sections from rats treated with PDC revealed no evidence of toxic effects.

Discussion

The present results provide direct evidence that ionotropic Glu receptors in the NAc have inhibitory actions on DA release. Application of the direct receptor agonists NMDA and Glu reduced extracellular DA, and this effect was blocked by concurrent local application of KYN. A high concentration of NMDA increased DA release, and this effect was also blocked by KYN. To examine the influence of endogenous Glu on DA release, the

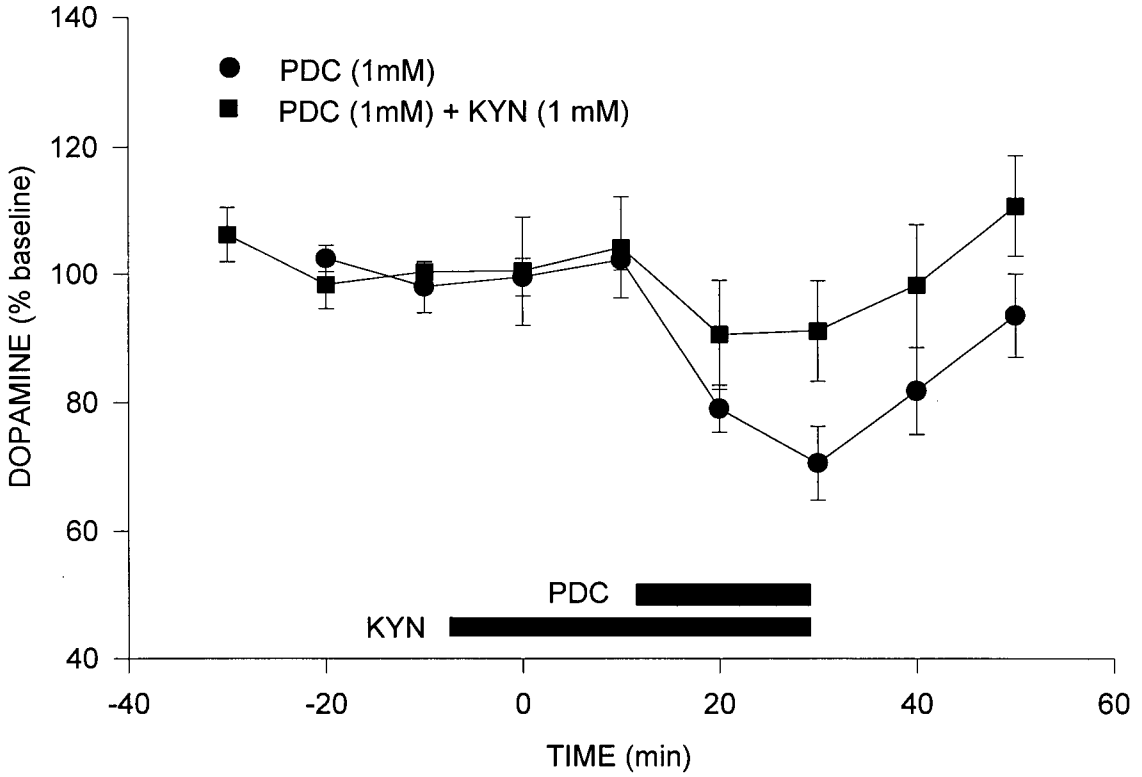


Figure 6.3. Dialysate dopamine concentrations sampled from the nucleus accumbens during local application of PDC (1 mM) with or without KYN (1 mM). Black bars indicate onset and duration of local drug application.

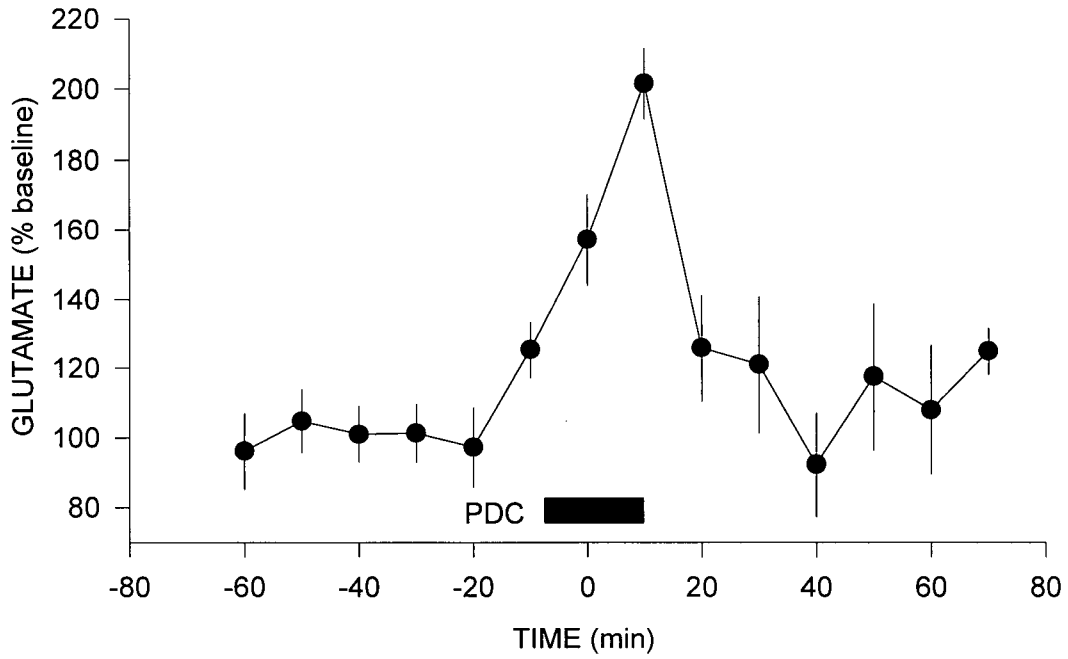


Figure 6.4. Dialysate glutamate concentrations sampled from the nucleus accumbens during local application of PDC (1mM). Black bar indicates onset and duration of local drug application.

specific Glu uptake inhibitor PDC was employed. As observed in previous studies, this compound increased extracellular Glu rapidly and effectively (Massieu et al. 1995; Bloc et al. 1995). When applied at the same concentration PDC decreased extracellular DA, and this effect was attenuated by KYN. Therefore, the decrease in DA was likely the result of increased extracellular Glu concentrations in the NAc. These findings indicate that endogenous Glu in the NAc inhibits DA release via actions at ionotropic Glu receptors.

All studies to date, including the present results, agree that DA release can be enhanced in vivo when local concentrations of Glu receptor agonists are sufficiently high in the NAc (Jones et al. 1987; Imperato et al. 1990; Youngren et al. 1993; Svensson et al. 1994) or striatum (Moghaddam et al. 1990; Keefe et al. 1992; Westerink et al. 1994.) This may be due to direct actions of these agents on Glu receptors located on DA terminals, as studies have demonstrated that Glu increases DA release from striatal synaptosomes (Desce et al. 1992). The question arises as to whether such increases are physiologically relevant, and the present data suggest that they are not. The following lines of evidence support this conclusion. 1) The observation that increased extracellular Glu produced by PDC decreases DA release strongly suggests that endogenously derived Glu decreases DA release. Furthermore, this effect was blocked by a concentration of KYN that did not itself affect DA release. 2) A physiologically relevant inhibitory action of Glu receptor agonists is consistent with the fact that Glu receptor antagonists consistently increase DA release in the NAc (Imperato et al. 1990; Youngren et al. 1993; Chapter III). 3) Control experiments were performed to confirm that the present results did not result from an experimental artifact relating to ionic composition, pH, or probe recovery. 4) None of the Glu receptor agonists

produced observable neurotoxic effects, and the effects of all of the agonists washed out quickly.

The reasons for the discrepancy between the present results and the studies showing only Glu-induced increases in DA release in vivo (Imperato et al. 1990; Youngren et al. 1993; Svensson et al. 1994) are unclear, but may relate to the issue of spreading depression. Some technical aspect of the present study may have provided protection against spreading depression, a phenomenon that has been proposed to underlie increases in DA release produced by Glu receptor agonists (Moghaddam et al. 1990; Svensson et al. 1994). For example, the present study used a faster flow rate than most other laboratories. Additionally, the present study was performed in unanesthetized rats 2 days after probe implantation, whereas other studies have used anesthetized rats and/or shorter intervals between surgery and dialysis. It is noteworthy that the concentration of NMDA (1 mM) which increased DA release in the present study has been shown by others to cause spreading depression, whereas the concentration of NMDA that decreased DA does not (Obrenovitch et al. 1994). The possibility that differences in experimental protocols may influence results pertaining to the glutamatergic regulation of DA release is further supported by the fact that two studies using the push-pull cannula method also found that Glu decreases DA release in the striatum (Cheramy et al. 1986; Leviel et al. 1990).

Studies of evoked DA release are not consistent with a potent local inhibitory influence of Glu. For example, chemical and electrical stimulation of the cortex increases extracellular concentrations of both Glu and DA in the striatum and NAc (Chapters II,III,IV; Godukhin et al. 1980; Palmer et al. 1989; Perschak and Cuenod 1990; Karreman and Moghaddam 1996), suggesting that evoked DA release persists in the presence of increased

extracellular Glu. Furthermore, Glu receptor antagonists applied locally in the striatum or NAc do not strongly affect the amplitude of DA release evoked by cortical stimulation (Karreman and Moghaddam 1994; Taber and Fibiger 1995) or stress (Keefe et al. 1992). In fact, data presented in Chapter III showed a trend toward a potentiation of cortical-stimulation induced DA release by local Glu receptor antagonists while feeding-evoked DA release was significantly potentiated (Chapter V). This agrees with a report that local application of a Glu receptor antagonist increases stress induced DA release in the NAc (Gratton and Doherty, 1994). These data are consistent with the possibility that while the net effect of cortical stimulation is to increase accumbal DA release, which occurs via excitatory projections to the VTA (see below), locally released Glu in the NAc has inhibitory effects (perhaps due to activation of NAc-VTA GABAergic projections, Fig. 6.5) which serve to attenuate the magnitude of the cortico-VTA driven increase. In disagreement with this formulation, one study has reported that locally applied NMDA antagonists blocked increases in DA release in the striatum produced by 5 min of tail pinch (Wheeler et al. 1995). However, the validity of these results is suspect due to the unprecedented increase in extracellular DA (100 %) that was produced by a mild stressor (tail pinch). Many studies have indicated that mild stress fails to influence DA release and metabolism in the striatum, and even severe stressors generally have only small effects (Abercrombie et al. 1989; Keefe et al. 1993). Additionally, the failure of Wheeler et al. (1995) to include Mg^{++} in the perfusion solution reduces the physiological relevance of their results.

Substantial evidence points to the VTA as the primary site of glutamatergic regulation of accumbal DA release. Application of Glu to the VTA increases extracellular DA in the

NAc and increases post-mortem tissue concentrations of DA metabolites (Kalivas et al. 1989), and Glu receptor antagonists applied to the VTA decrease extracellular DA concentrations in the NAc (Karreman et al., 1995). Stimulation of the PFC, which likely activates glutamatergic projections to the VTA, increases DA release in the NAc as well as burst firing in VTA neurons (Gariano and Groves, 1988; Murase et al., 1993). Furthermore, application of ionotropic Glu receptor antagonists to the VTA blocks the effects of cortical stimulation on DA release in the NAc. A similar finding has been obtained with respect to the cortical regulation of DA release in the medial striatum (Karreman and Moghaddam, 1996). Therefore, a glutamatergic projection from the PFC to the VTA appears to be the primary mechanism by which Glu regulates DA release in the NAc.

The mechanism by which Glu inhibits basal DA release, as observed in the present study, probably does not involve Glu receptors on DA terminals. Glowinski and colleagues have shown that stimulation of ionotropic Glu receptors increases DA release from striatal slices and synaptosomes (Giorguieff et al. 1977; Desce et al. 1992). These findings suggest that any ionotropic Glu receptors located on DA terminals facilitate DA release. This possibility is supported by the present and previous observations that high concentrations of Glu receptor agonists increase DA release (Imperato et al. 1990; Youngren et al. 1993; Svensson et al. 1994). It is unlikely that such receptors also mediate decreases in DA release produced by NMDA, Glu, or PDC as activation of ionotropic receptors coupled to cation channels should depolarize terminals. Another possibility is that activation of metabotropic Glu receptors on DA terminals inhibits DA release. This possibility is supported by the results of a previous study from this laboratory showing that the metabotropic Glu receptor agonist ACPD weakly reduces basal DA concentrations while blocking stimulation-evoked

DA release (Chapter III). However, in the present study the decrease in extracellular DA evoked by Glu could be fully blocked by the ionotropic Glu receptor antagonist KYN. Although this compound did not fully block PDC's effects, this may have been due to KYN's failure to reach the synaptic cleft where PDC would produce the greatest increases in Glu concentrations.

A multisynaptic model is required to explain the present results, and there are at least two possibilities that can be considered. These include inhibitory interneurons and long feedback loops. The primary candidate for an interneuron in the NAc with inhibitory actions on DA release would be a GABA-containing neuron. However, because extracellular DA concentrations in the striatum are not affected by local application of either the GABAA agonist baclofen or the GABAB antagonist bicuculline, it is unlikely that GABAergic receptors are located on DA terminals (Tanganelli et al. 1994; Westerink et al. 1994). This is further supported by the observation that local application of muscimol increases striatal DA release in intact rats but not in rats with a kainate-induced lesion of the striatum (Yoshida et al. 1992). It seems more likely, therefore, that the relevant Glu receptors are located on NAc projection neurons, activation of which would stimulate feedback inhibition of DA neurons in the VTA (Yim and Mogenson 1980). A model for such an organization is depicted in Figure 6.5. In this model ionotropic Glu receptors are located on GABA neurons that project back to the VTA and provide an inhibitory input to DA neurons. In support of this organization baclofen infusion into the VTA decreases DA release in the NAc while bicuculline in the VTA increases DA release in the NAc (Yoshida 1994; Westerink 1996). The latter observation indicates that the GABAergic projection to the VTA tonically inhibits DA release in the NAc. According to this model, locally applied Glu receptor agonists would

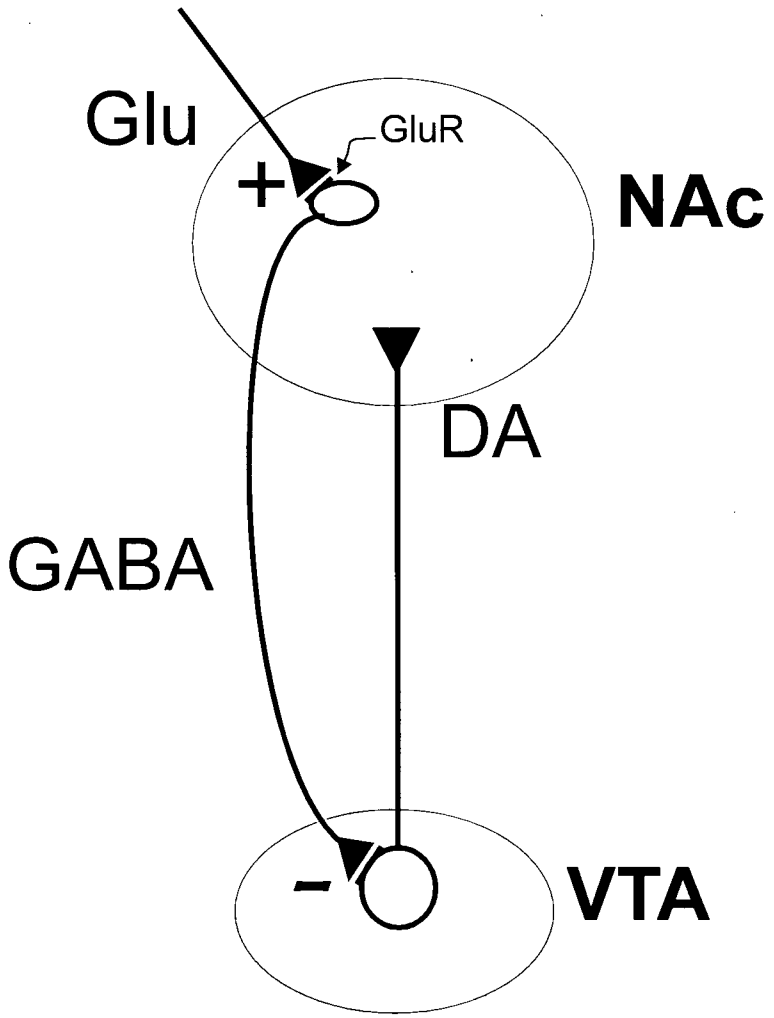


Figure 6.5. Schematic diagram illustrating the model proposed to account for the present results. See text for description.

increase inhibitory input to the VTA and reduce basal DA release in the NAc, while the antagonists would reduce the influence of this input, resulting in an increase in basal DA release. Furthermore, when very high concentrations of Glu receptor agonists are applied to the NAc, the pathological condition of spreading depression would occur, enhancing DA release as a result of abnormal terminal depolarization. Alternatively, high concentrations of locally applied Glu could shift the GABAergic projection neurons into a state of depolarization block (Zieglgansberger and Puil 1983), thus removing the inhibitory input to the VTA and increasing DA release in the NAc.

VII. General Discussion

The experiments presented in this thesis indicate that activation of the PFC has a facilitatory influence on subcortical DA neurotransmission. The mechanism mediating this response involves activation of glutamatergic afferents to the VTA and not to the NAc. Secondary to this arrangement, Glu acts in the NAc to inhibit DA release via both ionotropic and metabotropic receptors. The contribution of metabotropic receptors most likely occurs via local actions in the NAc, whereas the ionotropic receptors probably activate inhibitory feedback loops to the VTA. These results contribute to a growing body of evidence indicating that the functional anatomy of cortical regulation of subcortical DA release is not consistent with anatomical models proposed to underlie schizophrenia.

First, the observation that stimulation of the PFC increases subcortical DA release is not consistent with the notion that in the schizophrenic brain overactivity of subcortical dopamine systems could result from a loss of activity in the PFC, as proposed by Weinberger (1987). A second aspect of the Weinberger model that has come under scrutiny is the proposal that excess dopaminergic activity is present in schizophrenia. Even if dopaminergic hyperactivity occurred secondary to cortical dysfunction, one would still expect to see markers of enhanced DA activity in schizophrenic brains, but such evidence has not been consistently obtained (see Introduction). In fact, the evidence for increased DA receptors in the striata of schizophrenics (Wong 1986) implies the opposite condition, that the mesolimbic DA system is underactive, as DA receptor binding is known to increase in response to a loss of DA. These findings do not speak to the primary thrust of the Weinberger hypothesis which proposes that a lesion early in development results in hypofrontality and the negative

symptoms of schizophrenia. Nevertheless, taken as a whole the arguments outlined here argue against excess subcortical dopaminergic activity resulting from hypofrontality and the disinhibition of subcortical DA systems.

A second hypothesis of schizophrenia that implicates an abnormal subcortical response to a primary cortical deficit was proposed by Grace (1991). According to this model, glutamatergic afferents from the PFC maintain basal DA release in the NAc, presumably via receptors on DA nerve terminals. The mesolimbic projection, on the other hand, mediates acute, transient increases in DA release from phasic type stimuli. In schizophrenia, Grace hypothesizes, the cortical afferents are inactive, resulting in decreased basal DA release in the NAc. In response, DA receptors in the NAc are upregulated, resulting in an abnormally enhanced phasic post-synaptic response in the mesolimbic DA system. The present results contradict Grace's hypothesis on three counts. First, Glu in the NAc does not seem to have an important role in mediating basal DA release in the NAc, as glutamatergic antagonists applied in this region fail to reduce basal DA release. Indeed, high concentrations of the antagonists applied in the NAc increased DA release (Chapter III), a finding reported in other studies as well (Imperato et al. 1990a; Keefe et al. 1992; Youngren et al. 1993). Second, Glu in the NAc has inhibitory effects on basal and evoked DA release under physiological conditions (Chapters III,IV,VI). Finally, the observation that TTX or AP5+CNQX applied in the VTA decreases basal DA concentrations in the NAc indicates that this region is strongly involved in maintaining tonic DA concentrations. Taken together, these data refute the functional anatomy proposed by the Grace hypothesis to be dysfunctional in schizophrenia.

Models of schizophrenia that integrate PFC abnormalities with subcortical DA dysfunction have all focused on the cortico-striatal projection as the most likely site of dysfunction, omitting any contribution of the VTA (Carlsson and Carlsson 1990; Robbins 1990; Deutch 1993). The present results clearly indicate that the glutamatergic mechanisms in the VTA play a significant role in maintaining basal DA release in the NAc and also mediate increases in DA release in the NAc evoked by a variety of stimuli. For example, Chapters IV and V show that TTX application in the midbrain abolished basal DA concentrations in the NAc, and previous studies have shown that DA in the limbic and dorsal striatum are similarly affected (Keefe et al. 1993; Karreman and Moghaddam 1996). Furthermore, Glu in the VTA contributes to the maintenance of basal DA concentrations in the NAc as antagonists of Glu receptors applied in the VTA decrease basal DA release (Chapter IV,VI: Karreman and Moghaddam 1996). A previous study suggests that afferents from the PFC are specifically involved in this response as cooling of the PFC decreases DA cell firing in the VTA (Svensson and Tung 1989). Glutamatergic mechanisms in the VTA also mediate increases in DA release from acutely presented stimuli. For example, the experiments presented in Chapters IV and V implicate glutamatergic activity in the VTA in mediating DA release in the NAc evoked with electrical stimulation or feeding. Other studies support such an organization as Glu application in the VTA increases DA metabolism in the VTA (Kalivas et al. 1989), and electrical stimulation of the PFC increases burst firing in DA neurons in the VTA (Murase et al. 1993a).

The question then arises, if the VTA is the primary site at which Glu influences the mesolimbic DA system, then could abnormalities in this region be implicated in the cortical dysregulation of subcortical DA release hypothesized to occur in schizophrenia? To address

this question a second look at research examining the effect of PFC lesions on subcortical DA systems is merited. These studies find that a cortical lesion produced with 6-OHDA, excitotoxic agents, suction ablation, or knife cut results at most, in a weak transient potentiation of DA release and metabolism in the mesolimbic DA system (Scatton et al. 1982; Wilkinson et al. 1993; Whishaw et al. 1992; Bubser 1994). However, studies examining the effect of cortical 6-OHDA lesions on the activation of subcortical DA systems in response to acute stimuli generally find potentiated responses to stress, reward, and potassium application in the absence of a change in basal DA release or metabolism (Deutch et al. 1990; Mitchell and Gratton 1992; Roberts et al. 1994). Furthermore, the neurochemical response in the striatum to systemic application of DA receptor agonists remains unchanged, suggesting no deficit in the normal functioning of DA neurons (Scatton et al. 1982; Whishaw et al. 1992). These findings indicate an abnormal regulation of DA systems, in the absence of a dysfunction of DA neurons per se.

In thinking about what sort of mechanism in the VTA could underlie an increase in responsiveness of DA neurons resulting from a cortical lesion, compensatory mechanisms should be considered. If the glutamatergic input from the PFC is important for maintaining basal DA activity, as indicated in the present experiments, then it would not be surprising if the system were capable of compensating for a loss of this input. A lesion of the PFC could reduce the activity of glutamatergic inputs to the VTA, and DA neurons post-synaptic to the PFC afferents (Sesack and Pickel 1990) might then upregulate Glu receptors to maintain normal basal firing. Whether deafferentation of the VTA will result in upregulation of Glu receptors in this nucleus is unknown. However, the potential for such a mechanism is implicated by studies showing that PFC lesions lead to upregulation Glu receptors in the

striatum, resulting in increased [^{3}H]Glu binding and an enhanced post-synaptic Glu response (McLennan 1980; Barbeito et al. 1989; Samuel et al. 1990). As a result of such hypothetical Glu supersensitivity in the VTA, increases in glutamatergic input to this area following acute stimuli, such as stress or feeding (Chapter V), could lead to excess activity in DA neurons. The glutamatergic input could originate in remaining cortico-midbrain cells or from other glutamatergic inputs to the VTA. This organization is similar to that proposed by Grace (1991) for DA in the NAc, but in this case it is the Glu response rather than the DA response that is upregulated.

Regardless of such speculation, the present results add to an increasing body of evidence that fails to implicate either a primary or secondary role for DA in the etiology of schizophrenia. Thus, alternative explanations for the observations that a) amphetamine can evoke a schizophrenia-like state and b) D2 antagonists have antipsychotic efficacy are warranted. One possibility is that DA is in a state of functional opposition to some other system that is compromised in schizophrenia. This would result in a relative but not absolute elevation in DA tone, whereas in the chronic amphetamine condition, DA tone would be elevated relative to normal activity in the other system. As dopaminergic afferents project predominantly to cortical and striatal regions, circuitry within these areas may be involved. Anatomical and physiological evidence has combined to describe a series of loops that originate in the cortex, project to the striatum, then pallidal areas, thalamic areas and then back to the cortex (Alexander et al. 1986). These loops were originally delineated in primates to segregate various motoric functions based on electrophysiological evidence, and anatomical evidence supports their presence in rodent species as well (Alexander et al. 1986; Groenewegen et al. 1990). One of these circuits, the anterior cingulate circuit, represents a

prime candidate for pathophysiology in schizophrenia as it originates in a brain region often implicated as showing pathology in schizophrenia (Benes 1995; Shelton and Weinberger 1987), and projects to the NAc, a region thought to be an important site of action of neuroleptic drugs (White and Wang 1983; Moghaddam and Bunney 1990; Robertson and Fibiger 1992). Because two of the components of this circuit receive significant dopaminergic inputs, this loop also represents a reasonable substrate for dopaminergic drugs. Amphetamine treatment could push this system into a pathological state, evoking psychosis, whereas a DA receptor antagonist could perhaps return this circuit to normal activity.

Support for an important role for this circuitry in schizophrenia comes from a recent study using PET scanning to assess oxygen use in schizophrenics during auditory hallucinations. This study found that the thalamus, anterior cingulate, putamen, and NAc all showed significantly elevated activity relative to whole brain averages during an auditory hallucinations (Silbersweig et al. 1995). A number of PET studies have also found abnormalities in the basal ganglia at rest or during performance of a variety of tasks (Buchsbaum et al. 1992; Gur and Pearlson 1993). Thus, cortico-striatal dysfunction, independent of dopaminergic influence, may underlie significant aspects of the pathophysiology of schizophrenia.

In general, there appears to be a growing consensus that schizophrenia results from a disorganization of activity in broadly distributed forebrain regions centering on heteromodal association cortex but involving other regions as well (Gur and Pearlson 1993; Ross and Pearlson 1996). The specific interactions governing the fundamental disorganization are not understood, and the various models that have been proposed are inconsistent and sometimes mutually exclusive. As efferents from many of the implicated areas use Glu as a

neurotransmitter, the proposal that glutamatergic systems may represent the primary site of pathology in schizophrenia is garnering increasing support (Moghaddam 1994; Ellison 1995; Benes 1995, Olney and Farber 1995). This proposal remains difficult to test however, as few glutamatergic agents can be administered to patients. Indeed, Glu receptor agonists induce neurotoxic reactions and Glu receptor antagonists evoke psychosis and also have neurotoxic effects (Ellison 1995; Olney and Farber 1995; Rothman and Olney 1995). For these reasons the search has begun to find compounds that regulate Glu transmission with few side effects.

The DA receptor antagonists that are currently used for the treatment of schizophrenia are far from ideal as most produce a high incidence of extrapyramidal side effects such as severe parkinsonism, and long-term treatment can result in tardive dyskinesia (Reynolds 1992). It is possible that drugs with a different site of action (i.e. non-D2 receptor) could have the putatively therapeutic effect of reducing DA transmission without causing the side effects associated with the neuroleptics. The nonspecific metabotropic Glu receptor agonist ACPD may have such properties as the present results indicated that ACPD attenuates the DA response in the NAc to feeding, PFC stimulation, and VTA stimulation at a concentration that itself had little effect on basal DA release. Thus, this compound may serve as a novel antipsychotic agent. Unfortunately, the seven subtypes of metabotropic Glu receptor are distributed broadly throughout the brain and have diverse physiological functions (Pin and Bockaert 1995), so systemic administration of this non-specific agonist would very likely evoke significant side effects. For example, intra-cerebroventricular application of ACPD has been shown to evoke seizures in rats (McDonald et al. 1993). Thus, a more productive strategy would be to administer a compound serving as a specific agonist at the relevant subtype of metabotropic Glu receptor. Unfortunately, selective agonists for the metabotropic

receptor subtypes are not yet available. It will be interesting to consider subtype selective metabotropic receptor agonists in the context of schizophrenia when they do become available.

VIII. REFERENCES

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