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IN VIVO MODULATION OF STRIATONIGRAL PROJECTION NEURONS: 
THE EFFECTS OF AMPA AND D1 RECEPTOR ACTIVATION ON 
STRIATAL AND NIGRAL GABA EFFLUX

DISSERTATION

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

by

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The Ohio State University

1995

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Chapter I.
GENERAL INTRODUCTION

The basal ganglia refers to a group of neuronal structures all believed to be critical for the production and expression of voluntary movement. These structures include the thalamus, striatum, substantia nigra pars compacta and pars reticulata, internal and external globus pallidus, as well as the subthalamic nucleus. Of all these areas, the striatum appears to be the most crucial in terms of the integration of sensory and motor information (Alexander & Crutcher, 1990).

Much of the information regarding striatal function has been gleaned from studies examining pathologies that involve striatal afferents and efferents. Diseases such as Parkinson’s Disease and Huntington’s Chorea, the former characterized by a lack of volitional movement, the later by increased uncontrollable bursts of movement, present two distinct clinical manifestations of striatal dysfunction (Albin et al., 1989). Increased understanding of the changes
underlying these disease states has provided great insight into basal ganglia processes, especially within the striatum.

One particular experimental model of Parkinson's Disease (PD) has lead to numerous discoveries regarding the basal ganglia. PD is characterized by a progressive loss of the dopamine neurons that originate within the substantia nigra pars compacta and normally innervate the striatum. In laboratory animals, unilateral depletion of these dopamine (DA) neurons via the neurotoxin 6-hydroxydopamine (6-OHDA) provides a model of this debilitating disease (Zigmond & Stricker, 1989). However, not all of the findings that have emerged from the study of these depleted animals reflect the actual functioning of the striatum in normal animals. In spite of this fact, many have attempted to describe normal striatal function based upon these very subjects (Gerfen et al., 1990; for review, see Alexander & Crutcher, 1990). While in some instances their predictions have been validated in normal animals, other findings remain unconfirmed. At this point in time, continued study of the normal
intact striatum is needed to more fully and appropriately establish the function of these basal ganglia structures.

The purpose of the experiments presented within this document is to more fully elucidate neurotransmitter interactions within the intact striatum with an emphasis on striatal projection neurons. In particular, the transmitters to be studied are gamma aminobutyric acid (GABA), glutamate via activation of the AMPA receptor subtype and dopamine via activation of the D1 receptor subtype. The literature discussed below will examine the current data regarding striatal structure and circuitry as well as the role that the above mentioned neurotransmitters may play within that system.

**Striatal Projection Neurons: Anatomical, morphological, and electrophysiological characteristics**

The striatonigral and striatopallidal projections are the two major output pathways of the striatum (Graybiel & Ragsdale, 1979). The former is referred to as the direct pathway, projecting from the striatum to the substantia nigra pars reticulata and then up to the thalamus. The latter is referred to as the indirect
pathway, projecting from the striatum to the external globus pallidus; the globus pallidus then sends projections to the subthalamic nucleus which subsequently projects to the substantia nigra pars reticulata, or the globus pallidus neurons may project directly the substantia nigra pars reticulata, which finally projects up to the thalamus (see Figure 1). Both the striatonigral (direct) and striatopallidal (indirect) pathways utilize GABA as their neurotransmitter (Kita and Kitai, 1988). In addition, these two pathways are also distinguished by the specific neuropeptides that are colocalized with GABA. In the direct striatonigral pathway GABA is colocalized with substance P and dynorphin. In the indirect striatopallidal pathway GABA is colocalized with enkephalin (Haber and Nauta, 1983; Gerfen and Young, 1988).

The cell bodies of the GABAergic neurons from which these two pathways arise are medium spiny neurons and constitute 90-95% of the striatum (Kemp and Powell, 1971). Morphologically, these neurons are characterized by size (10-20 um in diameter), by their aspinous cell body and proximal dendrites, and by higher order dendrites densely covered with spines
(Nisenbaum et al., 1993). They also display a number of local axon collaterals that remain within the striatum and usually synapse around the dendrites of the neuron from which they originated (Kawaguchi, Wilson, and Emson, 1990). While most of the neurons contained in the striatum are of the medium spiny type, the striatum itself is not a homogeneous region. Rather, the striatum contains two heterogeneous compartments, termed the patch and the matrix compartments (Graybiel and Ragsdale, 1978; Herkenham and Pert, 1981; Gerfen et al., 1985). Patches can be distinguished by dense areas of mu opiate receptor binding (Pert et al., 1976), while the matrix, which comprises over 80% of the striatum, can be distinguished by dense somatostatin immunoreactive fibers (Gerfen, 1984). Both the direct and indirect projection neurons originate within the matrix (Gerfen, 1984, 1985; Jimenez-Castellanos and Graybiel, 1989).

Electrophysiologically, medium spiny neurons have a resting membrane potential of approximately -70 mV. They display an action potential that has a characteristic positive-negative waveform (Berger et al., 1987). In addition, they have a very low rate of spontaneous discharge (Berger et al., 1987; Nisenbaum
et al., 1993). The majority of excitatory afferents into the striatum arise from the neocortex and form asymmetric synaptic contacts with the dendritic spines of medium spiny neurons (Smith and Bolam, 1990). The transmitter utilized by these afferents is surmised to be the excitatory amino acid glutamate (Perschak & Cuenod, 1990). Stimulation of subcortical white matter elicits EPSP's in striatal cells, that can be reduced by 95% with the addition of the AMPA receptor antagonist CNQX (Nisenbaum et al, 1993), suggesting that glutamate-induced stimulation of striatal medium spiny neurons is mediated primarily via the activation of AMPA or a related receptor subtype.

AMPA receptors are just one of the subtypes of glutamate receptors found within the striatum. To date, there are three major classes of glutamate receptors, separated based upon their physiological characteristics (Monaghan et al., 1989). These classes include NMDA receptors, non-NMDA receptors, and metabotropic receptors, all of which can be observed within the striatum and many of which are believed to reside on medium spiny neurons (Albin et al., 1992). The AMPA receptor belongs to the non-NMDA class which also includes kainate and quisqualate-sensitive
receptors. All of these receptors produce their effects via fast activation of ligand gated ion channels. As discussed above, the corticostriatal inputs to the striatum activate the AMPA receptor subtype. The majority of AMPA receptors found within the striatum reside within the matrix (Dure et al., 1992), from which the projection neurons arise. Therefore, these receptors may be located on both the direct and indirect projection neurons as well as on striatal interneurons.

**Striatal Projection Neurons: Localization of DA receptors**

In addition to the characteristics described above, striatal projection neurons can be further segregated based on the presence or absence of dopamine receptor subtypes. The two receptors that provide the most prominent distinction are the dopamine D1 and D2 subtypes, which receive dopaminergic input from cells originating in the substantia nigra pars compacta (Gerfen et al., 1990). Autoradiographic studies examining D1 and D2 receptor binding provide evidence that both of these subtypes can be localized within the striatum. Both are densely distributed in a
heterogeneous manner with the highest densities observed in the dorsal medial aspect of the nucleus (Mansour et al., 1990). Studies examining the distribution of mRNA for the D1 and D2 receptors observed a segregation of these subtypes within the striatum, with most somata containing either the D1 receptor mRNA or the D2 receptor mRNA, but not both (Gerfen et al., 1990).

As stated previously, neurons projecting to the SNpr contain the peptides substance P and dynorphin while those projecting to the GP contain enkephlin. Moreover, mRNA for the D1 receptor is colocalized with substance P while mRNA for the D2 receptor is colocalized with enkephlin (Gerfen et al., 1990; Le Moine et al., 1990; Reiner & Anderson, 1990). Thus, it has been inferred, based on the colocalization of these peptides with mRNA for either the D1 or D2 subtype, that neurons possessing D1 mRNA project to the substantia nigra pars reticulata whereas those possessing D2 mRNA project to the globus pallidus. Additional studies have confirmed the colocalization of these peptides and mRNA's, and further combined this with retrograde labeling (Gerfen et al., 1990; Le Moine et al., 1991; Le Moine et al., 1990). These
colocalization studies have also been supported by selective lesion data. Application of a suicide transport agent to the globus pallidus leads to a significant decrease in both preproenkephalin mRNA and D2 receptor binding with no change in D1 binding in the striatum (Harrison et al., 1992). Further studies have shown that application of a different suicide transport agent to the SNpr leads to decreased D1 mRNA expression and receptor binding with no change in D2 mRNA or receptor density in striatum (Harrison, et al., 1990; Pollack et al., 1993). Taken together, these findings support the hypothesis that D1 receptors are selectively localized on neurons projecting to the SNpr (i.e. direct pathway) whereas D2 receptors are selectively localized on neurons projecting to the GP (i.e. indirect pathway).

Based on the findings described above, striatonigral projection neurons possess several distinctive characteristics. They utilize GABA as their transmitter, colocalized with substance P and dynorphin. They receive glutamatergic input from the cortex, and contain AMPA, NMDA, and kainate receptors (Albin et al., 1992). They also posses axon collaterals that remain within the striatum, usually
associated with the dendrites of the parent cell
(Misgeld et al., 1984; Wilson & Groves, 1980).
Furthermore, they express DA D1 receptors on their
somata. One additional fact about these neurons is
that they also express D1 receptors on their axon
terminals, both those that remain within the striatum
and those found within the SNpr (Barone et al., 1987;
Gingrich & Caron, 1993).

Striatonigral neurons: Effects of D1 agonists

The electrophysiological response of medium spiny
neurons to DA has been demonstrated. Iontophoretic
application of DA to the striatum has been shown to
decrease both spontaneous striatal neuronal activity
and evoked firing rates (Hu and Wang, 1988; Rolls et
al., 1984). This DA-induced decrease has been
demonstrated both at resting membrane potential and
more positive levels, but not when the membrane
potential is hyperpolarized (Calabresi et al., 1987).
The decrease in evoked firing rates is in part due to
the activation of release regulating D2 receptors on
the terminals of excitatory afferents, decreasing the
amount of striatal glutamate released (Donzati et al.,
1993; Yamamoto & Davy, 1992). The D1 component of this
decreased firing has also been explored. In vitro, application of the partial D1 agonist SKF 38393 (1-10 uM) to striatal cells increased the firing threshold and decreased the number of action potentials evoked by a depolarizing current (Calabresi et al., 1987; Twery et al., 1994). Single cell studies have demonstrated that SKF 38393 (1-5 uM) reduces the amplitude of Na+ currents evoked via depolarizing voltage steps (Surmeier et al., 1992). Thus, it appears that DA, acting at D1 receptors within the striatum, decreases the evoked firing rate of striatonigral neurons.

While the responsiveness of striatonigral neurons within the striatum has been investigated, the extent to which terminal D1 receptors are involved in altering neuronal firing rates has not been characterized. Terminal D1 receptors have been observed in several brain regions including the striatum, ventral tegmental area, internal globus pallidus, and the SNpr (Barone et al., 1987; Floran et al., 1990). These receptors are release regulating. However, unlike autoreceptors or other presynaptic receptors which normally act to decrease transmitter release, these receptors appear to increase transmitter release (Cameron & Williams, 1993; Floran et al., 1990). In vitro studies examining these
D1 receptors on terminals of striatal projection neurons have illustrated that activation of these receptors, both those within the striatum and SNpr, increases K⁺-induced GABA release (Floran et al., 1990). It has not been determined whether these receptors can be activated without the depolarization of the terminal. To what extent these receptors play a role on firing rates within the striatum is unknown. However, it has been demonstrated that D1 agonists lose their effectiveness when striatal neurons are hyperpolarized (Calabresi et al., 1987). Striatal release of GABA via terminal D1 receptors could serve to hyperpolarize the parent neuron following an initial or repeated depolarizing current within the terminal. Within the SNpr, D1 receptors are only located on striatonigral terminals (Gingrich & Caron, 1993). These receptors, when activated, increase the concentration of GABA, an effect that can be blocked via the D1 receptor antagonist SCH 23390 (Floran et al., 1990). In the VTA terminal D1 receptors on GABA terminals have also been demonstrated to increase evoked ipsp’s (Cameron & Williams, 1993). Furthermore, in the same study, the D1 antagonist SCH 23390 alone
was shown to decrease these evoked ipsp's, suggesting that dopamine may tonically mediate enhanced GABA release in the VTA.

Presently, there are few studies examining the effects of D1 stimulation on in vivo GABA release within either the striatum or SNpr of intact animals. One such study, utilizing push-pull cannulae to analyze the spontaneous release of [3H] GABA, observed an increase in striatal GABA release in the presence of the D1 agonist SKF 38393 (10 uM; Girault et al., 1986). Interpretation of these results, however, are problematic due to both the use of anesthesia and the absence of information regarding the level of Ca+ within the perfusion medium, both factors that could affect GABA release.

The effects of D1 activation on striatonigral GABA release have often been inferred from studies examining nigral firing rates. In support of decreased GABA release from striatonigral terminals following D1 receptor activation, studies examining systemic administration of the D1 agonist SKF 38393 (10 mg/kg i.v.) have reported an increase in the firing rates of 54% of SNpr neurons (>20% of baseline) in normal animals (Huang & Walters, 1994). However, not all
studies have demonstrated a consistent increase in nigral firing rates. Some studies, also utilizing systemic injection of the D1 agonist SKF 38393 (10 mg/kg), have observed SNpr firing rates that increased, decreased, or showed no change (Weick & Walters, 1987). Interestingly, when SKF 38393 is applied directly to the SNpr during concurrent stimulation of the striatonigral pathway, the result is either no change in the firing rate of SNpr neurons or only a slight decrease in the firing rate. Such a decrease may be the result of increased GABA efflux from striatonigral terminals (Waszczak, 1990). It is unlikely that this inhibitory effect is due to activation of GABA interneurons within the SNpr, as there is no evidence of post-synaptic D1 receptors within the SNpr (Jackson & Westlind-Danielsson, 1994). Thus, systemic D1 administration appears to primarily decrease GABA efflux from striatonigral terminals, while D1 administration directly to the substantia nigra may produce a slight increase in GABA efflux.

However, while the above cited evidence indicates a primarily inhibitory role for D1 receptors within the striatum, the most popular model of basal ganglia function proposes an excitatory D1 effect on
striatonigral terminals (Alexander and Crutcher, 1990; Gerfen, 1990). However, as discussed earlier, this proposal is based on several findings revealed in DA depleted animals.

Unlike intact animals in which D1 agonists produce few if any behavioral effects (Arnt, 1985; Malloy & Waddington, 1984), D1 agonists in DA depleted animals can induce numerous behaviors including rotation, locomotor activity, as well as oral stereotypy (Arnt, 1985; Arnt & Hyttel, 1985). In addition to behavioral changes, neurochemical effects have also been demonstrated. The D1-mediated activity of the proto-oncogene c-fos has been demonstrated to be enhanced in the denervated striatum (Robertson et al., 1988). In addition, increases in c-fos are correlated with increased dynorphin expression indicating that the Fos effects are mediated predominantly within the striatonigral neurons (Bronstein et al., 1994). Moreover, as opposed to intact rats in which systemic D1 agonists produce either variable changes or increases in SNpr activity, DA depleted rats treated with 10 mg/kg SKF 38393 display an overall decrease in nigral activity with 66% of the neurons sampled displaying either partial or complete inhibition as
would be predicted if D1 agonists activated striatonigral efferents (Weick & Walters, 1987). Finally, increases in the level of the peptides dynorphin and substance P, both utilized by striatonigral afferents, are observed in the striatum following D1 agonist administration in depleted animals (Gerfen et al, 1990; Li et al, 1990).

Based on these data many have concluded that D1 receptor activation produced an increase in striatonigral activity (e.g., Alexander & Crutcher, 1990). While this may be true in DA depleted animals it does not appear to be the case in normal intact animals. However, it is often concluded that even in normal animals D1 receptors excite the striatonigral pathway. The reason for this presumed functioning is two-fold. First, one of the most frequent changes observed following the removal of a particular neurotransmitter is the upregulation or supersensitivity of that transmitter's receptors. Thus, in the DA depleted animal, it has been proposed that the D1 receptor has become supersensitive and now produces even greater excitation than that observed in normals. Second, based on the known striatal connectivity we know that the expression of motor
behavior is mediated via a disinhibition of the thalamus. Such disinhibition can be achieved via the activation of the GABAergic striatonigral pathway. Thus, it has been concluded that since DA is involved in the production of locomotor behavior and striatonigral excitation may be necessary for behavioral expression, then DA acting at D1 receptors on striatonigral efferents must be excitatory. However, this may not be the true nature of D1 receptor induced striatonigral effects. By increasing our knowledge of how this projection pathway functions under normal conditions we may be able to displace the above model with one that better fits the data in non-depleted animals. Such an understanding would allow for a more accurate assessment of normal basal ganglia function as well as how it may be change in disease states involving the DA system.
SPECIFIC AIMS

Glutamatergic stimulation and dopaminergic modulation of GABA release from striatal projection neurons is presumed to be a major mechanism by which the basal ganglia fine tunes motor control. The in situ regulation of GABA output via specific DA receptor subtypes, however, has not been fully elucidated. Studies examining the functional output of the striatum in DA-depleted subjects have implied that D1 receptor activation increases the firing rate of GABAergic neurons while D2 activation decreases firing rate. Single cell electrophysiological studies in normal animals, however, observe the opposite, with D1 receptor activation decreasing and D2 receptor activation increasing or decreasing the firing rate of individual neurons. Moreover, this D1 effect is dependent upon corticostriatal activation, acting to dampen glutamate’s depolarizing effects through a reduction of Na⁺ currents. The effect of in vivo D1 activation under basal, non-stimulated conditions is equivocal. The purpose of the following studies is to further define the role of the D1 receptor subtype in the modulation of GABA efflux both within the striatum
(STR), from local collaterals and interneurons, and its main output area, the substantia nigra pars reticulata (SNpr). And furthermore, to examine these effects under conditions of basal vs high glutamatergic stimulation.
Chapter II.

GENERAL METHODS

The techniques utilized in the experiments to follow are explained below. Any variations from these general procedures will be noted in the experimental protocol discussed for each experiment. All conditions were in accordance with NIH guidelines and approved by the University Animal Review Committee.

Subjects

All subjects used were male Sprague-Dawley albino rats procured from Zivic-Miller. Subjects weight at the time of arrival at the Townshend Hall vivarium was 175-200 grams. All subjects were given at least seven days following transport to acclimate to their new environment. Subjects were housed multiply until one day prior to stereotaxic surgery at which time they were housed singly in plastic tubs (48 x 27 x 20 cm) containing Sanicel bedding. At all times food and water were available ad libitum. The vivarium was
temperature-controlled (21-23°C) and maintained a 12 hour light:dark (6:00 am to 6:00 pm) schedule throughout the experiments.

**In Vivo Microdialysis**

Male Sprague-Dawley (225-375 gm) were stereotaxically implanted with chronic unilateral guide cannulae (0.7 mm, o.d.) into the anterior striatum (+1.0 mm A, ±2.5 L, -5.0 V) or into the anterior striatum (as above) and ipsilateral substantia nigra pars reticulata (-5.7 mm A, ±3.8 L, -8.7 V at a 12° angle; all coordinates from Bregma). Subjects were allowed 3 days to recuperate from surgery and habituate to the cannulae. Following surgery animals were placed in the testing environment for at least 3 hr/day to allow them to be fully habituated prior to dialysis. All microdialysis procedures were conducted in plastic testing bowls (35 cm height, 38 cm diameter; Bioanalytical Systems, BAS). On the test day subjects were placed into the bowls and allowed to habituate for 1 hr. Probes (0.5 mm, o.d., 2 mm membrane tip; constructed in our laboratory) were inserted into the striatum or the striatum and the SNpr and continuously perfused with artificial cerebrospinal fluid (CSF) at a
rate of 2.0 ul/min. The artificial CSF (pH = 7.0) consisted of 155 mM Na⁺, 2.9 mM K⁺, 1.1 mM Ca²⁺, 0.83 mM Mg²⁺, 132.8 mM Cl⁻, and 5.9 mM glucose. Dialysates were collected every 15 min and immediately frozen for subsequent analysis via High Pressure Liquid Chromatography. Three hours after the initial probe insertion, 5 baseline GABA samples were collected from either or both regions, following these collections subjects received the test drugs (see text for specific details). Upon the completion of testing, probes were removed and tested for in vitro recovery. The recovery was only used to determine the viability of individual probes. Only those probes displaying at least a 7% recovery were used in subsequent sessions. At no time was the in vitro recovery used to adjust picomolar GABA values. While there was a range in the in vitro recovery values, this range does not reflect or account for the range in responses observed following the various drug treatments. Furthermore, it remains unknown to what extent in vitro recovery actually reflect the in vivo recovery of microdialysis probes. For these reasons, the data were not corrected for in vitro recovery.
Subjects were tested every other day for a total of 3 sessions. Subjects receiving multiple doses or drugs were tested with dose or drug order counterbalanced.

**High Pressure Liquid Chromatographic (HPLC) Analysis**

Microdialysates containing GABA were subject to precolumn derivatization with o-phthalaldehyde (OPA) and tertiary-butylthiol (TBT) to form an oxidizable thiol-substituted isoindole (BAS, Amino Acid Analysis Kit, 1993). This derivatization compound was prepared by dissolving 27 mg OPA in 10 ml of 0.1 M carbonate buffer (pH = 9.6) containing 50% methanol. Forty-five ul of TBT was then added to this solution to form the final product. Ten microliters of this reagent was added to a 20 ul dialysate sample and allowed to react for 3 minutes prior to injection into our HPLC-ED system. The mobile phase (pH = 4.96) contained 58% sodium acetate and 42% acetonitrile. All samples were compared to external GABA standards prepared and analyzed daily.
Behavioral Assessment

During each 15 min collection interval a behavioral assessment of each subject was recorded. Any behavior occurring during this time period was reported. Such behaviors included rotation (ipsi- or contralateral to probe), locomotion (forearms only, all 4 limbs, wall climbing, rearing), stereotypy (sniffing, licking, mouthing), and grooming (hands, face, full body). The frequency of such behaviors was noted as either absent = 0; present but discontinuous = 1; or present continuously = 2. Any behavior observed more than once and lasting for more than 30 sec was considered continuous.

Verification of probe sites

Three to five days following the final dialysis session, rats were given a lethal dose of sodium pentobarbitol and decapitated. Brains were rapidly removed and stored in formalin at 4° C until sectioning. Some subjects were perfused transcardially with saline followed by formalin prior to decapitation. Brains were subsequently mounted on a microtome and 40 um sections were taken and wet mounted on gelatin coated slides. Once sections dried they underwent
cresyl violet staining. Probe placements were then confirmed in these Nissl stained sections.

**Statistical Analyses**

Sample sizes were 5-6 animals per cell unless stated otherwise. A two factor (drug, drug dose, or session as the between-subject factor; time as the within-subject factor) analysis of variance (ANOVA) was used to determine the effect of the various manipulations on GABA efflux. The analysis only include the drug perfusion time points as compared to the last two baseline points. The three collections following termination of the drug were not used, as inclusion of this data would serve to obscure any drug effects.

All statistics were performed on data converted to percent change from baseline. Percentage data was calculated using the mean baseline. The percent change data was used to decrease the variability caused by individual differences in basal levels of GABA and differences in the recovery of probes. T-tests were performed on the raw data comparing the mean basal values for each experimental group. There were no conditions in which basal values were significantly
different. In addition ANOVAs of selected conditions performed on the raw data for both AMPA- and SKF 81297-mediated changes in GABA efflux produced results similar to those observed following analysis of the percent change data. At no time did the percent change data violate the law of homogeneity of variability.

The effects of individual drugs were then analyzed separately using a one-way ANOVA with time as the within-subject factor. Initial analyses included all four drug perfusion points. However, since the duration of some of the drug effects appeared to dissipate following only two or three collection intervals, subsequent analyses only included the time points during which the drug was still active. Post-hoc analyses were conducted using planned t-test comparisons. Statistical significance was defined as $p < 0.05$.

**Drugs**

The drugs to be used in the studies discussed below include the following: AMPA, a glutamate agonist selective for the AMPA receptor subtype; Kainate, a glutamate agonist selective for the kainate receptor subtype; SKF 81297, a full dopamine agonist
selective for the D1 receptor subtype; SCH 23390, a dopamine antagonist selective for the D1 receptor subtype; Nipecotic acid, a GABA uptake blocker; Tetrodotoxin (TTX), a Na+ channel blocker.
Chapter III.
Construction of Microdialysis Probes

Rationale
The technique of in vivo microdialysis allows for the sampling of extracellular neurotransmitters within the brain of an awake unrestrained animal. This sampling procedure requires the placement of a microdialysis probe possessing a dialysis membrane into the brain region of interest. This membrane allows for the diffusion of a transmitter substance with a molecular cutoff weight of 13,000 kd. The probes are constructed to allow the inward flow of artificial cerebrospinal fluid (aCSF) to continuously perfuse the dialysis membrane, as well as to allow the outward flow of aCSF (plus substances under the molecular weight cutoff, including neurotransmitters) to an outlet from which dialysate samples can be collected. The design and construction of such dialysis probes used in all the studies to follow will be the subject of this chapter.
The microdialysis probes used in these particular studies are designed for subjects who were to receive multiple dialysis sessions. Multiple sessions and thus repeated probe insertion requires the surgical implantation of a chronic guide cannula. The guide is stereotaxically positioned to lie directly above the region to be sampled. In the experiments to be presented in subsequent chapters, the cannula were placed in either the anterior striatum and/or the SNpr. Guide cannulae were purchased from CMA (Acton, MA) and consisted of a stainless steel shaft (i.d. 0.9mm, length 1cm) fused to an acrylic body (length 1.7cm). The body of the cannula had a 2.5mm opening at the top which was designed to allow a cap to screw into the guide holding either a dummy cannula or a probe securely in place. When a subject was not being dialyzed, the stainless steel dummy rod (length 1.5mm) was placed within the cannula. The tip of this dummy was flush with the end of the guide shaft.

Based on the above description, it was necessary to design probes that could be inserted and held in placed within this guide. These particular guide cannula were originally designed to fit the CMA 10 microdialysis probe. The probe to be discussed below is based upon this model and in fact uses some recycled parts from damaged CMA 10 probes.
Prior to the development of probe making in our laboratory, CMA 10 probes were purchased from Bioanalytical Systems (BAS; Lafayette, IN). After a few insertions leaks would develop in the dialysis membrane of these probes making them inoperable. Sometimes a tear in the membrane would occur after a single insertion. Such damage was even more likely in active animals. Given the high cost of these probe ($58 each) and their limited utility, it was essential to learn to either make new dialysis probes or be able to repair damaged manufactured probes. In the end the probes to be discussed are a combination of the two approaches. Using the acrylic body of the CMA 10 probe, the remainder of the probe is built around this one piece. These new probes have several advantages. First, they are far more cost effective than purchased probes (averaging about $2.00 each). Second, the dialysis membrane is half the diameter of the CMA 10 probe, reducing mechanical damage caused by probe insertion through the guide cannula. Third, the membrane is less electroactive than that utilized in the CMA 10 probes, and is thus more conducive to the diffusion of amino acids such as GABA. Thus, the
probes to be described below are not only more economical, but are also better suited for the analysis of GABA efflux.

**Microdialysis Probe Construction**

Figure 2 illustrates the CMA 10 probe design. It is dismantled by filing off the outer cannula just below the acrylic body of the probe. The inlet tubing is then removed and the inner cannula can be pulled out of the body. At this stage all that remains is the body of the probe with attached outlet arm and tubing. To prepare the new probe a 5 cm piece of cappilary tubing (o.d. 150um, i.d. 75um) must be cut. This tubing is very fragile and therefore is supported by threading it through a .25cm piece of 30 gauge stainless steel tubing. Once the support is attached, the inner cannula can be inserted through the top of the body. The inlet support through which the cappilary tubing cannula has been threaded must be firmly imbedded into the body of the probe to assure that the inner cannula will stay attached and intact. With the inlet support affixed, the inner cannula should extend from approximately 2mm above the support, through the body, and about 4cm below the body.
The next step is to attach the new outer cannula. This outer cannula consists of a 26 gauge stainless steel hypodermic tubing cut to a length of 2cm. It should slip up over the inner cannula and inside the body of the probe as far as it will reach (approximately 1-1.5cm beyond the joint). The junction between the outer cannula and the body can then be glued. The adhesive used for the construction of these probes is Loctite Superbonder. This glue dries in less than 2 minutes and forms a tight leak free bond. The best seal is achieved by placing the glue near the outer top portion of the outer cannula and then sliding it up into the body.

Once the outer and inner cannula have been assembled, inlet tubing must be attached to the inlet support. The tubing used is 30 gauge teflon tubing cut to a length of 25cm. Glue should be placed around the inlet support described above, after which the inlet tubing is slipped down over the support until it presses against the body.

The next step involves correctly sizing the tip of the probe. In the experiments to follow the length of the probe tip extending beyond the guide shaft was 2mm. Length was adjusted by placing the probe into a guide
cannula and cutting the inner cannula 2mm from the bottom of the shaft. After removing the probe from the guide shaft, the dialysis membrane is now attached. The membrane used in this design was Spectra/Por Hollow Fiber Bundles (Fisher, Inc.). This membrane has a molecular weight cutoff of 13,000 kd with an inner diameter of 200 um. To affix the membrane a 1cm piece of membrane must be cut. The membrane is then gently slipped over the inner cannula and then up under the outer cannula. The membrane is pushed under the outer cannula until only 1mm remains below the inner cannula. The junction of the outer cannula and the membrane is then be glued.

The final step in the preparation of the probe is sealing the tip of the dialysis membrane. The difficulty in gluing the tip is in limiting the amount of glue that may permeate along the membrane, reducing the area available for diffusion or possibly blocking the flow of liquid out of the inner cannula. To limit the area of membrane glued, a drop of glue is first placed onto the tip of a syringe, and then applied to the membrane with the syringe. Often, several applications of glue are necessary to completely prevent leaks from the membrane tip. To determine if
the membrane is completely sealed alcohol may be perfused through the inlet tubing. If no leaks are present and the alcohol flows from the outlet tubing the probe is ready for in vivo use.

In Vitro Recovery

When probes are completed the membranes must be kept wetted in 0.5% Kathon dissolved in distilled water. This prevents the membrane from drying out. To test the recovery of these probes, the membrane is placed into a 10 pmol GABA standard solution. Following a 20 min perfusion with aCSF, a 15 minute sample is collected and analyzed via HPLC. The percentage of the 10 pmol recovered in this sample is indicative of the in vitro recovery of the probe. The probes used in the following studies had recoveries ranging from 4% to 22%, with an average recovery of 9.8% (± 0.09). This recovery is similar to that observed in manufactured probes.

Conclusions

In summary, these probes assembled within the laboratory have proven effective, efficient, and economical. No longer do leaks in the membrane end the
productivity of a probe. Relatively quickly, a new membrane can be attached and once again the probe is functional. This rapid turnover was crucial in those studies where drugs produced robust behavioral effects. In these subjects, removal of the probe at the end of a session often led to damaged membranes. Such damage was remedied often the same day with probes ready for subsequent dialysis sessions. While assembly can be time consuming and the fine detail work may at times be frustrating, clearly these concerns are far less disconcerting than spending $58.00 on a probe that may work for a single dialysis session.
Chapter IV

Validation of Repeated Microdialysis Sessions for the Measurement of GABA efflux

Rationale

The in vivo measurement of the neurotransmitter GABA is complicated by several issues. As a relatively recent technique, GABA microdialysis can be limited by several factors, the first and foremost being the abundance of amino acids both in brain and in the environment. Due to the ubiquitous nature of these amino acids it was crucial to confirm that the chromatographic peak produced from an in vivo dialysate was in fact GABA and not some other amino acid. This issue was addressed by administering a specific GABA uptake blocker in vivo to selectively elevate GABA levels.

The second point to be considered when measuring GABA efflux is the relative contributions of neuronal vs non-neuronal pools of GABA efflux. It is well known that within the central nervous system (CNS) there are two pools of GABA, one neuronal and one glial. To
ensure that effects are not due to changes in glial release we used the sodium channel blocker tetrodotoxin (TTX) to block neuronal depolarization, inhibiting all neuronal release of GABA while leaving glial release unaffected. Any changes in GABA efflux observed in the presence of TTX can be assumed to be the result of changes in glial release.

The third and final issue to be discussed in this chapter is the validation of a repeated microdialysis sessions design. Specifically we examined the validity of employing repeated dialysis sessions for the measurement of AMPA- and D1- mediated GABA efflux within the striatum. Statistically this design can be more powerful than a between-subject strategy, allowing for the assessment of various drug/dose effects within the same animal and against its own basal efflux, decreasing variance between animals. It has been suggested, however, that repeated testing may interact with the effects of drugs, distorting the appropriate interpretation of any observed effects (Robinson, 1990). In addition, changes in GABA efflux, both basal and stimulated, may be affected by repeated insertion of the probe over multiple sessions. Some of the possible effects of repeated testing over a number of
days could include changes due to increased gliosis around the probe, GABA terminal damage, increased glial uptake of GABA as well as changes in drug availability. Therefore, it was necessary to justify the appropriateness of the design with regard to repeated drug administration and repeated sessions.

**Procedures**

Male Sprague-Dawley (275-350) rats were stereotaxically implanted with unilateral stainless steel guide cannulae (o.d. 0.9 mm) terminating in the anterior striatum (1.0 A, 2.5 L, 4.0 V; all coordinates from Bregma). Following a 3 day recovery period, concentric dialysis probes were inserted and basal and drug-induced GABA efflux was measured in freely-moving animals.

The procedure for any given dialysis session was as follows: After 60 min of habituation to the testing bowls the probe was inserted and continuously perfused with artificial cerebrospinal fluid (aCSF) at a rate of 2 ul/min (see Chapter II for details). Three hours after probe implantation, 5 baseline samples were collected from the STR at 15 min intervals. Following this baseline period, either nipecotic acid (500 uM),
TTX (10 uM), AMPA (100 uM), SKF 81297 (10 uM), or aCSF was administered via the probe directly into the STR. In those subjects receiving multiple dialysis sessions, each session was separated by a day in which dialysis was not performed. Probe placements were determined as described in Chapter II and are illustrated in figure 3.

Nippecotic Acid:

In those subjects receiving nippecotic acid (500 uM), the drug was perfused directly into the striatum for 60 minutes following baseline and dialysates were collected at 15 minute intervals. These subjects only received a single dialysis session.

TTX:

In those subjects receiving TTX (10 uM), the drug was perfused directly into the striatum for 45 minutes following baseline and dialysates were collected at 15 minute intervals. This initial 45 minute period was followed by a subsequent 45 minute perfusion with TTX (10 uM) + AMPA (100 uM). Subjects were separated into two groups. The first group received TTX only on the first dialysis session (3 days following surgery),
while the second group received aCSF during the first two dialysis sessions and TTX on the third session only.

Repeated Sessions:

In those subjects receiving the glutamate agonist AMPA (100 μM) or SKF 81297 (10 μM), drugs were perfused directly into the striatum for 60 minutes following baseline with collections made at 15 minute intervals. The probe was then perfused with aCSF without drug for 45 minutes and 3 additional dialysates were collected.

Subjects in the repeated sessions experiments were separated into two distinct groups. The first group of animals (Group 1) received intrastriatal perfusion of AMPA (100 μM) via the probe to stimulate striatal GABA release on all 3 sessions. The second group of animals (Group 2) were similarly treated except that their perfusion medium contained aCSF alone on sessions 1 and 2 with AMPA (100 μM) perfused into the STR only on session 3. Similarities in the ability of AMPA to increase GABA efflux on session 1 (Group 1) vs session 3 (Group 2) determined the appropriateness of the within-subject design. Comparison of the effects of AMPA on session 1 (Group 1) vs session 3 (Group 1)
addressed the impact of repeated administration of AMPA. Those subjects receiving the D1 agonist SKF 81297 (10 uM) were similarly treated however subjects in Group 1 only received the agonist on session 1 and session 3 with aCSF perfused during session 2.

**Results**

The effects of nipecotic acid (500 uM) can be observed in figure 4. There was a significant increase in the maximum percent change in GABA from baseline ($t[2] = -19.37; p < 0.003$) following the administration of this specific GABA uptake blocker. These effects were observed as soon as the first collection interval and were maintained during the entire drug perfusion period (60 minutes). The maximum percent increase observed was 1598% of basal GABA. These findings indicate that the chromatographic peak determined by external standards was in fact GABA.

The administration of TTX (10 uM) significantly decreased GABA efflux within the striatum. The effect of TTX on striatal GABA efflux was immediate and lasted throughout perfusion. As can be observed in figure 5, there were no differences observed between those subjects tested on the first versus the third dialysis
session neither in terms of basal ($F_{[1,4]} = 3.03, p< 0.16$) nor TTX-induced ($F_{[1,4]} = 2.83, p< 0.17$) GABA efflux. As all subjects were found to be similar, the data presented below represent the combined results of both sessions.

Striatal GABA efflux was significantly reduced following the administration of TTX ($F_{[4,20]} = 12.58, p< 0.0001$), for all perfusion periods. The maximum percent change was 74%, indicating that the majority of GABA being measured was of neuronal origin. Moreover, in both groups the co-perfusion of TTX with the excitatory amino acid AMPA (100 uM) blocked the stimulatory effect normally observed following AMPA perfusion (see figure 5). Thus, even with the addition of AMPA, GABA in the presence of TTX remained significantly below basal values ($F_{[4,20]} = 11.67, p< 0.0001$). These data indicate that the majority of both basal and AMPA-mediated GABA efflux is of neuronal origin even following multiple perfusions.

Repeated Sessions (AMPA):

AMPA (100 uM) induced a significant increase in striatal GABA efflux in all of the sessions in which GABA efflux was measured. Subjects receiving AMPA
during all three dialysis sessions (Group 1) displayed similar basal GABA levels on both session 1 and session 3 ($t[5] = -.55, p< 0.6$). Additionally, subjects receiving AMPA only on session 3 (Group 2) displayed baselines similar to those of subjects in Group 1 ($t[16] = -1.67, p< 0.12$), as can be seen in Figure 6. Therefore, neither repeated insertion of the microdialysis probe, nor repeated AMPA administration altered basal GABA values.

In order to determine the effects of repeated probe insertion on AMPA-mediated GABA efflux comparisons were made between Group 1 session 1 and Group 2 session 3 as can be observed in Figure 7. Analysis of variance of the final two baseline points against all four drug perfusion points revealed a significant drug effect ($F[5,35] = 3.42, p< 0.01$). There was, however, no significant effect of session ($p< 0.13$), nor a significant session by drug interaction ($p< 0.41$). Individual ANOVA’s on the final two baseline points against drug perfusion time points were performed for each individual session to determine the nature of the observed drug effect. An ANOVA on Group 1 session 1 was significant when only the first drug perfusion point (15 minutes) was included in the
analysis ($F[2,8] = 4.49, p< 0.049$). An ANOVA on Group 2 session 3 revealed a significant drug effect when comparing the final two baseline points against all four drug perfusion points (15, 30, 45, 60 minutes) ($F[5,25] = 2.95, p< 0.031$). Planned comparisons of the first drug perfusion time point (15 min) and the final drug perfusion time point (60 min) against the final baseline point revealed significant drug effects ($t[5] = -2.35, p< 0.03$ and $t[5] = -2.23, p< 0.038$, respectively) at both time points. Thus, AMPA administration was able to increase GABA efflux in both sessions and while the effect was of increased duration in Group 2 session 3, this increased duration did not lead to significant differences between the sessions.

The above comparisons indicate that repeated probe insertions do not affect AMPA-mediated GABA efflux. However, in order to determine whether repeated administration of AMPA altered GABA efflux, comparisons were made between Group 1 session 1 and Group 1 session 3 (Figure 8). Analysis of variance of the final two baseline points against the first three drug perfusion points (15, 30, 45 min) revealed a significant drug effect ($F[4,20] = 3.27, p< 0.032$). However, neither a significant session effect ($F[1,5 = 0.39, p< 0.56]$ nor
a significant drug by session interaction was observed $(F[4,20] = .43, p< 0.78)$. An ANOVA on Group 1 session 3 revealed a significant drug effect during the first three drug perfusion time points $(F[4,20] = 3.49, p< 0.026)$. Thus, during both session 1 and 3 there was a significant although brief increase in GABA efflux as can be observed in Figure 8. Again, no difference between the two sessions were observed indicating that there was no effect of repeated drug perfusion.

Repeated Sessions (SKF 81297):

SKF 81297 (10 uM) induced a significant decrease in GABA efflux in all of the sessions in which GABA efflux was measured. Subjects receiving SKF 81297 on sessions 1 and 3 (Group 1) displayed similar baseline GABA levels on session 1 and session 3 $(t[5] = .13, p< 0.90)$. Additionally, subjects receiving SKF 81297 only on session 3 (Group 2) displayed baselines similar to those of subjects in Group 1 $(t[16] = -1.43, p< 0.20)$, as can be seen in Figure 9. Therefore, as was observed with AMPA perfusion, neither repeated insertion of the microdialysis probe, nor repeated SKF 81297 administration altered basal GABA values.
In order to determine the effects of repeated insertion of the probe over multiple sessions on SKF 81297-induced GABA efflux, comparisons were made between Group 1 session 1 and Group 2 session 3 as can be observed in Figure 10. Analysis of variance of the final two baseline points against all four drug perfusion time points revealed a significant drug effect (F[5,50] = 3.22, p < 0.013). There was, however, no significant effect of session (F[1,10] = .32, p < 0.58), nor a significant session by drug interaction (F[5,50] = 0.27, p < 0.92). Individual ANOVAs on the final two baseline points against drug perfusion time points were performed for each individual session to determine the nature of the observed drug effect. An ANOVA on Group 1 session 1 revealed a significant drug effect when comparing the final two baseline points against the first three drug perfusion points (15, 30, 45 min) (F[4,20] = 3.71, p < 0.021). An ANOVA on Group 2 session 3 was significant when all four of the drug perfusion points (15, 30, 45, 60 min) were included in the analysis (F[5,25] = 3.34, p < 0.019). Thus, SKF 81297 was able to decrease GABA efflux in both sessions and while the effect was of a slightly increased duration
in Group 2 session 3, this increased duration did not lead to significant differences between the sessions.

The above comparisons indicate that repeated probe insertions do not effect SKF 81297-mediated GABA efflux. However, in order to determine whether repeated administration of SKF 81297 altered GABA efflux, comparisons were made between Group 1 session 1 and Group 1 session 3. Analysis of variance of the final two baseline points against all four drug perfusion points (15,30,45,60 min) revealed a significant drug effect (F[5,50] = .68, p< 0.001). However, neither a significant session effect (F[1,10] = 0.50, p< 0.51) nor a significant drug by session interaction was observed (F[5,50] = 1.74, p< 0.16). An ANOVA on Group 1 session 3 revealed a significant drug effect during all four drug perfusion time points (F[5,25] = 18.14, p<0.0001). Thus, during both sessions 1 and 3, there was a significant decrease in GABA efflux which was of a slightly more long lasting duration in Group 1 session 3 as can be observed in Figure 11. Again, while there was no difference observed between the sessions, there was a tendency for those subjects that received SKF 81297
more than once to display a decrease in GABA efflux lasting slightly longer than that observed in subjects only treated once.

Conclusions

The results reported above indicate that the amino acid being measured via HPLC is in fact GABA. They also indicate that the majority of this GABA is of neuronal origin, even following multiple dialysis sessions. Finally, they indicate that there does not appear to be a detrimental effect of session on either basal or drug mediated GABA efflux. The absence of significant session effects following either AMPA or SKF 81297 perfused directly into the striatum indicates that repeated insertion of a microdialysis probe and subsequent perfusion with aCSF does not change the manner in which these drugs effect GABA efflux. Similar findings have been reported for the D2 antagonist clebopride with regard to the measurement of striatal acetylcholine (ACh) efflux and for the benzodiazapine inverse agonist FG 7142 on cortical ACh (Bruno et al., 1994). To my knowledge, this study is the first to demonstrate that striatal GABA efflux can be measured repeatedly within the same subject.
The data regarding multiple perfusion with the excitatory amino acid agonist AMPA demonstrated the absence of a significant session effect. As excitatory amino acids are well known to induce excitotoxicity and cell death at high doses, it was crucial to show that AMPA perfused directly into the striatum was not damaging to the area around the probe. Since no session effect was observed following multiple perfusion with AMPA and histological verification of the probe placement revealed no excessive damage around the probe tip in subjects treated with AMPA, it is likely that AMPA at the dose given was not excitotoxic, even when administered during multiple sessions.

A similar lack of session effects was observed with the D1 agonist SKF 81297. It has been suggested that SKF 38393 at certain doses acts as a neurotoxin within the striatum (Kelly et al., 1988). Thus, it was important to demonstrate that the full D1 agonist SKF 81297 could be administered to the striatum during multiple sessions without damaging the area around the probe. Again, both the lack of a session effect observed in those subject tested repeatedly, and the lack of damage observed in these subject upon histological verification indicates no such toxicity.
These data are critical for the analysis of subsequent studies examining glutamate-DA interactions within the striatum in which AMPA, SKF 81297 and AMPA + SKF 81297 are administered often within the same animal. Thus, while others have demonstrated that multiple perfusions of some drugs (i.e. amphetamine) alter the response of particular neurotransmitters in the striatum, no such effects were observed when examining either basal or drug-induced changes in GABA efflux following multiple sessions or multiple drug perfusions within the striatum.
Chapter V.

AMPA and DA D1 Mediation of Striatal and Nigral GABA Efflux

Rationale

Data from electrophysiological studies within the striatum indicate that D1 receptor activation serves to decrease the number of action potentials observed in striatonigral neurons in response to depolarizing pulses (Calabresi et al., 1987; Twery et al., 1994). This effect has been shown to be the result of dampening Na+ current activity in response to excitatory inputs (Surmeier, et al., 1992). The in vitro GABA release data, however, do not appear to corroborate this electrophysiological data. In release studies using striatal slices, D1 receptor activation within the striatum has been shown to increase GABA release (Floran et al., 1990). This effect, which has only been observed during K+-induced depolarization, has been used to support the position that D1 receptor activation increases striatonigral activity. However,
as discussed in Chapter 1, D1 receptors are present not only on the cell bodies of striatonigral neurons, but also on the terminals of these projection neurons both within the striatum and the SNpr (Barone et al., 1987; Floran et al., 1990). Furthermore, these receptors have been shown to be release regulating receptors that appear to increase GABA release from terminals when activated (Cameron & Williams, 1993). Thus, increased GABA release following D1 receptor activation, which has only been observed when striatal neurons are depolarized, may be due to activation of terminal D1 receptors and may depend upon the level of corticostriatal activation.

The experiments to be discussed in this chapter examined the effects of intrastriatal perfusion of the D1 agonists SKF 81297 (10 or 100 uM), the non-NMDA glutamate agonist AMPA (100 uM) or the combination of AMPA (100 uM) + SKF (10 or 100 uM) on STR and SNpr GABA efflux. These studies were proposed in order to examine D1-mediated release of GABA from striatonigral neurons in vivo. Furthermore, since most of the in vitro studies were performed under depolarized conditions, studies examining the co-perfusion of AMPA with the D1 agonists served to replicate some of the in
vitro studies in an intact system. Thus, these studies examined the effects of D1 receptor activation under basal and stimulated conditions within the striatum and the substantia nigra pars reticulata of an awake animal.

Finally, studies suggest that there is a very low level of tonic glutamatergic corticostriatal activation (Neisenbaum et al., 1993). To determine whether such basal stimulation exists in awake animals, an additional group of subjects were treated with the AMPA antagonist DNQX (100 uM) in an attempt to block this corticostriatal excitation.

**Procedures**

Male Sprague-Dawley rats (225-375 grams) were implanted with unilateral guide cannulae terminating in the anterior striatum and the substantia nigra (see Chapter II for details). The animals were divided into 2 treatment groups with one group receiving aCSF or aCSF + SKF 81297 (10 or 100 uM) perfused via the dialysis probe into the striatum and the other group receiving AMPA (100 uM) or AMPA (100 uM) + SKF 81297
(10 or 100 μM) also directly perfused into the striatum. Both STR and SNpr GABA release was determined in all subjects.

After 5 baseline collections, animals were administered SKF 81297 via the probe into the striatum. In half of these animals, CSF containing AMPA (100 μM) was then co-infused into the STR with the SKF 81297. GABA efflux was measured every 15 minutes for one hour following SKF 81297 administration. During subsequent 45 minutes aCSF was perfused once again with collections at 15 minute intervals. Subjects receiving the glutamate antagonist DNQX (100 μM) were treated similarly. However, only 3 subjects were studied.

Since we were limited to 3 dialysis sessions, separated by 1 day each, dose was maintained as a within-subject factor with AMPA activation as the between-subjects factor. Subjects were randomly assigned to the above conditions. In order to perform a more conservative statistical analysis, all factors were treated as between-subject factors working against detection of treatment effects.

Probe placements were determined as described in chapter II and are illustrated in figure 3 (striatum) and figure 12 (substantia nigra).
GABA Efflux within the Striatum:

Striatal GABA release data from those subjects treated with SKF 81297 under basal conditions are illustrated in Figure 13. A mixed two-factor ANOVA was performed with dose (0, 10, or 100 uM) as the between-subject factor and collection interval (time) as the within-subject factor. Comparisons were made using the final two baseline points against all four drug perfusion time points. In these subjects, there was a trend for a dose effect ($F[2,10] = 20.37, p < 0.06$) as well as a trend for a time effect ($F[5,25] = 2.15, p < 0.09$). Furthermore, a significant dose by time effect was revealed ($F[10,50] = 3.32, p < 0.002$). In order to further characterize these findings, the data were dissected to examine the effects of the individual doses on striatal GABA efflux. Artificial CSF alone had no significant effect on GABA efflux as was the prediction ($F[5,25] = 0.47, p < 0.80$). SKF 81297 (10 uM) was found to significantly reduce striatal GABA efflux ($F[2,25] = 3.07, p < 0.027$). Interestingly, a bidirectional effect of SKF 81297 on GABA efflux was observed with the lower dose decreasing release while
the higher dose (100 uM) produced a significant increase in striatal GABA (F[5, 25] = 2.99, p < 0.03).

Striatal GABA efflux in those subjects co-perfused with AMPA and SKF 81297 are shown in Figure 14. Again a mixed two-factor ANOVA was performed with drug and/or dose (AMPA 100 uM, AMPA 100 uM + SKF 81297 10 or 100 uM) as the between-subject factor and collection interval (time) as the within-subject factor. Comparisons were made using the final two baseline points against all four drug perfusion time points. A significant effect of time was observed in these subjects (F[5, 20] = 4.45, p < 0.007). However, neither a significant drug/dose effect (F[2, 8] = 1.77, p < 0.231), nor a significant drug/dose by time interaction (F[10, 40] = 0.94, p < 0.510) was observed. The effects of the individual treatments were subsequently examined with one way ANOVAs with time as a within-subject factor. As predicted AMPA (100 uM) alone produced a significant time effect, increasing GABA efflux within the striatum (F[5, 30] = 2.80, p < 0.03). The co-perfusion of AMPA + SKF 81297 (10 uM) also induced a significant increase in GABA (F[5, 30] = 3.69, p < 0.01). While AMPA + SKF (100 uM) also increased striatal GABA release, this effect was
not significant when examining all four drug perfusion points. However, t-test comparisons reveal that AMPA + SKF 81297 (100 uM) did significantly increase GABA efflux during the first two time points (15, 30 minutes) as compared to the final baseline point (t[4] = -4.45, p < 0.011 and t[4] = -3.21, p < 0.03, respectively).

Comparisons of AMPA alone with AMPA + SKF 81297 (10 uM), as illustrated in figure 15, reveal that the low dose of SKF 81297 reduces the increase in striatal GABA efflux observed when AMPA is given alone, however this reduction is not significant (F[1, 6] = 4.09, p < 0.19). Additional comparisons between AMPA alone and AMPA perfused with the higher dose of SKF 81297 (100 uM) are demonstrated in figure 16 and do not display any significant differences between the two groups. Finally, comparisons between the high dose of SKF 81297 alone and when co-perfused with AMPA demonstrate similar responses in both groups of subjects following these different treatments again shown in figure 16.

GABA Efflux within the Substantia Nigra:

The results for GABA efflux in the substantia nigra pars reticulata following perfusion of drugs
within the striatum produced highly variable effects as can be observed in Figures 17 and 18. None of the striatal drug manipulations produced any significant changes in striatal GABA efflux. This was not due to poor probe placement as only those subjects with at least one millimeter of dialysis membrane perfusing the nigra were analyzed. Figure 12 displays a montage of nigral probe placements for subjects used in this study. Thus, even when GABA could be detected within the substantia nigra, neither AMPA nor SKF 81297 within the striatum produced a correlated increase in nigral GABA efflux.

**Effects of AMPA Antagonists on Striatal GABA Efflux:**

The administration of the glutamate antagonist DNQX (100 uM) into the striatum did not produce the predicted decrease in basal GABA efflux. In fact what was observed was an increase in GABA efflux that marginally approached significance during the first drug perfusion period. The lack of significance may be due to the small sample number as only three subjects were tested. However, these data, which can be observed in figure 19, indicate that simply blocking AMPA receptors does not lead to a reduction in GABA.
It is possible that the observed increase is due to a long loop feedback that serves to further increase the firing rate of striatal cells in response to inhibition via the antagonist. It has been demonstrated that the glutamate antagonist CNQX blocks corticostriatal stimulation of medium spiny neurons in striatal slices (Nisenbaum et al., 1993), however, it is unclear how our increased GABA efflux would relate to striatal firing rates.

Behavioral Effects of Striatal AMPA and SKF 81297:

Prior to drug administration animals tended to be quiet, often sleeping or lying with eyes open. On no occasion were animals highly active during baseline collections. The perfusion of AMPA induced robust locomotor behavior during the entire perfusion period and even after the drug had been turned off. The most often observed behaviors were rotation, wall climbing, rearing, and sniffing. These behaviors were often all performed at a high frequency during any one collection period. These animals appeared highly agitated and some repeatedly attempted unsuccessfully to jump out of the testing bowl. Those subjects perfused with AMPA + SKF 81297, also displayed similar behaviors.
Animals perfused with the D1 agonist SKF 81297 at either dose did not exhibit any observable changes in behavior. These animals tended to sleep or remain immobile during the entire session with only the occasional discontinuous bout of grooming.

Finally, those subjects perfused with the AMPA antagonist DNQX (100 uM) also demonstrated increased locomotor activity, however, they did not display behavioral activation until after the drug had been removed. Thus, during the final three collection intervals, when only aCSF was being perfused, these subjects demonstrated rotation and wall climbing that dissipated after about 30 minutes.

As is demonstrated in Table 1., changes in striatal GABA efflux could not be dissociated from observable behavior. While both AMPA and SKF 81297 (100 uM) produced increases in striatal GABA, only AMPA administration produced increases in locomotor activity. Furthermore, while different doses of SKF 81297 induce bidirectional changes in GABA efflux, neither induced increased behavior. Thus, changes in GABA efflux do not appear to reflect similar changes in behavior. Additional evidence for lack of dissociation
between increases in behavior and increases in striatal GABA efflux are illustrated in figure 20.

**Conclusions**

These experiments clearly demonstrate that D1 modulation of GABA efflux within the striatum is quite complex whether examined under basal conditions or under conditions of increased glutamatergic tone. The low dose of SKF 81297 decreased GABA efflux both under basal and stimulated conditions, seemingly in agreement with the electrophysiological data that would explain such a decrease in release based on a dampening of excitatory inputs. However, the high dose of SKF 81297 seems to agree with the *in vitro* slice data, increasing the release of GABA within the striatum. It is interesting that this SKF 81297-induced increase in striatal GABA was not further enhanced by the addition of AMPA to the perfusion medium. All of these interactions underscore the complexity of striatal circuitry as well as the differences observed when working in whole animals as opposed to slices.

The lack of an effect within the substantia nigra pars reticulata was unexpected. It was unexpected that the AMPA manipulation which is known to enhance
striatonigral firing rates (Nisenbaum et al., 1993) did not produce increases in GABA efflux within the SNpr. This lack of effect is even more troubling due to the increase in behavioral activation observed in these animals. As the final output to the thalamus, inhibition of nigrothalamic neurons is necessary for excitatory inputs from the thalamus to effect cortical circuits necessary for the expression of behavior. This inhibition of these nigrothalamic neurons is presumably due to increase GABA release within the SNpr. Why we failed to see such an increase in release while clearly observing enormous increases in behavioral activity remains unknown. It is possible that as a structure that receives numerous inputs from the globus pallidus (internal and external), the subthalamic nuclei, the striatum, as well as from some brainstem nuclei, GABA efflux within the substantia nigra is too variable for one to measure small changes induced by all striatal manipulations. Perhaps a larger increase in firing rates is necessary before changes can be observed within the SNpr. The ability
of these methods to detect changes in the substantia nigra following direct perfusion of drugs within the nigra is examined in Chapter VII.
Chapter VI.

Effects of the D1 Antagonist SCH 23390 on SKF 81297-induced Changes in Striatal GABA Efflux

Rationale

These experiments were conducted to confirm that the observed D1 agonist-induced changes in GABA efflux could be blocked by a selective D1 antagonist. This should ensure that the D1 agonist is acting selectively at the D1 receptor subtype and not producing its effects via non-selective activation of another DA receptor subtype. These experiments are especially important considering the bidirectionality of the D1 effect observed within the striatum. It is possible that the reason for the change in the direction of the effect with the high dose is due to the non-specific activation of additional DA receptor subtypes. Thus, the ability to block both the high and low doses of SKF 81297 with the well characterized selective D1 antagonist SCH 23390 was examined.
Procedure

Male Sprague-Dawley rats (225-375 grams) were implanted with unilateral guide cannulae terminating in the anterior striatum (see Chapter II for details). The animals were divided into 2 treatment groups. The first group received either SCH 23390 (10 uM), SKF 81297 (10 uM), or SCH 23390 (10 uM) + SKF 81297 (10 uM) perfused directly into the striatum. The second group received SCH 23390 (10 uM), SKF 81297 (100 uM), or SCH 23390 (10 uM) + SKF 81297 (100 uM) also directly perfused into the striatum. Striatal GABA efflux was determined in all subjects.

After 5 baseline collections, animals were perfused with the drugs listed above. GABA efflux was measured every 15 minutes for one hour following drug administration. During the subsequent 45 minutes aCSF was perfused once again with collections at 15 minute intervals.

Subjects were randomly assigned to the above conditions. In order to perform a more conservative statistical analysis, all factors were treated as between-subject factors working against detection of treatment effects.
Probe placements were determined as described in chapter II and are illustrated in figure 3.

Results

The data for subjects receiving SKF 81297 (10 uM) and SCH 23390 (10 uM) are shown in figure 21. A two-way ANOVA with drug as the between subject factor and time as the within subject factor was performed examining the final two baseline points against the four drug perfusion time points. While there was no significant effect over time ($F[5,25] = 1.10, p< 0.384$), the analysis did reveal a significant effect of drug treatment ($F[2,10] = 5.48, p< 0.025$), as well as a significant drug by time effect ($F[10,50] = 2.52, p< 0.015$). Upon closer examination of the individual drug effects, one way ANOVAs with time as the within-subject factor revealed a significant decrease in striatal GABA efflux in those subjects receiving SKF 81297 ($F[5,25] = 5.18, p< 0.002$) as is consistent with those studies discussed above. However, neither SCH 23390 nor SCH 23390 + SKF 81297, produced significant changes in GABA efflux. Planned comparisons of subjects receiving SKF 81297 alone with those receiving SCH 23390 + SKF 81297 at the first
(t[5] = -3.92, p< 0.011) and final (t[5] = -2.83, p< 0.037) drug perfusion time points indicated that SCH 23390 significantly antagonized the SKF 81297-induced decrease in striatal efflux throughout the perfusion period.

Similar analyses were performed on results from those subjects receiving the higher dose of SKF 81297 (100 uM) in combination with SCH 23390 (10 uM; Figure 22). The initial analyses revealed a significant effect of time (F[5,25] = 4.59, p< 0.009) as well as a significant drug by time effect (F[10,50] = 2.34, p< 0.037, however there was no significant drug effect (F[2,10] = .15, p< 0.886). Analyses of the individual drug effects demonstrated that only SKF 81297 (100 uM) induced a significant increase in GABA efflux (F[5,25] = 6.69, p< 0.001). However, post hoc analysis comparing the final baseline point to subsequent drug perfusion points revealed that only the first 15 min time point was significantly different from baseline (t[5] = -4.03, p< 0.01). Neither of the other groups, SCH 23390 alone nor SCH 23390 + SKF 81297, induced significant changes in striatal GABA efflux. However, there was a trend for an increase in GABA efflux following SCH 23390. Planned comparisons between SKF
81297 alone and SCH 23390 + SKF 81297 in which the first and only significant drug perfusion period was examined revealed a significant effect ($t[5] = 3.01, p < 0.03$), indicating antagonism of the high dose of SKF 81297 with the D1 antagonist SCH 23390. Therefore, during the one perfusion period in which SKF 81297 induced a significant increase in GABA efflux, SCH 23390 was apparently able to antagonize that effect as can be observed in figure 22.

**Conclusions**

The results in both those subjects administered the low or the high dose of SKF 81297 once again demonstrate the bidirectionality of the D1 effect on GABA efflux with the low dose decreasing and the high dose increasing the release of GABA within the striatum. In both groups, SCH 23390 induced an increase in GABA efflux, however, this increase did not prove to be significant. Interestingly, while SCH 23390 alone tended to increase GABA efflux, it was still able to antagonize both the decrease and increase induced by the different doses of the D1 agonist. The lower dose of SKF 81297 induced a longer lasting effect on GABA efflux, which was blocked completely by the
addition of SCH 23390. In those subjects receiving the higher dose of SKF 81297 the observed increase in GABA efflux was significant but relatively brief. The brevity of this effect may be due to increased variability in individual responses to the agonist which precluded the detection of significance. However, at the one time point during which the significant increase was observed, the addition of SCH 23390 to the perfusion medium was able to block the expected increase. Thus, the D1 antagonist SCH 23390 (10 μM) was able to fully block the effect of either dose of the D1 agonist. These results indicate that SKF 81297 at both doses tested are acting at D1 receptor subtypes and are therefore not the result of some non-specific receptor activation.
Chapter VII

Future Directions: Administration of the Glutamate Agonist Kainate and Direct Drug Manipulations within the Substantia Nigra pars Reticulata

Rationale

The purpose of this chapter is to present some preliminary data on both striatal and nigral manipulations using an additional glutamate agonist. As the studies presented earlier (see Chapter V) did not reveal significant changes in the substantia nigra following striatal manipulations with the glutamate agonist AMPA, it was postulated that perhaps an agonist acting at the kainate receptor which is also present in large numbers within the striatum (Albin et al., 1992), may induce changes GABA efflux within the substantia nigra. Indeed, a recent dialysis study examining the effect of kainic acid on striatonigral GABA efflux demonstrated significant increases in both striatal and nigral release following intrastriatal kainate (Bianchi et al., 1994).
In addition, to ensure that alterations in nigral GABA levels can be detected with this method, kainate was also perfused directly into the nigra. As there are glutamate receptors present on neurons within the substantia nigra (Albin et al., 1992), and these particular neurons, like those in the striatum, possess local axon collaterals (Daniau et al., 1982), glutamate agonists should increase GABA efflux in the nigra. Finally, as there has been much discussion as to the presence of release regulating terminal D1 receptors within the substantia nigra (Cameron and Williams, 1993; Floran et al., 1990), the ability of the D1 agonist SKF 81297 to stimulate release from these receptors under basal conditions in the awake animals was also tested.

**Procedures**

Male Sprague-Dawley rats (225-375 grams) were implanted with unilateral guide cannulae terminating within the anterior striatum and substantia nigra pars reticulata (see Chapter II for details). The animals were treated with either the glutamate agonist kainate (100 uM) directly perfused into the striatum, kainate (100 uM) perfused into the substantia nigra, or the
high dose of the D1 agonist SKF 81297 (100 uM) also perfused into the substantia nigra. Striatal and nigral GABA efflux was determined for those subjects receiving intrastriatal kainate. During sessions in which drugs were perfused into the substantia nigra, only nigral GABA efflux was measured.

After 5 baseline collections, animals were perfused with the drugs described above. GABA efflux was measured every 15 minutes for one hour following drug administration with the exception of kainate delivered into the nigra which was only perfused for 30 minutes to avoid the possibility of seizure activity. During the subsequent 45 minutes (75 minutes for those receiving intranigral kainate) aCSF was perfused once again with collections at 15 minute intervals. Subjects received all of the above conditions in a counterbalanced order.

Probe placements were determined as described in chapter II and are illustrated in figure 3 (striatum) and figure 12 (substantia nigra).

Results

Subjects treated with kainate delivered directly into the striatum (n=3) displayed increase GABA efflux
within the striatum as well as concomittant increases within the substantia nigra (figure 23). A one-way ANOVA was performed using time following drug perfusion as the within-subject factor. Comparison of the final two baseline points against the first three drug perfusion time points revealed a significant effect of kainate on striatal GABA efflux ($F_{[4,8]} = 5.28$, $p < 0.022$). A similar analysis against all four drug perfusion points revealed a significant effect of kainate on nigral GABA efflux ($F_{[5,10]} = 4.11$, $p < 0.027$). T-tests revealed that both striatal and nigral GABA efflux was significantly increased by the first 15 minute drug perfusion time point ($t_{[2]} = -5.16$, $p < 0.036$ and $t_{[2]} = -11.70$, $p < 0.007$, respectively). Furthermore, this increase in GABA efflux had dissipated in both areas by the final 60 minute time point ($t_{[2]} = -2.28$, $p < 0.150$ and $t_{[2]} = -2.12$, $p < 0.168$, respectively). Thus the kainate-induced increase in striatal GABA, as well as the concomitant increase in nigral GABA efflux, both depicted in figure 23, are maintained throughout the drug perfusion period, although the increased efflux is only significantly different from baseline during the first 45 minutes of kainate perfusion.
As within the striatum, kainate perfused directly into the substantia nigra (n=4) also induced increased GABA efflux (figure 24). Although the drug was only perfused for 30 minutes, a one-way ANOVA comparing the last two baselines against the four time points following the initiation of kainate (60 minutes post drug) revealed a significant drug effect which was present even after the drug had been removed (F[5,15] = 3.92, p< 0.018). Thus, the glutamate agonist was capable of increasing GABA efflux, and the effect was long lasting within the substantia nigra as can be seen in figure 24.

The results following intranigral perfusion of the D1 agonist SKF 81297 (100 uM) did not reveal any significant effects (n=3). However, there was a trend toward an increase in nigral GABA efflux following perfusion of the agonist as can be observed in figure 25. This trend was most apparent in the first 15 minute time point which is demonstrated with a t-test comparing the final baseline point to that first drug perfusion point (t[2] = -2.76, p< 0.11). Perhaps a larger sample size would produce a significant increase in nigral GABA efflux following SKF 81297. Based on the data presented it seems clear that the
direction of the GABA efflux following this dose of SKF 81297 is an increase which is of a relatively brief duration.

Behaviorally, kainate perfused into either the striatum or the substantia nigra induced locomotor activity. This activity included continuous wall climbing, fast locomotion, rearing, sniffing and occasionally wet dog shakes. The form of locomotion induced was primarily contralateral rotation away from the side of drug perfusion. None of the subjects tested exhibited the tremor, rigidity, or salivation frequently observed during seizures. Thus, it was concluded that while kainate produced an extremely robust behavioral response, no seizure activity was present.

Conclusions

The results presented above indicate that the glutamate agonist kainate is able to induce increases in GABA efflux within the striatum and nigra. These changes in nigral GABA efflux following kainate perfusion into the striatum are the first data offered within this dissertation to provide evidence for glutamatergic activation of the striatonigral pathway
leading to subsequent increases in the release of GABA within the substantia nigra. The lack of effect of striatal AMPA to stimulate nigral GABA efflux remains unknown. The relative increases in striatal GABA were not different between the two agonists, thus changes in striatal GABA efflux are not the determining factor. Furthermore, studies examining glutamate receptor distribution following destruction of striatonigral neurons revealed a similar loss for AMPA and kainate receptors, 18% and 15% respectively (Tallaksen-Greene et al., 1992). Additional studies suggest that the kainate subtype is more densely localized within the patches or striosome regions of the striatum while AMPA receptors are found in greater number in the matrix (Dure et al, 1992). Perhaps it is this distinction that underlies the different nigral effects observed in these studies.

The data regarding kainate-induced GABA efflux following nigral perfusion as well as those results discussed above, demonstrate that indeed it is possible to detect changes in nigral GABA efflux with this particular microdialysis method. Kainate receptors have been documented within the substantia nigra (Monaghan & Cotman, 1982; Albin et al, 1992) and are
believed to be necessary for transmitting signals from glutamatergic afferents originating within the subthalamic nucleus (Kita & Kitai, 1987). Indeed, increased excitation in the substantia nigra is believed to be responsible for the generation of seizures (Bonhaus et al., 1986). While these subjects did not show signs of seizures, they did demonstrate increased motor activity following the administration of kainate as well as increased GABA efflux.

The lack of a significant effect of SKF 81297 may in fact be due to the low sample size. The trend toward an increase in GABA efflux following the D1 agonist corresponds with the belief that there are release regulating D1 receptors on striatonigral terminals that act to increase GABA release. In fact, Cameron and Williams (1993) suggest that, with regard to the VTA, these terminal D1 receptors may be tonically activated by dopamine. If many of the receptors are already occupied by endogenous dopamine, it is possible that the SKF 81297 effect was not significant due to a ceiling effect. Further studies investigating the effects of increased doses of SKF 81297 as well as examining the effect of the D1
antagonist on nigral GABA to determine if there is a

tonic D1 activity could help resolve these issues.

In summary, these studies indicate that GABA
efflux within the substantia nigra can be regulated by
excitatory amino acid agonists both within the striatum
and applied to the nigra itself. Further, they suggest
that D1 receptor agonists may indeed increase GABA
efflux in vivo within the nigra, in agreement with the
in vitro literature. Finally, these studies indicate
that differences exist between the effects of AMPA
versus kainate in terms of the regulation of
striatonigral GABA efflux.
Chapter VIII.

General Discussion

The basal ganglia are a group of structures involved in the transmission and processing of cortical inputs regulating sensory and motor information. In fact, these structures are crucial for the production and expression of voluntary movement. Anatomically, the primary input area of this cortical information is the striatum (caudate and putamen) and as such, this area has been the focus of an enormous amount of research in the past twenty years (For review, see Alexander & Crutcher, 1990). Initially, the striatum was described as a homogeneous region which acted as a funnel for a wide variety of cortical information. More recently, this structure has been redefined as a heterogeneous structure receiving cortical inputs that may be somatotopographically organized in some regions, as well as dissociated in terms of the layer of cortical origin. In addition, distinct output pathways from the striatum serve to further segregate incoming
information. Thus, the striatum appears to filter and modulate cortical inputs rather than merely funneling such information.

The function of the striatum remains the focus of much research. Of primary interest are the dopaminergic afferents that origiate within the substantia nigra pars compacta. Dopamine itself is a neuromodulator which serves to regulate the activity of fast acting neurotransmitters. Dopamine produces its effects via the activation of receptors, of which there are at least five subtypes, all believed to utilize G proteins as their effector mechanisms. These receptors can be segregated into two major families, the first family is referred to as the D1-like family and consists of the D1 and D5 receptor subtypes, the second is referred to as the D2-like family and it consists of the D2, D3, and D4 receptor subtypes (Gingrich & Caron, 1993). Both D1 and D2 receptors are found in high density within the striatum (Boyson et al., 1986). In fact, the striatum is the brain region most richly innervated by dopamine and containing the largest number of dopamine receptors. In addition, these two receptor subtypes appear to be segregated from one another, with the majority of D1 receptors located on
medium spiny neurons projecting directly to the substantia nigra pars reticulata, while the majority of post-synaptic D2 receptors are found on medium spiny neurons projecting to the external globus pallidus (Gerfen et al., 1990). Thus, dopaminergic modulation via D1 and D2 receptors plays a major role in the processing of cortical information within the striatum.

The primary importance of dopamine is illustrated by the neurodegenerative condition, Parkinson’s Disease, in which the dopaminergic cell bodies of the substantia nigra degenerate, decreasing the amount of dopamine available for release within the striatum. The major symptoms of Parkinson’s Disease include rigidity, resting tremor, postural abnormalities, and bradykinesia. In those patient’s displaying symptoms, the loss of dopamine is often 80-90% of that normally found in the striatum (Horneykiewicz & Kish, 1986). These patient’s are generally unable to produce or control movements as a result of this striatal dopamine loss. The motor impairments that result from severely decreased striatal dopamine have been replicated in animal models of Parkinson’s Disease. Indeed, many of
the discoveries made regarding striatal function have come from studies that utilize such dopamine depleted animals.

One of the most well known and often cited models of striatal dopamine function is based upon results from these dopamine depleted animals. Briefly, this model asserts that dopamine acting at D1 receptors increases the firing rate of the GABAergic output neurons in the direct pathway, while activation of D2 receptors decreases the firing rate of the GABAergic output neurons in the indirect pathway. Therefore, either D1 or D2 activation should decrease substantia nigra pars reticulata firing rates which would result in the increase of thalamocortical excitation, leading to cortical excitation and the subsequent production of movement (see figure 1). However, while this may hold true for dopamine depleted animals, the evidence in normal animals does not seem to support this model. In fact, in normal animals activation of the D1 receptor doesn’t lead to the expression of motor behavior. Moreover, electrophysiological studies in normal animals indicate that D1 activation decreases the firing rate of medium spiny neurons.

Gaining a better understanding of the role of D1 receptors in the
normal striatum as well as how corticostriatal glutamatergic inputs may effect D1 activation was the goal of the work presented in the preceding chapters.

The technique of in vivo microdialysis was chosen for several reasons. First, this technique allows one to observe the relatively immediate neurochemical effects of drug manipulations within a particular brain region in an awake animal. It also allows one to compare changes in neurotransmitter levels with changes in behavior. Furthermore, the use of more than one probe can also determine how changes in one region may affect transmitter levels in an additional projection area. Finally, the chronic cannula preparation allows sampling to take place without anesthesia, eliminating a potential confound that often must be used in electrophysiological studies. Thus, this technique gives one a window on neurochemical changes without introducing the confounds of restraint or anesthesia.

The use of the in vivo microdialysis technique for the measurement of neuronal GABA levels is reasonable, as demonstrated in those studies presented in chapter III. The Na⁺ channel blocker tetrodotoxin, which eliminates nerve impulses, is able to block the majority of GABA measured. In addition, the specific
GABA uptake blocker, Nipecotic acid, increased GABA efflux by over 1000%. Thus, microdialysis is capable of measuring changes in neuronal GABA efflux. Furthermore, the results of the repeated sessions studies, also presented in Chapter III, demonstrate that it is reasonable to measure GABA efflux over three consecutive sessions separated by an off day.

As might be predicted the inclusion of the glutamate agonist AMPA induced an increase in GABA efflux within the striatum. This increase is presumed to be the result of an increase in the firing rate of medium spiny neurons increasing the release of transmitter from axon terminals. While these projection neurons do primarily innervate either the substantia nigra pars reticulata or the external globus pallidus, they also have a large number of axon collaterals that remain within the striatum. GABA released from these collaterals is believed to be the cause of the increase in striatal GABA efflux observed following AMPA perfusion. However, it is also possible that the small number of GABAergic interneurons within the striatum are contributing to this effect.

The duration of the AMPA-mediated GABA increase is relatively brief. While the drug is perfused for an
entire hour, significant increases were primarily observed during the first 15 to 30 minutes. This lack of a prolonged effect could be the result of several different mechanisms. It is possible, for instance, that the AMPA receptor becomes desensitized following 30 minutes of constant activation and depolarization. Since we are testing an intact animal, it is also possible that long loop feedback decreases normal corticostriatal glutamate release and thereby reduces the effect. In fact, a large proportion of NMDA receptors are located on striatal projection neurons (Tallaksen-Greene et al., 1992). Their activation by endogenous glutamate may be necessary for the observed AMPA effect. Since depolarization via activation of fast acting excitatory amino acid receptors is a necessary condition for NMDA receptor activation, increases in AMPA may activate NMDA receptors tonically stimulated by glutamate. When long loop changes decrease the tonic glutamate, the NMDA receptors would no be longer stimulated and this might account for the cessation of the AMPA-mediated GABA increase. Furthermore, increases in other neurotransmitters within the striatum may serve to inhibit the AMPA-mediated increase. For instance, dopamine acting at D2
receptors located on corticostriatal glutamate terminals can inhibit glutamate release (Maura et al., 1988). In addition, the GABA released within the striatum by the AMPA activation may provide a form of feedback inhibition that serves to prevent excessive activation of these medium spiny neurons. Moreover, activation of GABAergic interneurons may also function to decrease the effect of AMPA on these medium spiny neurons. Regardless of the mechanism, it is clear that increased glutamate stimulation via the AMPA receptor does not lead to prolonged increases of striatal GABA.

The effects of striatal AMPA on GABA efflux within the substantia nigra pars reticulata were surprising. There were no significant changes in GABA efflux and not even anything resembling coordinated release between the striatum and the nigra. Increasing the firing rate of medium spiny neurons in the direct striatonigral pathway would be expected to lead to an increase in GABA released within the substantia nigra. However, activation of medium spiny neurons in the indirect striatopallidal pathway may lead to a decrease in GABA via the inhibition of pallidonigral GABA neurons (see figure 1). Thus, it is possible that the lack of effect was due to the stimulation of both
pathways resulting in opposite effects on GABA efflux and leading to the lack of a net change in GABA efflux. Indeed, the substantia nigra is a point of convergence within the midbrain receiving inputs from the globus pallidus, subthalamic nucleus, striatum, and possibly even the cortex. In addition, this structure contains GABAergic interneurons as well as axon collaterals of GABAergic projection neurons (Deniau et al., 1982). It is possible that increased striatonigral GABA efflux inhibits tonically active GABA interneurons within the substantia nigra. Therefore, the absence of a nigral effect following striatal AMPA, could be due to a simultaneous increase in the release of GABA from striatonigral terminals, in conjunction with a decrease in the release of GABA from axon collaterals and interneurons within the nigra. Finally, it is possible that the dose of AMPA utilized in these studies was enough to mediate local changes within the striatum, but was not sufficient to produce robust increases in nigral GABA efflux. While lower doses were used in preliminary studies, higher doses were not tested due to the possibility of excitotoxicity within the striatum.

In order to address the lack of nigral effect observed in these studies, further examination with an
additional glutamate agonist was warranted. A recent microdialysis study using the glutamate agonist kainate observed changes in nigral GABA efflux following striatal kainate perfusion (Bianchi et al, 1994). In a replication of this study, kainate was perfused into the striatum and both striatal and nigral GABA efflux were measured. The results demonstrated that after the administration of kainate into the striatum, both striatal and nigral GABA release were increased. Thus, it is possible to selectively increase nigral GABA efflux by perfusing a glutamate agonist into the striatum.

The reason for the differences between kainate and AMPA are unclear. Binding studies within the rat striatum reveal a greater number of AMPA receptors as compared to kainate receptors (2.5 vs 0.95 pmol/mg protein, respectively; Albin et al., 1992). Studies examining the relative number of these receptors located on striatonigral neurons, using a suicide transport toxin to specifically damage these cells, found similar decreases in receptor number. Twelve days following the toxin the number of AMPA receptors was reduced by 18%, while the number of kainate receptors was reduced by 15% (Tallaksen-Greene et al.,
1992). Additional studies have described differences in the localization of these receptors in terms of segregation into the patch and matrix regions of the striatum (Dure et al., 1992). The majority of AMPA receptors are located within the matrix region. It is from this region that the projection neurons, including the striatonigral neurons, arise. The majority of kainate receptors, however, are located within the patches of the striatum (Dure et al., 1992). Previous studies have suggested that patch neurons project back to the substantia nigra pars compacta (Gerfen, 1984). Thus, perfusion of the glutamate agonist kainate, may simultaneously stimulate the striatonigral pathway as well as causing inhibition of the substantia nigra pars compacta. The proposed kainate-induced inhibition of dopamine neurons could lead to decreased dopamine release within the striatum. If, as the electrophysiological data suggests, dopamine via D1 receptors normally inhibits striatonigral firing, then decreased dopamine release via kainate may serve to further increase striatonigral firing by decreasing dopamine induced inhibition. In addition, D2 activation has been shown to tonically inhibit corticostriatal glutamate release (Maura et al., 1988;
Yamamoto & Davy, 1992). If kainate produces a decrease in striatal dopamine, this decrease in D2 stimulation, could result in the disinhibition of corticostriatal glutamate terminals, thus producing increased glutamate release. Thus, the proposed decrease in striatal dopamine release via kainate activation, could lead to increased glutamate release as well as decreased D1 inhibition of striatonigral neurons. In this manner, kainate could induce striatonigral activity more specifically and to a greater degree than AMPA receptor activation. Further studies investigating the effects of dopamine ligands (D1 and D2) on kainate-induced GABA efflux both within the striatum and the substantia nigra pars reticulata may provide evidence for the above model.

The results observed with the D1 agonist SKF 81297 were unexpected. While the low dose of the D1 agonist decreased GABA efflux, a higher dose led to significant increases in striatal GABA. Both effects have support within the literature. The electrophysiological data supports the decreased GABA efflux following the low dose of agonist (Surmeier et al., 1992; Twery et al., 1994). In previous work, the partial D1 agonist SKF 38393 has been shown in normal animals to decrease the
firing rate of medium spiny neurons in response to a depolarizing pulse (Calabresi et al., 1987). This effect has been demonstrated to be due to a dampening of sodium currents via D1 receptor activation (Surmeier et al., 1992). In fact, preliminary studies in our laboratory using the partial agonist SKF 38393 (10 μM) found that this compound induced a decrease in GABA efflux similar to that observed following SKF 81297 (10 μM) when perfused into the striatum (unpublished observations). Thus, based on these studies one would predict that administration of a D1 agonist may reduce GABA efflux in the striatum by decreasing the firing rate of medium spiny projection neurons. The predominant region of activity for the D1 agonist is presumed to be at D1 receptors located on the cell body of striatonigral neurons. While it is true that there are also striatal GABAergic interneurons, it is unlikely that the D1 agonist is producing the observed effects via inhibition of these interneurons, as recent studies indicate that these neurons possess the mRNA for the D2 receptor subtype, but not the D1 receptor subtype (Lenz et al., 1994). Therefore, the most logical explanation for the changes in GABA efflux observed following the perfusion of the low dose of SKF
81297 is a decrease in the excitation of striatonigral neurons, reducing the amount of GABA normally release into the striatum by collaterals.

The effect observed following the perfusion of the high dose of the D1 agonist also has some support in the literature (Floran et al., 1990; Girault et al., 1986). Previous release data on striatal slices have demonstrated increased GABA efflux following the administration of dopamine in the presence of the D2 antagonist sulpiride (Floran et al., 1990). In vivo data also demonstrate an increase in GABA efflux in anesthetized animals following the intrastriatal administration of 10 uM SKF 38393 (Girault et al., 1986).

The fact that there is support in the literature for the D1-mediated increase in GABA efflux observed in the striatum still dose not explain the differences observed following the two different doses of SKF 81297. It seems likely that these doses are acting via separate mechanisms. As discussed above, the low dose is believed to be having its effects via D1 receptors located on the cell body of striatonigral neurons. The effect observed following the high dose of SKF 81297, however, may be due to activation of D1 receptors other
than those on striatonigral cell bodies. The increased amount of drug available may lead to the recruitment of additional D1 receptors.

In addition to the D1 receptors located on striatonigral cell bodies, there are also D1 receptors located on the terminals of these striatonigral neurons (Barone et al., 1987; Jackson & Westlind-Danielsson, 1994). These terminal receptors are release regulating and when activated serve to increase the amount of GABA released (Floran et al., 1990). Furthermore, while striatonigral neurons project to the substantia nigra pars reticulata, they also have axon collaterals that remain within the striatum (Misgeld et al., 1984; Wilson & Groves, 1980). These collaterals may also possess terminal D1 receptors.

Recent evidence suggests that the second messenger system used by these terminal receptors may be different from those located on the cell body. It has long been established that the D1 receptor is linked to the adenylyl cyclase system (Kebabian & Calne, 1979). However, more recent evidence has also linked these receptor to the phospholipase C system. These data find that D1 activation increases inositol phosphate formation (Undie & Friedman, 1990). Moreover, this
effect has been observed in the substantia nigra where only terminal D1 receptors are located (Martin & Waszaczak, 1992). These results do not preclude the coupling of some of these terminal D1 receptors to adenylyl cyclase. Indeed, studies using antibodies raised against the Gs protein have observed this protein in the substantia nigra, however the concentration within the nigra is more than five-fold lower than that of the striatum (Herve et al., 1992). Thus, the different actions of these D1 receptors may be due to the utilization of different second messenger systems.

It is possible that the increased dose of SKF 81297 leads to the activation of these terminal receptors causing an increase in the amount of GABA released within the striatum. Indeed, this same dose of SKF 81297 perfused into the nigra, which possesses only terminal D1 receptors (Gingrich & Caron, 1993) tended to increase GABA efflux, although the small number of animals tested did not reveal a significant effect. Thus, it is possible that D1 agonist activation of terminal D1 receptors is responsible for the enhanced GABA efflux following the high dose of SKF 81297.
The data on nigral GABA efflux following intrastriatal administration of the high dose of SKF 81297 does not appear to reflect an increase in striatonigral firing rates. If, as some in the literature have suggested, D1 administration increases striatonigral firing (Gerfen et al., 1990) and subsequently increases GABA release within the nigra, then changes in nigral efflux should have been observed following D1 administration. As no changes in nigral efflux were observed, it is unlikely that the GABA efflux observed in the striatum was due to increased firing of striatonigral projection neurons. However, if, as suggested above, the high dose of the D1 agonists acts at D1 terminal receptors within the striatum, then the increase in striatal GABA would not necessarily be reflected by nigral GABA release. In fact, it has been proposed that many of these collaterals may synapse close to the parent neuron (Penny et al., 1988). If this arrangement holds true, then increased GABA efflux via terminal D1 receptors within the striatum may serve to reduce striatonigral firing via a collateral feedback inhibition. Thus, the decreased GABA release observed following the low dose of the D1 agonist and the increased GABA release
following the higher dose, may both served to lessen striatonigral firing rates through two independent mechanisms.

As stated above, D1 receptors have not been demonstrated on GABA interneurons within the striatum. Therefore, as with the low dose of SKF 81297, it is unlikely that the D1-induced increase observed following the high dose of SKF 81927 is mediated via recruitment of D1 receptors located on these interneurons. However, it is possible that other striatal interneurons may play a role in this effect. While still controversial, some studies have demonstrated a direct effect of D1 agonists on acetylcholine (ACh) release within the striatum (Consolo et al., 1992). These studies indicate that D1 applied to the striatum can increase ACh release (Consolo et al., 1992). Further studies have demonstrated that up to 30% of striatal ACh interneurons may possess D1 receptors (Guennoun & Bloch, 1992). In addition, it has also been shown that ACh agonists can significantly increase striatal GABA efflux (Galli et al., 1994). It is possible that the higher dose of the D1 agonist recruits D1 receptors localized on ACh interneurons, thereby increasing ACh
release which could ultimately serve to increase GABA efflux. This proposed multisynaptic effect of D1 agonists on GABA efflux could be tested by simultaneously perfusing a cholinergic antagonist with the high dose of SKF 81297 and measuring striatal GABA efflux. If the cholinergic blocker prevents or decreases the observed increase in GABA efflux, then the above model would have some support.

The interaction between these doses of SKF 81297 and AMPA receptor activation did not attain significance. There was a trend, however, for the addition of the low dose of SKF 81297 to decrease AMPA-mediated GABA efflux. This dampening of an AMPA effect is predicted by both the electrophysiological data and the effects of the D1 agonist alone on GABA efflux. The fact that these effects were not significant may be due to AMPA-induced increases in striatal GABA efflux mediated by activation of non-striatonigral neurons. The D1 inhibition would only effect the portion of GABA efflux due to striatonigral excitation.

The lack of an interaction between AMPA and the high dose of SKF 81297 may also be due to non-striatonigral stimulation. While the AMPA may increase striatal GABA efflux via the activation of projection
neurons, the SKF 81297 may increase striatal efflux via the activation of terminal D1 receptors. Why the activation of these two receptors does not lead to an additive effect may be due to the negative feedback of the axon collaterals. That is, terminal D1 receptor activation could increase striatal GABA which may then dampen the AMPA-mediated excitation of striatonigral projection neurons. Perhaps that is why there are no differences observed between SKF 81297 (100 uM) given alone and that same dose of the agonist given together with AMPA.

As indicated in chapter VII, kainate induced an increase in striatal GABA efflux that correlated with an increase in nigral GABA efflux. It appears as though this glutamatergic agonist is better able to specifically stimulate the striatonigral pathway then AMPA. Future studies attempting to understand the interactions between D1 receptor activation and striatonigral activation via glutamate may be better served by using kainate. Thus, while the results discussed in this thesis offer insight as to the effects of both D1 and AMPA receptor activation alone, the impact of striatonigral activation on D1 receptor mediated GABA efflux remain equivocal. The fact that
AMPA could not induce an increase in nigral GABA may indicate that this agonist does not selectively stimulate striatonigral projection neurons. Thus, as discussed above, the lack of interaction effects observed in this thesis may be due to activation of non-striatonigral pathways. Clearly, many studies remain to be done to better understand this complicated striatonigral system in the normal animal.
Table 1. Table 1 depicts the lack of a correlation between activity and changes in GABA efflux. Both AMPA and SKF 81297 increased GABA efflux, however, only AMPA produced any behavioral activation. Furthermore, neither dose of SKF 81297 produced behavioral activation.
# Locomotor Behavior

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>GABA Efflux</th>
<th>Locomotion</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMPA</td>
<td>100 uM</td>
<td>INCREASE</td>
<td>INCREASE IN CIRCLING, REARING, WALL CLIMBING</td>
</tr>
<tr>
<td>SKF 81927</td>
<td>100 uM</td>
<td>INCREASE</td>
<td>NO ACTIVITY</td>
</tr>
<tr>
<td>SKF 81297</td>
<td>10 uM</td>
<td>DECREASE</td>
<td>NO ACTIVITY</td>
</tr>
</tbody>
</table>
Figure 1. Schematic diagram of the circuitry and neurotransmitters of the basal ganglia-thalamocortical circuitry

Figure 1 depicts the circuitry of the basal ganglia including inputs from the cortex and efferents to the thalamus. Both the direct and indirect pathways are demonstrated as well as the neurotransmitters they utilize.
Figure 1.
Figure 2. Illustration of a CMA 10 microdialysis probe

Figure 2 is an illustration of the CMA 10 microdialysis probe with each individual component labeled.
Membrane diameter 0.5 mm
Stainless-steel shaft diameter 0.64 mm
Shaft length 20 mm
Inlet internal volume negligible
Outlet internal volume 4 μl

Figure 2.
Figure 3. Probe placement in animals undergoing microdialysis of striatal GABA

Figure 3 illustrates the microdialysis probe placements in all animals undergoing microdialysis within the striatum. Probe placements for individual experiments are designated by a particular symbol. While placement of probes into individual hemispheres was counterbalanced between animals during the actual experiments, placements are depicted within only one hemisphere for each individual experiment for the purpose of clarity. Coordinates from Bregma are illustrated in the upper right portion of the sections.

Symbols:  
- AMPA + SKF 81297  
- DNQX  
- Kainate  
- Repeated Sessions: AMPA  
- Repeated Sessions: SKF 81297  
- SCH 23390  
- SKF 81297
Figure 3.
Figure 4. Striatal nipecotic acid (500 uM)

Figure 4 depicts the effects of nipecotic acid (500 uM) on the mean (±S.E.M) GABA efflux following direct administration into the striatum via the microdialysis probe. The nipecotic acid maximally increased basal GABA levels by 1598% within the striatum.
GABA (pmol)/20 ul

STRIATAL NIPECOTIC ACID (500 uM)

Figure 4.

GABA (pmol)/20 ul

0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8

CSF

CSF + NIP

1598%
Figure 5. Striatal TTX (10 μM)

Figure 5 depicts the effects of TTX (10 μM) on the mean (±S.E.M.) GABA efflux following direct administration to the striatum via the microdialysis probe. Subjects were either treated with TTX during session 1 (solid circles) or session 3 (solid squares). Both groups received TTX (10 μM) alone followed by perfusion with TTX (10 μM) and AMPA (100 μM). Data from subjects receiving AMPA (100 μM) alone are also presented for comparison (solid triangles). Both TTX and TTX + AMPA reduced striatal GABA efflux. TTX completely blocked the normally observed AMPA effect. Mean (±S.E.M.) baseline GABA levels for each group were as follows: Session 1 (0.58±0.28), Session 3 (0.27±0.08), AMPA (0.47±0.11).
Figure 5. Change in GABA efflux over time following the onset of drug perfusion. The graph shows the effect of TTX (10 μM) and TTX + AMPA (100 μM) on GABA efflux. The x-axis represents time (min) following onset of drug perfusion, while the y-axis represents % change in GABA efflux. The graph includes data from two sessions: Session 1 (TTX) and Session 3 (TTX + AMPA).
Figure 6. Basal efflux: AMPA repeated sessions

Figure 6 depicts the mean (± S.E.M.) basal striatal GABA efflux in those subjects receiving repeated microdialysis sessions. Subjects shown were either treated with AMPA (100 uM) on session 1 (open bar), AMPA (100 uM) on session 3 only (upward hatches), or AMPA (100 uM) during all 3 sessions (downward hatches). No significant differences were observed between the three groups.
Figure 6.

GABA (pmol)/20 ul

0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

DIALYSIS SESSION

1
3
3

BASAL EFFLUX

AMPA perfused during all 3 sessions

AMPA perfused only on 3rd session
Figure 7. Repeated insertions of microdialysis probe in subjects receiving striatal AMPA (100 uM)

Figure 7 depicts the effect of repeated probe insertion on mean (±S.E.M.) striatal GABA efflux in subjects receiving striatal AMPA (100 uM) on session 1 (solid circles) or subjects receiving AMPA (100 uM) only on session 3 (solid squares). In both groups AMPA produced an increase in striatal GABA efflux lasting for approximately 45 minutes. No significant differences were seen between the two groups. Mean (±S.E.M.) baseline GABA levels for each group were as follows: Session 1 (0.48±0.12), Session 3 (0.20±0.06).
Figure 8. Repeated perfusion of AMPA (100 uM) into the striatum

Figure 8 depicts the effect of repeated probe insertion on mean (±S.E.M.) striatal GABA efflux in subjects receiving striatal AMPA (100 uM) on session 1 (solid circles) or subjects receiving AMPA (100 uM) on all 3 sessions (solid squares). In both groups AMPA produced an increase in striatal GABA efflux lasting for approximately 45 minutes. No significant differences were seen between the two groups. Mean (±S.E.M.) baseline GABA levels for each group were as follows: Session 1 (0.48±0.12), Session 3 (0.64±0.27).
Figure 8.

% CHANGE IN GABA EFFLUX

TIME (MIN) FOLLOWING ONSET OF DRUG PERFUSION

SESSION 1: GROUP 1
SESSION 3: GROUP 1

DRUG OFF
Figure 9. Basal efflux: SKF 81297 repeated sessions

Figure 9 depicts the mean (±S.E.M.) basal striatal GABA efflux in those subjects receiving repeated microdialysis sessions. Subjects shown were either treated with SKF 81297 (10 μM) on session 1 (open bar), SKF 81927 (10 μM) on session 3 only (upward hatches), or SKF 81927 (10 μM) during sessions 1 and 3 (downward hatches). No significant differences were observed between the three groups.
Figure 9.

GABA (pmol) / 20 ul

- Basal efflux
- SKF 81297 perfused only on 3rd session
- SKF 81297 perfused during sessions 1 and 3

Dialysis session 3
Figure 10. Repeated insertions of microdialysis probe in subjects receiving striatal SKF 81927 (10 uM)

Figure 10 depicts the effect of repeated probe insertion on mean (±S.E.M.) striatal GABA efflux in subjects receiving striatal SKF 81927 (10 uM) on session 1 (solid circles) or subjects receiving SKF 81927 (10 uM) only on session 3 (solid squares). In both groups SKF 81927 produced a decrease in striatal GABA efflux lasting for approximately 45 minutes. No significant differences were seen between the two groups. Mean (±S.E.M.) baseline GABA levels for each group were as follows: Session 1 (0.31±0.08), Session 3 (0.43±0.10).
**STRATIAL SKF 81297 (10 uM)**

- **SESSION 1: GROUP 1**
- **SESSION 3: GROUP 2**

**% CHANGE IN GABA EFFLUX**

**TIME (MIN) FOLLOWING ONSET OF DRUG COPERFUSION**

- **DRUG OFF**

---

**Figure 10.**
Figure 11. Repeated perfusion of SKF 81297 (10 uM) into the striatum

Figure 11 depicts the effect of repeated probe insertion on mean (±S.E.M.) striatal GABA efflux in subjects receiving striatal SKF 81927 (10 uM) on session 1 (solid circles) or subjects receiving SKF 81927 (10 uM) on sessions 1 and 3 (solid squares). In both groups SKF 81927 produced a decrease in striatal GABA efflux lasting for approximately 60 minutes. No significant differences were seen between the two groups. Mean (±S.E.M.) baseline GABA levels for each group were as follows: Session 1 (0.31±0.08), Session 3 (0.28±0.09).
Figure 11.

% CHANGE IN GABA EFFLUX

STRIATAL SKF 81297 (10 uM)

SESSION 1: GROUP 1
SESSION 3: GROUP 1

TIME (MIN) FOLLOWING ONSET OF DRUG COPERFUSION

DRUG OFF
Figure 12 illustrates the microdialysis probe placements in all animals undergoing microdialysis within the substantia nigra. Probe placements for individual experiments are designated by a particular symbol. While placement of probes into individual hemispheres was counterbalanced between animals during the actual experiments, placements are depicted within only one hemisphere for each individual experiment for the purpose of clarity. Coordinates from Bregma are illustrated in the upper right portion of the sections.

Symbols:  
- AMPA  
- Kainate  
- SKF 81297
Figure 13. Effects of D1 receptor activation on striatal GABA efflux

Figure 13 depicts the effect the D1 agonist SKF 81297 on mean (±S.E.M.) striatal GABA efflux. SKF 81297 (10 uM; solid circles) induced a decrease in GABA efflux that lasted approximately 45 minutes. SKF 81297 (100 uM; solid squares) induced an increase in GABA efflux that reached its maximum level at 15 minutes. CSF perfused into the striatum produced no change in GABA efflux. The mean (±S.E.M.) baseline values for each of the groups were as follows: SKF 81297 (10 uM; 0.34±0.10), SKF 81297 (100 uM; 0.15±0.03), and aCSF (0.38±1.6).
Figure 13.

STRITAL GABA EFFLUX

% CHANGE IN GABA EFFLUX

TIME (MIN) FOLLOWING ONSET OF DRUG PERFUSION

-100
0
100
200
300
400
500

SKF 81297 (10 uM)
SKF 81297 (100 uM)
CSF

DRUG OFF

0 15 30 45 60 75 90 105
Figure 14. Effects of AMPA and D1 receptor activation on striatal GABA efflux

Figure 14 depicts the effect the AMPA alone and AMPA + the D1 agonist SKF 81297 on mean (±S.E.M.) striatal GABA efflux. AMPA (100 uM; solid circles) induced an increase in GABA efflux that peaked at 30 minutes. AMPA + SKF 81297 (10 uM; solid squares) also induced an increase in GABA efflux that reached its maximum level at 15 minutes, although the increase was less than AMPA alone. AMPA + SKF 81297 (100 uM; solid triangles) again increased striatal GABA efflux, an effect that lasted for approximately 60 minutes. No significant differences between the groups were observed. The mean (±S.E.M.) baseline values for each of the groups were as follows: AMPA (100 uM; 0.29±.07), AMPA + SKF 81297 (10 uM; 0.30±0.13), AMPA + SKF 81297 (100 uM; 0.26±0.11).
Figure 14

STRIATAL GABA EFFLUX

% CHANGE IN GABA EFFLUX

TIME (MIN) FOLLOWING ONSET OF DRUG PERFUSION

-100 0 100 200 300 400

AMPA (100uM)
AMPA + SKF 81297 (10uM)
AMPA + SKF 81297 (100uM)

DRUG OFF
Figure 15. Effects of AMPA and SKF 81297 (10 uM)
on striatal GABA efflux

Figure 15 depicts the effect the AMPA (100 uM) or SKF 81297 (10 uM) alone as compared to AMPA + the D1 agonist SKF 81297 (10 uM) on mean (±S.E.M.) striatal GABA efflux. AMPA (100 uM; solid circles) induced an increase in GABA efflux that peaked at 30 minutes. AMPA + SKF 81297 (10 uM; solid squares) also induced an increase in GABA efflux that reached its maximum level at 15 minutes, although the increase was less than AMPA alone. SKF 81297 (10; solid triangles) decreased striatal GABA efflux, an effect that lasted for approximately 45 minutes. No significant differences between the AMPA and AMPA + SKF 81297, although SKF 81297 alone was significantly different from both groups. The mean (±S.E.M.) baseline values for each of the groups were as follows: AMPA (100 uM; 0.29±.07), AMPA + SKF 81297 (10 uM; 0.30±0.13), SKF 81297 (10 uM; 0.34±0.10).
STRNATAL GABA EFFLUX

% CHANGE IN GABA EFFLUX

-100
0
100
200
300
400

AMPA (100 uM)
AMPA + SKF 81297 (10 uM)
SKF 81297 (10 uM)

TIME (MIN) FOLLOWING ONSET OF DRUG PERFUSION
Figure 16. Effects of AMPA and SKF 81297 (100 uM) on striatal GABA efflux

Figure 16 depicts the effect the AMPA (100 uM) or SKF 81297 (100 uM) alone as compared to AMPA + the D1 agonist SKF 81297 (100 uM) on mean (±S.E.M.) striatal GABA efflux. AMPA (100 uM; solid circles) induced an increase in GABA efflux that peaked at 30 minutes. AMPA + SKF 81297 (100 uM; solid squares) also induced an increase in GABA efflux that lasted approximately 45 minutes. SKF 81297 (100; solid triangles) increased striatal GABA efflux, with its maximal effect at 15 minutes. No significant differences between the AMPA, AMPA + SKF 81297, or SKF 81297 were observed. The mean (±S.E.M.) baseline values for each of the groups were as follows: AMPA (100 uM; 0.29±0.07), AMPA + SKF 81297 (100 uM; 0.26±0.11), SKF 81297 (100 uM; 0.15±0.03).
Figure 16.

STRIATAL GABA EFFLUX

\% CHANGE IN GABA EFFLUX

TIME (MIN) FOLLOWING ONSET OF DRUG PERFUSION

- AMPA (100 uM)
- AMPA + SKF 81297 (100 uM)
- SKF 81297 (100 uM)

DRUG OFF
Figure 17. Effects of striatal SKF 81297 on nigral GABA efflux

Figure 17 depicts the effects of striatal SKF 81927 on mean (±S.E.M.) nigral GABA efflux. Neither SKF 81297 (10 uM; solid circles), SKF 81927 (100 uM; solid squares), nor aCSF (solid triangles) produced any meaningful changes in nigral GABA efflux. The mean (±S.E.M.) baseline values for each of the groups were as follows: SKF 81297 (10 uM; 0.40±0.10), SKF 81297 (100 uM; 0.35±0.13), and aCSF (0.34±.15).
Figure 17.

NIGRAL GABA EFFLUX

- % CHANGE IN GABA EFFLUX

- TIME (MIN) FOLLOWING ONSET OF DRUG PERFUSION

SKF 81297 (10 uM)
SKF 81297 (100 uM)
CSF

DRUG OFF
Figure 18. Effects of striatal AMPA and SKF 81297 on nigral GABA efflux

Figure 18 depicts the effects of striatal AMPA alone or AMPA + SKF 81927 on mean (±S.E.M.) nigral GABA efflux. Neither AMPA (100 μM; solid circles), AMPA + SKF 81297 (10 μM; solid squares), nor AMPA + SKF 81927 (100 μM; solid triangles) produced any meaningful changes in nigral GABA efflux. The mean (±S.E.M.) baseline values for each of the groups were as follows: AMPA (100 μM; 0.39±0.19), AMPA + SKF 81297 (10 μM; 0.36±0.15), and AMPA + SKF 81297 (100 μM; 0.23±.06).
NIGRAL GABA EFFLUX

TIME (MIN) FOLLOWING ONSET OF DRUG PERFUSION

-100
0
100
200
300

% CHANGE IN GABA EFFLUX

AMPA (100uM)
AMPA + SKF 81297 (10uM)
AMPA + SKF 81297 (100uM)

DRUG OFF

Figure 18.
Figure 19. Effects of striatal DNQX (100 uM)

Figure 19 depicts the effect of the AMPA antagonist DNQX (100 uM) on mean (±S.E.M.) striatal GABA efflux. DNQX (solid circles) increased GABA efflux within the striatum. While not statistically significant, a maximal increase in GABA efflux peaked at 30 minutes. At no time after drug infusion did GABA efflux return to basal levels. The mean (±S.E.M.) baseline value for DNQX subjects was 0.76±0.26.
Figure 19.

% CHANGE IN GABA EFFLUX

Time (min) Following Onset of Drug Perfusion

STRIATAL EFFLUX

DRUG OFF
Figure 20. Behavioral effects of striatal GABA efflux

Figure 20 depicts the behavioral effect accompanying changes in GABA levels. Behaviors were designated as either present or absent during a particular collection and then the correlated with changes in GABA efflux observed during that collection period. Behavior was monitored throughout the entire collection interval. The frequency of these behaviors was not dissociated. The four behaviors examined were those most often observed during testing; sniffing, wall climbing, grooming, and locomotor behavior. As can be observed, no behavior could be predicted based on the percent of GABA increase. Increased GABA efflux was often observed without correlated increases in behavior.
Figure 20.
Figure 21. Effects of SKF 81297 (10 uM) and SCH 23390 (10 uM) on striatal GABA efflux

Figure 21 depicts the effect the D1 agonist SKF 81297 and the D1 antagonist SCH 23390 on mean (±S.E.M.) striatal GABA efflux. SKF 81297 (10 uM; solid circles) decreased GABA efflux, an effect that lasted approximately 60 minutes. SCH 23390 (10 uM; solid squares) induced a small increase in GABA efflux although this was not significant. Finally, SKF 81927 + SCH 23390 (solid triangles) produced no change in GABA efflux. Thus, the SCH 23390 appeared to block SKF 81297-induced decrease. The mean (±S.E.M.) baseline values for each of the groups were as follows: SKF 81297 (100 uM; 0.28±0.08), SCH 23390 (10 uM; 0.28±0.08), and SKF 81927 + SCH 23390 (0.31±.11).
Figure 21. % change in GABA efflux following onset of drug perfusion. The graph shows the time course of GABA efflux following the administration of different drugs. The x-axis represents time in minutes (0-105) following the onset of drug perfusion, while the y-axis represents the percentage change in GABA efflux. The lines represent different drug treatments: SKF 81297 (10 μM), SCH 23390 (10 μM), and SKF 81297 (10 μM) + SCH 23390 (10 μM).
Figure 22. Effects of SKF 81297 (100 uM) and SCH 23390 (10 uM) on striatal GABA efflux

Figure 22 depicts the effect the D1 agonist SKF 81297 and the D1 antagonist SCH 23390 on mean (±S.E.M.) striatal GABA efflux. SKF 81297 (100 uM; solid circles) induced an increase in GABA efflux that lasted approximately 45 minutes, although was only significantly different from baseline during the first 15 minutes. SCH 23390 (10 uM; solid squares) also induced an increase in GABA efflux although this was not significant. Finally, SKF 81927 + SCH 23390 (solid triangles) produced no change in GABA efflux. Thus, the SCH 23390 appeared to block the SKF 81297 effect. The mean (±S.E.M.) baseline values for each of the groups were as follows: SKF 81297 (100 uM; 0.31±0.08), SCH 23390 (10 uM; 0.24±0.02), and SKF 81927 + SCH 23390 (0.22±0.11).
SKF 81297 (100 μM)
SCH 23390 (10 μM)
SKF 81297 (100 μM) + SCH 23390 (10 μM)

Time (min) following onset of drug perfusion

% Change in GABA efflux

Drug Off
Figure 23. Effects of striatal kainate (100 μM)

Figure 23 depicts the effects of striatal kainate (100 μM) on mean (±S.E.M.) striatal (solid circles) and nigral (solid squares) GABA efflux. Kainate produced increase GABA in both the striatum and the nigra. This increase was maintained during the entire perfusion period. The GABA efflux in the nigra was well correlated with GABA efflux within the striatum. The mean (±S.E.M.) baseline values were as follows: striatal efflux (0.37±0.22), nigral efflux (0.33±.26).
EFFECTS OF STRIATAL KAINATE (100 μM)

% CHANGE IN GABA EFFLUX

TIME (MIN) FOLLOWING ONSET OF DRUG PERFUSION

DRUG OFF
Figure 24. Effects of kainate (100 um) perfused into the substantia nigra

Figure 24 depicts the effects of nigral kainate (100 uM) on mean (±S.E.M.) nigral (solid circles) GABA efflux. Kainate produced a significant increase GABA within substantia nigra. This increase was greatest during the first 15 minutes of perfusion. The mean (±S.E.M.) baseline value for subjects receiving kainate was 0.10±.02.
Figure 24.

% CHANGE IN GABA EFFLUX

TIME (MIN) FOLLOWING ONSET OF DRUG PERFUSION

NIGRAL KAINATE (100 uM)

DRUG OFF
Figure 25. Effects of SKF 81297 (100 um) perfused into the substantia nigra

Figure 25 depicts the effects of nigral SKF 81297 (100 uM) on mean (±S.E.M.) nigral (solid circles) GABA efflux. SKF 81297 induced an increase GABA within substantia nigra, although there was only a trend toward significance. This increase was greatest during the first 15 minutes of perfusion. The mean (±S.E.M.) baseline value for subjects receiving SKF 81297 (100) was 0.20±.12.
Figure 25.

% CHANGE IN GABA EFFLUX

TIME (MIN) FOLLOWING ONSET OF DRUG PERFUSION

NIGRAL SKF 81297 (100 uM)

DRUG OFF
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