Grant Proposal:
Cognitive impairment in schizophrenia: site-specific investigation of the role of dopaminergic and glutamatergic pathways
Executive Summary

We propose the following hypothesis: local perturbations to activity in the cortico-striatal-thalamic loop (here designated the C-S-T circuit) can produce global effects, including the cognitive deficits present in schizophrenia. We outline an experimental plan which investigates this hypothesis using a primate model of schizophrenia, involving local perfusion of drugs onto the striatum and dorsolateral prefrontal cortex. Since this hypothesis includes features of other hypotheses of schizophrenia, notably the dopamine and glutamate hypotheses, we will study modulators of the dopaminergic and glutamatergic pathways in the C-S-T circuit. The specific aims of this proposal are:

Develop the primate model by locally administering an NMDA antagonist and a dopamine agonist to dorsolateral prefrontal cortex (dIPFC) and dorsal caudate (dC), and confirm the cognitive deficit using a delayed match-to-sample (DMS) task;

a) Perform electrophysiological investigation into the dC and dIPFC in chronically impaired monkeys;

b) Investigate the electrophysiology of monkeys for effective drug/brain region combinations over the course of drug administration;

Perform anatomical investigations into treated, impaired monkeys.

Background

Schizophrenia is a psychotic disease with a lifetime prevalence of approximately 1% in all human populations. Its hallmarks are hallucinations and delusions, although a range of positive and negative symptoms can occur, and some of these symptoms can occur in other diseases. Despite this heterogeneity and overlap with other conditions, cognitive impairment is a unifying feature of the disease, and one aspect of this impairment—formal thought disorder—is unique to schizophrenia.

Working memory is directly relevant to these cognitive disturbances, and indeed, schizophrenics perform poorly on tests of working memory (reviewed in Bunney and Bunney, 2000). The dorsolateral prefrontal cortex (dIPFC; areas 9 and 46) is particularly critical for working memory (reviewed in Petrides, 2000). Imaging studies have revealed decreased activation of dIPFC during working memory tasks in schizophrenics (Perlstein et al., 2001). This decrease is correlated with the severity of cognitive disorganization, but not with positive or negative symptoms, suggesting that dIPFC dysfunction relates to working memory deficits in schizophrenics.

dIPFC dysfunction may result from abnormalities within the dIPFC itself. Early anatomical work, reviewed by Bunney and Bunney (2000), found evidence for neuronal loss or damage throughout the PFC of schizophrenics. More recent evidence suggests that schizophrenics have fewer thalamic projections to dIPFC (Lewis et al., 2001; Pierr et al., 2001). Abnormalities of GABAergic transmission (Akbarian et al., 1995) and changes in the expression of genes that encode proteins involved in presynaptic function (Mirnics et al., 2000) are also present in dIPFC.

How might abnormalities within the dIPFC impair working memory? Recent experiments that use animals treated with phencyclidine (PCP) as models for schizophrenia suggest two
possibilities. The first involves dysregulation of dopamine in the dlPFC. Monkeys that have been treated systemically with PCP for 2 weeks are impaired on a working memory task 7 days after the cessation of PCP infusion (Jentsch et al., 1997). Along with this enduring cognitive deficit, these monkeys have decreased dopamine utilization within the dlPFC, while dopamine utilization in other parts of the frontal lobe, striatum, and nucleus accumbens as well as serotonin utilization are unaffected. Furthermore, the monkeys' deficits are rescued by the atypical antipsychotic clozapine, which increases dopamine release in dlPFC, and has a similar cognitive benefit for human schizophrenics. NMDA receptor antagonists such as PCP seem to affect dopamine levels in dlPFC indirectly, by increasing glutamate release from PFC neurons, which feed back onto dopaminergic neurons in the ventral tegmental area (Svensson, 2000).

The second possibility is that the increased release of glutamate itself disrupts dlPFC function, independently of dopamine. In rodents, a single dose of PCP disrupts working memory, induces locomotion, and increases glutamate and dopamine levels in the PFC. At a dose that normalizes prefrontal glutamate but not dopamine levels, the metabotropic glutamate receptor agonist LY354740 attenuates the behavioral effects of PCP (Moghaddam and Adams, 1998).

A third hypothesis distinct from either of these is that dlPFC dysfunction and working memory deficits result from abnormalities elsewhere in a cortico-striatal-thalamic loop. dlPFC projects to the head of the caudate nucleus (Giguere and Goldman-Rakic, 1988), which is also involved in working memory tasks (Monchi et al., 2001; Levy et al., 1997; Owen et al., 1996). The caudate regulates activity in the dorsomedial globus pallidus interna (dmGPi; Tremblay and Fillion, 1989). dmGPi contains neurons that respond selectively during working memory tasks (Mushiake and Strick, 1995), and project, via the thalamus, to dlPFC (Middleton and Strick, 1994). Glutamatergic and dopaminergic inputs converge on the striatum, where they have a profound influence. This has led Carlsson et al. (2000) to propose that schizophrenia may involve an imbalance between these neurotransmitters in the striatum. Indeed, a role for the basal ganglia in working memory is supported by imaging studies and behavioral deficits in individuals with basal ganglia lesions (reviewed in Middleton and Strick, 2000).

In summary, the enduring working memory deficits in PCP-treated monkeys are an appealing model of cognitive impairment in schizophrenia. However, two major questions remain. First, while PCP is being infused, at what anatomic and neurochemical loci does it act? And second, once infusion ceases, what are the enduring anatomic and electrophysiological alterations that presumably underlie the working memory deficit? We propose to answer these questions using a combination of behavioral, electrophysiological, and anatomical experiments on monkeys that undergo local infusion of selective pharmacological agents.

Specific Aim 1: Investigate the effects of locally disrupting glutamatergic and dopaminergic neurotransmission in the dorsolateral prefrontal cortex and striatum by correlating microinfusion of MK-801 and apomorphine with decreased performance on a delayed match to sample task.

Hypothesis:

Most hypotheses about neurotransmitter-related cognitive deficits in schizophrenia center around dopamine and glutamate effects in the dlPFC and dorsal caudate (dC). We expect that locally altering dopaminergic and glutamatergic activity in these areas will be sufficient to cause cognitive dysfunction.
Experimental Design:
We will locally microperfuse MK-801, an NMDAR antagonist which is more specific than PCP, and apomorphine, a D1/D2 receptor agonist that induces psychotic behavior (Hitri et al., 1993) into the dC and dlPFC of rhesus monkeys trained to perform a delayed match to sample (DMS) task. In this working memory task, subjects are shown a visual “target” stimulus followed by a delay and then asked to select the target from among distractors by making an eye movement toward the matching stimulus. We will progressively increase each drug dosage until we reach a level that acutely impairs performance on the DMS. The length of the delay period in the DMS task will be varied for each monkey, and the delay at which the monkeys perform above a pre-set threshold will be used as a measure of performance. The experimental design can be summarized as follows:

(MK-801, apomorphine, saline) x (dC, dlPFC) x (various delay lengths in DMS task).

Performance will be tested before injections (baseline), during administration of the drug (acute) and after administration of the drug (chronic). We are most interested in which dosages of which drugs in which areas produce chronic effects; these will define those combinations which will be studied further in Specific Aim 2.

Expected Results and Interpretations:

The following table summarizes the predictions of the four major hypotheses of neurotransmitter dysfunction.

<table>
<thead>
<tr>
<th></th>
<th>hypo-DA in PFC</th>
<th>hyper-Glu in PFC</th>
<th>dC</th>
<th>Multifactorial</th>
</tr>
</thead>
<tbody>
<tr>
<td>dC MK-801</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Apomorphine</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>PFC MK-801</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
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<tr>
<td>Apomorphine</td>
<td>✓</td>
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</tbody>
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These hypothesized results assume that local administration of a drug will affect only that region. Because the C-S-T circuit is so interconnected, this may not be the case. This table provides a starting point for evaluating these hypotheses; mixed results will allow us to rule out some hypotheses and investigate indirect effects of the drugs using electrophysiological and anatomical methods in Aims 2 and 3.

Possible Problems and Limitations

It is possible (but unlikely) that none of our local drug administration paradigms will produce chronic deficits in DMS. In this case, we can revert to systemic administration of a drug such as PCP that is known to cause chronic working memory deficits (Jentsch et al, 1997). Another possibility is that the effects we see will be so strong that the monkeys will be unable to perform the DMS task even at very short delays, indicating a more fundamental motor or sensory
dysfunction. Funahashi et al (1993) showed that even monkeys with complete prefrontal lesions are able to complete a DMS at short delays (but not longer ones).

Specific Aim 2: Characterize the electrophysiological changes that underlie the persistent psychosis developed in Aim 1, as a function of perfusion dose and duration.

Hypothesis: Chronic schizophrenic behavior, induced by microperfusions, result from specific, persistent electrophysiological changes in either or both the dorsal caudate (dC) of the basal ganglia or the dorsolateral prefrontal cortex (dlPFC).

Procedure:
Overview: We will simultaneously record from the dC and the dlPFC while monkeys perform the DMS task described in Aim 1. All recordings will be compared to within-animal controls performed before the administration of drugs in Aim 1.

Set One: Set one will be performed on monkeys whose drug/region combination produced a behaviorally measured psychosis persisting one week after microperfusion termination. This set will enable us to characterize the electrophysiological changes that underlie the persistent psychosis developed in Aim 1. These results will indicate what final electrophysiological conditions create psychosis in a particular monkey. Additionally, if multiple conditions induced psychosis in Aim 1, these results will determine what they have in common. It may be that administration of different drugs at different sites result in persistent psychosis but do so through electrophysiological changes in the same area. For example, it may be that drug interactions in the dlPFC actually cause permanent changes in the activity of the dC, or vice versa.

Potential Results:

PFC: When analyzing recordings from the dlPFC, we will focus on neural activity during the delay period of the DMS task. Neurons in the dlPFC provide a neural basis for short term memory and hence are critical in this task (Miller et al., 1996). These neurons’ activities are increased by specific stimuli and this stimulus specific increase is maintained throughout the delay period. Additionally, these cells show abrupt modulation when the test stimulus matches the sample. Although we expect that many drugs which act in the dlPFC result in hypofrontality, it is difficult to predict what specific effect the drugs will have on task related neural responses. However, we anticipate either a loss of stimulus specificity, disruption of delay activity, or a failure to respond when the test stimulus matches the sample.

Caudate: We predict that both drugs will change baseline and task related firing rates in the caudate. For example, the D2 dopamine receptor, present on the medium spiny output neurons of the caudate (Mink,1996; Carlsson et al., 2000), activates K+ channels leading to membrane hyperpolarization and thus a decrease in neuron excitability. Conversely, the NMDA receptor (also present in the caudate: Carlsson et al., 2000), when activated, permits an influx of cations that depolarizes the membrane and increases neuronal excitability.

Set Two: We will record from a second set of monkeys as they undergo treatment with the successful drug/region combinations from set one. The same recordings will be performed, however, they will be concurrent with systematic administration of the appropriate drug in the appropriate areas. This set of recordings will allow us characterize the changes within and between areas as a function of perfusion dose and duration. We will investigate the dose at which
local electrophysiological changes begin, and that at which they correspond with changes in the other area and task performance. This will allow us to potentially select one of these two areas as more influential in this task. For example, while drug administration to the dlPFC might immediately change activity locally, behavioral effects might not appear until the activity of the dC changes. This would indicate that while the administration of drugs to the dlPFC induces psychosis, it does so indirectly; and the more direct cause is change in the caudate.

**Pitfalls:** The largest potential pitfall in these experiments is ensuring that we have selected the right areas in which to record. Although a preponderance of evidence suggests that the dC and dlPFC are particularly important components of the C-S-T loop, there are many other components that are involved. It is possible that while administration of the drugs to these areas induces psychosis other areas, e.g. dmGPi or MD thalamus, could show the persistent electrophysiological changes. If we fail to find persistent changes in either dC or dlPFC these areas will be investigated. Additionally, as in Aim 1, local administration might not be sufficient to induce psychosis, in which case, as in Aim 1, traditional systemic administration will be used.

**Specific Aim 3:** Characterize the molecular changes that underlie each chronic psychosis developed in Aim 1 to assess the functional significance of post-mortem human observations.

**Hypothesis:** Chronic schizophrenic behavioral and electrophysiological abnormalities, induced by microperfuasions, are correlated with specific molecular changes in the mediodorsal thalamus (mdTh) and/or the dorsolateral prefrontal cortex (dlPFC).

**Experiment 1:** Multislice proton magnetic resonance spectroscopy imaging ($^1$H-MRSI) has successfully revealed decreases in neuronal tissue volume in dlPFC and mdTh through an estimation of N-acetylaspartate (NAA: a putative neuronal marker) and choline (Cho: a putative marker of synthetic membrane formation) levels in discrete volumes (Bertolino et al., 1996, Omori et al., 2000). By anesthetizing control and experimental animals and imaging NAA and Cho levels before drug administration, at onset of acute symptoms, and intermittently throughout the chronic phase, we can correlate the progression of neuronal membrane loss with alterations in cognitive and electrophysiological measurements.

**Experiment 2:** Microarray gene expression analysis of the PFC in schizophrenic patients post-mortem reveals a selective decrease in transcripts encoding proteins involved in presynaptic function, glutamatergic, and GABAergic transmission (Mirmics et al., 2000). Additionally, a robust and ubiquitous downregulation of RGS4, a regulator of G-protein signaling that shortens the postsynaptic response was discovered (Mirmics et al., 2001). These changes imply reduced synaptic connections within the PFC and a corresponding attempt to maintain activity levels despite loss of synaptic interconnectivity. Because human gene chips have been empirically used for non-human primate controls of chronic haloperidol treatment in the afore-mentioned experiments, the molecular changes that occur within the dlPFC of chronically 'schizophrenic' animals developed in Aim 1 can be compared to control monkeys and correlated to specific electrophysiological changes observed in Aim 2 and the results of human post-mortem analysis.

**Experiment 3:** At the cellular level, evidence for reduced cortico-cortical and thalamo-cortical connectivity has focused on deep layer 3 pyramidal neurons within dlPFC. These cells receive afferent excitatory drive from mdTh and their axon collaterals provide the abundant excitatory connections within the PFC that are critical to prefrontal cognitive processes (Giguere and
Goldman-Rakic, 1988; Kritzer and Goldman-Rakic, 1995). Evidence for reduced thalamo-cortical connectivity within the middle layers dlPFC was discovered immunohistochemically by quantifying parvalbumin-immunoreactive varicosities (putative axon terminals of mdTh afferents; Lewis et al., 2001). Additionally, it may be possible that alterations in inhibitory prefrontal circuitry might underlie the cognitive and electrophysiological changes revealed in Aims 1 and 2. Previous findings demonstrate that the density of GABA membrane transporter (GAT-1)-immunoreactive chandelier neuron axon terminals is specifically reduced (50%) in the middle layers of dlPFC in schizophrenic subjects (Pierri et al., 1999). Post-mortem immunohistochemistry using these markers will facilitate an examination of these circuits anatomically in discrete regions.

**Pitfalls:** It is possible that statistically significant correlations will be difficult to establish given the limited number of animals we will first use. Because 1H-MRSI is non-invasive, within-animal analysis pre- and post-treatment will greatly increase our ability to establish significance. Our microarray expression analysis examines changes in large groups of transcripts and of substantial magnitude in the human post-mortem study, greatly increasing our probability of detection. The immunocytochemical research examines the magnitude of changes between superficial and middle layers of dlPFC, again facilitating within-animal control. Additionally, it is possible to create within-animal controls by severing the corpus callosum and administering drugs to only one hemisphere. Regardless of any perceived limitations, success in Aims 1 and 2 will ensure medical research-level access to enough animals to perform the powerful molecular analyses proposed here.

**Conclusions**

Despite its high incidence and the tremendous amount of research devoted to it, schizophrenia remains poorly understood. While antipsychotic drugs are effective at treating some of the more prominent positive symptoms of the disease, e.g., hallucinations, there is very little that can be done to treat cognitive deficits, and progress in treating the disease as a whole remains elusive. Most vitally, there is at best a weak link between most attempts to assess the etiology of schizophrenia, and attempts to treat it. We believe the proposed study can help to bridge this gap. If our assertions are correct that a) local perturbations to the C-S-T circuit can induce global effects, and b) certain loci within the loop are particularly sensitive to perturbation, then we can look for very specific targets on which to focus treatment, based on new knowledge of disease progression. This in turn may open new opportunities for very focused, site-specific treatments which do not neglect the multifactorial, global nature of the disorder.

Currently, the most effective and widely used course of treatment for schizophrenia centers around the use of pharmacotherapy. A focus on restoring perturbations in the C-S-T circuit to treat schizophrenia may inspire a more targeted approach to drug therapy, based around the development of drugs whose activity is confined to particular anatomic and pharmacological loci. For example, if outputs from dC are found to be particularly sensitive to dopaminergic or glutamatergic perturbation, drugs can be targeted towards a downstream target in the loop, e.g. dorsomedial GPi, by developing drugs particular to the specific GABA_\textsubscript{A} receptor subunit composition of that location. Compared with attempting to directly restore the glutamate/dopamine alterations, and doing so on a global scale, this approach can be much more effective at dealing with schizophrenia-specific symptoms.
In addition, the specific drug/location combinations may allow re-examination of non-pharmacological approaches, such as electroconvulsive therapy or repetitive transcranial magnetic stimulation (McCall, 2001; Rollnik et al., 2000; Klein et al., 1999). More decisively beneficial treatments may result from using this approach on a local scale, for instance by deep-brain stimulation in carefully selected regions of the C-S-T circuit (e.g., dmGPi in the previous example).

It is also possible that other symptoms of the disorder, in addition to the cognitive deficits examined here, may result from disruptions in parallel regions of the C-S-T circuit. Our approach can then be used to assess the relative time course of behavioral, electrophysiological and anatomical changes in the progression of schizophrenia. This in turn may aid early diagnosis in the prodromal stage of the disease, currently a controversial prospect (McGlashan, 2001). In searching for an integral common pathway for diverse symptoms in schizophrenia, we can generate a powerful animal model for future study. In summary, the approach proposed here has the potential to significantly improve patient outlook for this mysterious and crippling disease.
References:


