Title: Striatal Effective Connectivity to Predict Treatment Response in Cocaine Misuse

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Research Strategy:

A. Significance

A.1. Cognitive enhancement as target for pharmacotherapy of cocaine dependence. Cocaine dependence is associated with several cognitive deficits [33, 95-97]. Cognitive enhancement has been suggested as a target for pharmacotherapy of stimulant addiction [97]. Investigation of the neurobiological mechanisms of therapeutic interventions to improve cognition has the potential for predicting and/or evaluating treatment outcomes, improving pharmacotherapy, and aiding the development of new treatment protocols [44]. Several studies [35, 98-100] have investigated the relationship between baseline cognitive functions and treatment outcome, and indicate that treatment outcome is influenced by baseline neuronal functioning. Thus, methods are needed to measure accurately alterations in neuronal systems underlying cognitive functions. This would allow development of potential biomarkers that could be predictive of results from more time consuming and costly clinical trial involved in the development of new treatments for cocaine dependence. There is a pressing need to develop clinically useful biomarkers to characterize subpopulations among disease populations and to assess efficiency of candidate therapies [101, 102]: To date, however, it is still one of the major challenges in drug addiction research to identify such biomarkers [103]. In this proposal, we will use an fMRI-based brain connectivity approach that may ultimately lead to biomarkers for cocaine dependence treatment response.

A.2. Regional fMRI activation studies in cocaine dependence. One of the traditional applications of fMRI in drug addiction is to identify regional neural substrates mediating impaired cognitive functions by using the analysis of regional activations (please see [90, 104] for review). Several research groups [7, 34-36] have used this method to study cognitive deficits in cocaine dependence, and found differences in fMRI blood oxygenation level dependent (BOLD) signal between CDs and controls while the subjects performed cognitive tasks. For example, using a working memory task, Moeller et al. [35] found that CDs showed significantly lower working-memory load-dependent activation in several cortical and subcortical regions compared to controls, including caudate nucleus, DLPFC, medial orbitofrontal cortex, and thalamus. Using a Go/NoGo task, Kaufman et al. [7] found that, relative controls, cocaine users had significant hypoactivity in cingulate, pre-supplementary motor and insula regions, during both successful response inhibition and errors of commission. FMRI analysis of regional activations has been used to measure cognitive functions in CDs and showed that baseline regional activations in CDs were predictive of treatment outcome [35, 99].

A.3. Brain connectivity analysis in cocaine dependence. Although traditional analysis of regional fMRI activation has been widely used in characterizing the pathology and treatment response of cocaine dependence and has produced valuable findings, this method has several limitations when it is applied to study disease or medications. First, cognitive processes essentially depend on interactions among brain regions [92, 105-110], however the analysis of regional activation does not directly measure these interactions. In addition, the sensitivity of traditional fMRI analysis to the presence or severity of disease and/or medications can be lower than that of connectivity analysis (please see [93] for review). Moreover, changes in the BOLD signal could be confounded by the possible disruption by disease or medications on neurovascular coupling and/or hemodynamic response [94], and therefore caution should be exercised when comparing populations with different physiologic or pharmacologic conditions [111, 112]. A previous study [113] has shown that the direct effects of dopamine (DA) upon the vasculature need to be considered when measuring the hemodynamic coupling associated with dopaminergic drugs. This is especially relevant in cocaine dependence, for which studies have suggested is a disorder associated with reduced DA neurotransmission in the brain [43, 44]. *These limitations suggest that methods are needed that measure the connectivity between brain regions [114] and that are more closely associated with the underlying neuronal activity* [79, 93, 115, 116].

Functional connectivity and effective connectivity analyses are two methods that can be used to measure functional integration (interactions among brain regions) [78]. Functional connectivity refers to correlations among spatially remote neurophysiological events [78]. Unlike functional connectivity, effective connectivity measures the causal (hence directional) effect that one region's neuronal activity has on another region [78]. Several functional connectivity studies [117-123] have been conducted to investigate cocaine dependence. These studies demonstrated abnormal functional connectivity in cocaine users during tasks [119, 121, 122].

A.4. Dynamic Causal Modeling of effective connectivity. One limitation of functional connectivity is that it is unable to establish causal relationships because it is unable to measure directional changes between brain regions. The DCM measurement of effective connectivity that will be used in the proposed study can overcome t/Jis limitation [78]. Positron emission tomography (PET) studies have demonstrated a significant correlation between dopamine D2 receptor function and glucose metabolism in orbitofrontal cortex, anterior cingulate, and

OLPFC in subjects with cocaine dependence and other addictions [44, 83]. Because these were correlational analyses, *it is unclear whether PFC is causing changes in striatum, or striatum is causing changes in PFC. This question is relevant because* a *prefrontal-striatal glutamatergic pathway may regulate striatal OA, and thus decreased function of this pathway may lead to* a *hypodopaminergic state in COs* [44, 86]. Low OA may disrupt frontal inhibition, resulting in poor inhibitory control over compulsive drug intake [84, 85]. Thus, prefrontal-striatal connectivity may be a potential target for developing new pharmacotherapies for COs. *Using OCM to establish the directionality of this connectivity and its degree of impairment relative to controls is* a *first step towards answering this clinically significant question.*

The possible confounding effects by disease or medications are reduced in OCM, as reflected by the following three points: (a) Effective connectivity in OCM is modeled at the underlying neuronal level rather than the observed hemodynamic level [75, 91]. (b) The parameters of the effective connectivity (modeled at the underlying neuronal level) and the hemodynamic response functions are jointly optimized in OCM [91]. (c) Stochastic OCM [77, 124], an advance in OCM technique that has been recently implemented by the authors of OCM! takes into account hidden and random fluctuations in neuronal and vascular responses that may have profound effects on effective connectivity [77].

A.5. OA enhancement as a *target for pharmacotherapy of cocaine dependence.* The impaired cognitive functions observed in COs may be related to decreased OA function [54, 125, 126], which is associated with cocaine dependence [43, 44, 127-129]. The hypodopaminergic state is considered as one of the main factors that triggers drug-seeking and taking [130]. PET studies in humans suggest that decreased DA function in COs contribute to repeated drug intake [43, 44, 46, 84]. This is supported by PET studies in primates, which have shown that there is an inverse relationship between 02 receptor availability and vulnerability to the reinforcing effects of cocaine [45, 131]. Studies in rodents indicate that reduced DA function is a major contributor to the transition from drug use to drug dependence [132].

OA enhancement has been proposed to be a target for pharmacotherapy of stimulant addiction [81, 88, 89]. A recent PET study by Martinez et al. [87] showed that low pretreatment striatal OA transmission in COs was associated with treatment failure from behavioral therapy alone (without any pharmacological treatment). It has been theorized that COs with low pretreatment OA, such as in [87], would benefit from the addition of OA enhancing medication to the behavioral therapy [44]. The results of Martinez et al. [87] in addition to our preliminary findings (section C.2.4) are consistent with the theory that preexisting differences between COs in OA circuits may underlie variability in responsiveness to different treatments [44].

Previous published studies in our center [133-135] and other studies [136, 137] showing feasibility, safety and tolerability of levodopa/carbidopa in treatment of cocaine dependence. Previous studies using the DA drugs levodopa/carbidopa [135] have showed significant treatment effects, as evidenced by significal)tly reduced cocaine positive urine and craving. In the current project, it is hypothesized that the effects of levodopa/carbidopa [133-135] will have effects on therapeutic action (OA enhancement) and lead to optimal treatment efficacy. *This clinical trial provides an opportunity for the present proposal to investigate the role of effective connectivity in the mechanism of treatment response to OA medications in COs.*

A.6. Striatal gating effects and DA function. An important issue that is relevant to the development of new treatments for cocaine dependence is how OA neurotransmission interacts with striatum and PFC during cognitive tasks. *One possible way that OA regulates cognition is via gating effects (i.e., positive modulation effect) of striatum that regulate connections between the PFC and posterior regions* [22, 23]. A cognitive model developed by O'Reilly and Frank [22] addresses this striatal gating mechanism. In brief, OA exerts its effects via firing of the "Open Gate" neurons in the dorsal striatum [22]. When "Open Gate" neurons in the dorsal striatum fire, they facilitate connections between frontal cortex and posterior cortical regions [22]. *Nonlinear OCM can be used to evaluate this striatal gating model [76] as an indicator of OA function. We propose to use OCM with the stochastic and nonlinear extensions (snOCM) to test our hypotheses.*

We propose to test snDCM on working memory and response inhibition in separate fMRI studies within the same CD subjects. Working memory is chosen for the task as it is well documented that DA plays a key role in working memory, and previous studies found altered working memory in COs. Cocaine use is associated with altered working memory function at both the behavioral [32, 33] and neuronal [34-37] levels in humans, and in animal studies [38-41]. Cocaine use is associated with altered DA function [42-51]. DA neurotransmission is involved in working memory [52-58]; supporting evidence comes from human [59-68] and animal [69-73] studies. There is evidence that working memory function is predictive of DA synthesis capacity in humans [74]. Our published data [35] and

unpublished pilot data (Section C.2.4) have shown that the baseline fMRI working memory activation and the effective connectivity elicited by the working memory task are predictive of treatment outcome with DA enhancing medications in COs. Previous studies have shown that cocaine use can induce impaired response inhibition in both human ([1-8] and see [9] for review) and animals ([10-12) and see [13] for review). In addition, there is evidence ([15-19) and see [20, 21] for review) supporting the hypothesis that DA modulates response inhibition. Furthermore, impulsive personality traits predict DA-dependent changes in frontostriatal activity during component processes of working memory [117). A study [100) conducted by our group has shown that trait impulsivity is predictive of cocaine addiction treatment outcome. *The relationship between cognitive domains and DA is relevant because the major objective of this study is to predict treatment outcome of DA enhancing medications.*

Summary of Significance: (a) Establishing the directional relationship between PFC and striatum and the role of striatal gating in cocaine treatment response will significantly improve our understanding of dopaminergic and pharmacotherapeutic mechanisms in COs. (b) The pilot data (Section C.2.4) suggest that treatment outcome in COs with low pretreatment striatal OA can be improved with OA enhancing treatments by increasing striatal OA function, which we theorize increases striatal gating of cortico-cortico connections. Thus the evidence supporting the hypothesized striatal gating mechanism will have significant implication in cocaine addiction treatment. (c) An issue in cocaine dependence research is the lack of clinically useful biomarkers for pharmacotherapies. Effective connectivity as measured by snOCM may ultimately lead to clinically useful biomarkers for cocaine dependence and its treatment.

B. Innovation:

There is a paucity of effective connectivity studies in the field of cocaine dependence, and we are unaware of any published studies of cocaine dependence that have used effective connectivity in predicting treatment response in COs. Thus, the proposed study is novel and will provide evidence on whether cocaine-related alterations in effective connectivity are related to treatment outcomes. In addition, the testing of the striatal gating model and the application of snOCM to cocaine dependence are also novel.

C. *Approach:*

C.*1 Overview of Study Design. The objective of this study is to use baseline (pretreatment) snDCM to predict the treatment response to DA medication.*

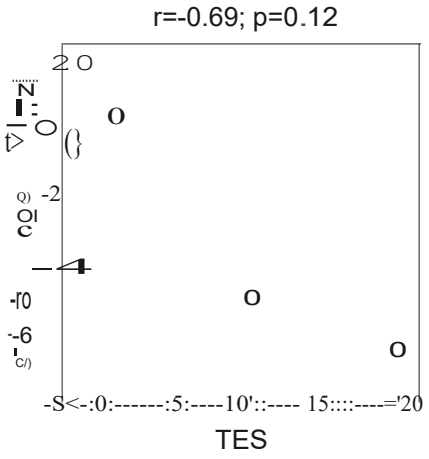
C.2 Preliminary Studies: C.2.1. *An analysis of regional fMRI activations showed that pretreatment thalamic activation predicted treatment response in COs* [35]. This study examined the relationship between pretreatment working-memory elicited regional activations and subsequent treatment response in treatment-seeking early abstinence COs. Nineteen COs who were randomized to treatment studies and 14 non-drug using controls underwent fMRI while performing a working memory task with variable memory load, as parameterized by two levels of memory delay and three levels of digit load (number of digits presented in each visual stimulus). Group comparison showed that COs had significantly lower regional activation in caudate, putamen, cingulate gyrus, middle and superior frontal gyri, inferior frontal gyrus pars triangularis and pars opercularis, medial orbitofrontal cortex, precentral gyrus, and thalamus compared to non-drug using controls. These brain regions are known to be part of a circuit associated with reward, conditioning/memory, executive control, and motivation/drive [92]. Within COs, pretreatment thalamic activation significantly correlated with the subsequent treatment response. Subjects with pretreatment thalamic deactivation showed the poorest treatment response, possibly related to thalamic involvement in mesocortical and mesolimbic OA projections. *This study supports our research group's capacity to carry out fMRI studies in COs and our research group's expertise in treatment response prediction research.*

C.2.2 *A DCM effective connectivity study showed that working memory load modulated brain connectivity in a parieto-frontal network in normal healthy subjects* [138]. This study used fMRI-based OCM to investigate how working memory load modulates effective connectivity in normal working memory system. FMRI data were acquired from eighteen non-drug-using healthy subjects while they performed the aforementioned working memory task [35]. Based on regional activations and consistent with working memory literature, eight regions of interest, i.e., bilateral (LR) OLPFC, LR anterior cingulate cortex (ACC), LR inferior frontal cortex (IFC), and LR posterior parietal cortex (PPC), were chosen for OCM analyses. Bayesian inference indicated that a bilinear OCM in which the digit load (3, 5, or 7 digits) modulated several parieto-frontal connections was the optimal model. Analysis of model parameters showed that higher digit load enhanced connection from left (L) PPC to L IFC, and lower digit load inhibited connection from right (R) PPC to L ACC. These findings

suggest that working memory load modulates effective connectivity in a parieto-frontal network, and may reflect' altered neuronal processes, e.g., information processing or error monitoring, with the change in working memory load. *This study supports our research group's expertise in the use of OCM.*

C.2.3 *A stochastic OCM effective connectivity study showed that the DLPFC-striatal effective connectivity was compromised in COs during working memory task* [37]. This study used fMRI-based stochastic DCM to test whether there is altered effective connectivity of working memory pathways in cocaine dependence. Nineteen COs and fourteen non-drug using controls were scanned while performing the aforementioned working memory task [35]. Nodes for DCM analysis were chosen based on areas that significantly activated common to both groups, according to Seghier et al [79]. Bayesian inference on model parameters showed that the COs had large and reliable differences compared to the control subjects in the strength of the modulation effects on prefrontal-prefrontal and prefrontal-striatal connections exerted by the memory delay conditions. The strength of the modulation effects on connection from DLPFC to striatum was lower in the COs compared to controls (Baysian confidence probability > 0.999). These findings are consistent with altered cortical-striatal networks related to reduced OA function in cocaine dependence. As far as we know, this is the first between-group effective connectivity study using stochastic OCM. *This study supports our research group's expertise in the use of stochastic OCM in addiction research.*

C.2.4 *Our pilot snOCM study showed: (a) causal (i.e., directional) relationship from OLPFC to striatum in COs; (b) the strength of the endogenous connection from OLPFC to ventral striatum was lower in COs compared to controls; and (c) low pretreatment gating by the dorsal striatum on the IFC-PPC connection predicted subsequent improvement after treatment wih OA enhancing medications.* Six treatment-seeking COs and six age- and sex-matched controls, who had been included in our previous OCM study [37], were included. The baseline (pretreatment) fMRI data acquired while the subjects performed the aforementioned working memory task [35] were used for testing Hypotheses 1 and 2. The COs subsequently received DA enhancing treatment (d-amphetamine: 1 subject, levodopa: 2 subjects, and modafinil: 3 subjects). Based on the significant regional activations, seven regions of interest were chosen as nodes for the snDCM analysis: L OLPFC, L IFC, L PPC, R PPC, LR dorsal striatum, LR ventral striatum, and LR thalamus. OCMs were constructed according to Ma et al. [37] with the following additions: (a) for each CD, the following putative OCMs were constructed: a model in which DLPFC causally (directionally) affects dorsal and ventral striatum, and a model in which the dorsal and ventral striatum causally affects the OLPFC.



Anatomically, OLPFC projects to the caudate, whereas limbic and paralimbic regions project to the ventral striatum [139, 140]. However, snOCM effective connectivity can be validly determined by indirect anatomical connectivity [141]. Thus, the connection from OLPFC to ventral striatum can be indirect via corticocortical intermediate connections [142, 143]. (b) The activity in the dorsal striatum was hypothesized to gate (positive modulation effect) the connectivity between the L IFC and the L PPC, based on O'Reilly and Frank [22]. Bayesian inference on model structure showed that the model in which the OLPFC causally affects the dorsal and ventral striatum had higher Bayesian exceedance probability (<1>=0.98) than the model in which the ventral and dorsal striatum causally affects the OLPFC. The strength of the endogenous connection from DLPFC to ventral striatum was lower in the COs (-0.025 Hz) compared to controls (0.019 Hz) (Baysian confidence probability> 0.999; Cohen's d = 1.89), but there was no significant difference between groups for dorsal striatum. These results are consistent with the corticostriatal circuits in addiction proposed in [85], in which increasingly repetitive or compulsive cocaine use is associated with progressively greater involvement of dorsal striatum but stable involvement of ventral striatum [144, 145). Thus it is possible that in COs there is relatively less heterogeneity as a group in ventral striatum and relatively more heterogeneity in dorsal striatum. Heterogeneity in dorsal striatum is consistent with the above figure. This may explain why a significant average *group* difference was found in the connection from OLPFC to ventral striatum but not in the connection from OLPFC to dorsal striatum. Based on evidence [146-150] showing that glutamate has an excitory effect on OA in striatum, it can be speculated that the lower (negative) strength of the connection from OLPFC to striatum in COs reflects

decreased DA caused by decreased prefrontal-striatal glutamatergic neurotransmission [44, 85, 86, 92]. Nonparametric Spearman correlation analysis showed that the pretreatment gating effect by the dorsal striatum on the connection from IFC to PPC was inversely related to the Treatment Effectiveness score (TES), a measure of relative abstinence from cocaine use, after 8 weeks of treatment with OA enhancing medications ($r = -0.69$, $p=0.12$, see figure in this section). These preliminary findings are consistent with the theory that OA enhancement may be a useful target for pharmacotherapy of cocaine dependence in COs who have pretreatment hypodopaminergic state [81, 88, 89]. A recent study by Martinez et al. [87] showed that low pretreatment striatal OA transmission in COs was associated with treatment failure from behavioral therapy alone (without any pharmacological treatment). One may speculate that COs with low pretreatment OA in that study would benefit from the addition of OA enhancing medication [44]. The results of [87] and our preliminary findings are consistent with the theory that preexisting differences between COs in OA circuits may be a mechanism underlying variability in responsiveness to different treatments [44]. *This pilot study supports our expertise in the use of snDCM and Hypotheses 1 and 2.*

C.2.5 *Our pilot fMRI study testing response inhibition in COs showed: (a) activation in an orbitofrontal-striatal-thalamica/ circuit; and (b) positive correlation of caudate activation with performance, and negative correlation of caudate activation with severity of cocaine addiction.* Eleven COs (cocaine group) and ten age-matched controls (control group) were included in an event-related fMRI study of response inhibition. The fMRI data were acquired while the subjects performed a Go/NoGo task [1], in which there are two levels of difficulty in NoGo trials (Easy NoGo and Hard NoGo trials), (see section C.4.6 and reference [1] for description). An SPM univariate 2nd level analysis of the fMRI data across both groups revealed that the main effects of correct NoGo trials relative to Go trials showed activation in four clusters (uncorrected 2-tail cluster $P < 0.05$, cluster size > 238 voxels) that were found in portions of LR thalamus, L posterior cingulate, LR supplemental motor area, L superior frontal gyrus (g), R superior parietal g, R angular g, R inferior parietal lobule, L fusiform g, and L parahippocampal g. There was no significant interaction of activation with task difficulty. Within COs, whole brain voxel-wise SPM regression analysis showed positive correlation of NoGo activation with NoGo accuracy (d') in portions of L caudate, R middle cingulate, R inferior parietal lobule, R medial orbital g, R putamen, R pallidum, R rectus g, and R lateral orbitofrontal cortex (cluster 2-tail false discovery rate [FOR] corrected $P < 0.10$, cluster size> 465 voxels). Whole brain voxel-wise SPM regression analysis also showed negative correlation of NoGo activation with severity of cocaine addiction (total KMSK score [151]) in portions of L caudate, L fusiform g, L angular g, L insula, L olfactory g, L medial orbital g, L rectus g, L putamen, L parahippocampal g, L middle temporal g, and L inferior temporal g (cluster 2-tail FOR corrected $P < 0.10$, cluster size> 443 voxels). This pilot study supports our expertise in the use of a Go/NoGo fMRI task in studying response inhibition in COs, and the results support the regions of interest selected for snDCM analysis to test Hypothesis 3 (C.4.19.2).

C.3 *Description of the Clinical Trial.* The objective of this proposal is to use pretreatment snOCM to predict the treatment response to OA enhancing medication in COs.

C.3.*1 Participants and Recruitment for the Clinical Trial:* Male and female treatment-seeking individuals, 18 to 55 years old, who meet current OSM-IV criteria for cocaine dependence and report using cocaine within the past 30 days are enrolled. Eligible subjects are required to submit at least one positive urine toxicology screen for the cocaine metabolite benzoylecgonine (BE) > 300 ng/ml,during the one-week screening and pretreatment assessment period. Subjects currently dependent on any psychoactive substance other than cocaine, nicotine, or marijuana are excluded. 60 treatment-seeking COs will be recruited.

C.3.2 Screening, Pretreatment, Randomization for the Clinical Trial: Candidates signing written informed consent enter a one-weeintake and assessment phase to evaluate eligibility and administer baseline pretreatment measures. Those who fail to return for required visits during this period are not randomized to treatment so as to reduce early treatment dropout rates [152]. An *urn randomization [153] procedure assigns subjects to one of the four treatment groups.* This procedure balances groups on pretreatment characteristics that may influence measures of cognitive performance and/or treatment outcome. The variables include (a) severity of cocaine use, based on days using cocaine in past 30 days and KMSK score [151]; (b) lifetime duration of cocaine use; (c) level of marijuana use, based on days using in past 30 days; (d) Barratt Impulsiveness Scale (BIS) [202], measuring trait impulsivity.

In the Clinical Trial, there is one active medication arm and one placebo arm. Equal allocation to treatment conditions is expected, as follows: 30 COs assigned levodopa/carbidopa 400/100 BID, and 30 COs

assigned placebo. Treatment assignment is concealed to all staff, including the investigators. Only the study pharmacist who dispenses the study medications is unblinded to participants' assigned condition. At each clinic visit (Monday, Wednesday, Friday) subjects are administered the medication at the dispensing window and given take-home doses for intervening days.

C.3.3 Treatment for the Clinical Trial: During the 12-week active trial participants are scheduled to attend 3 clinic visits per week (Monday, Wednesday, and Friday). Weekly cognitive behavioral therapy (CBT), an evidenced-based psychosocial treatment for cocaine dependence, is used as the behavioral therapy platform. Participants receive individual cognitive behavioral therapy in 50-minute weekly sessions. The CBT is based on the model of relapse proposed by Marlatt and Gordon [154].

C.3.4 Treatment Outcome: The primary outcome measure of cocaine use/nonuse is based on *urine drug screen* levels (acquired 3 x per week) of the cocaine metabolite benzoylecgonine (BE), coded as "positive" for cocaine use if BE ;;;: 300 ng/ml.However, urine-based definitions of cocaine use/nonuse have limitations in terms of data loss (due to missed visits) and carryover effect. Recent procedures for combining self-report and urine BE have been recommended [159, 160]. Thus, as a secondary measure of cocaine use/nonuse, the new SRPHK1 (SelfReportPHarmacoKinetic1) coding method is used. This classifies daily cocaine use based on evaluation of self-report (SUR) (obtained 3 x per week), quantitative urine BE levels, and participants' concordance rate (agreement between self-report and urine result). Retention in treatment is measured in two ways: (a) As a binary variable, defined as "completing" 12 weeks of cognitive behavioral treatment, since this cutoff is regarded as standard duration of treatment [161] and predictive of better outcome [162]. (b) A secondary continuous measure of retention is the total number of weeks a subject remained in treatment.

C.3.5 Retention of Participants: As part of participation in the clinical trial, participants receive incentives for attending the in-person intake ($50.00 in gift cards) and end of study ($50.00 in gift cards). They also receive $5 for each medication pack returned and bus/parking tokens as needed to help defray the costs of travel. Also during treatment, for each visit attended, subjects earn a draw from the attendance voucher "fishbowl", with prizes ranging in value from $0 to $100 (one slip). Prizes are exchanged for vouchers. In addition, they receive approximately $50.00 in gift cards for completing behavioral/cognitive laboratory assessments. All parking is free. Subjects receive an additional $130 for completion of the MRI scans.

C.3.6 Assessment of Medication Compliance: Riboflavin (50 mg) is added to the medication (and placebo) capsules and used as a marker to monitor compliance. The compliance to the treatment regimens is assessed by pill-counting and by urine riboflavin determination.

C.4 Experimental Design and Methods for the snDCM Study: C.4.1 Subjects for the snDCM Study: There are 3 groups in the snDCM study: (a) medication group, {b) placebo group, and (c) control group. Conservatively estimating approximately 20% unusable scans in each group because of excessive head motion, clinical structural abnormalities, artifacts, or subject no-show for the scan, this project will need to recruit a total of 30 treatment-seeking CDs for the medication group (15 male and 15 female), 30 treatment-seeking CDs for placebo group (15 male and 15 female), and 30 age-, handedness-, and education-matched non-drug-using healthy control subjects (15 male and 15 female) in order to achieve *24 subjects per group for final analysis* (Power Analysis section C.4.21). Thus, we need to recruit 24 subjects each year over the first 3 years and 18 subjects during Year 4. Fewer subjects will be recruited during Year 4 because more time in Year 4 will be devoted to final data analysis, write-up, presentation of findings, and subsequent grant applications.

C.4.2 Subject Recruitment and Screening: The medication group will consist of 30 subjects assigned to levodopa/carbidopa. The *placebo group* will consist of 30 subjects assigned to placebo in the clinical trial. The 30 non-drug-using healthy control subjects (control group) will be recruited through advertising in local newspapers and word of mouth. Written informed consent form will be obtained from all subjects.

All control subjects undergo an initial screening that consists of a physical examination, routine blood work, urinalysis, electrocardiogram (EKG), metal screening, structured diagnostic interview (SCID), addiction severity index (ASI), and human immunodeficiency virus (HIV) screening. We anticipate screening 45 potential control subjects in order to recruit 30 control subjects (yielding 24 control subjects in the final analysis).

C.4.3 Subject inclusion/exclusion criteria: The patients who meet DSM-IV criteria for current cocaine dependence, have at least 1 positive urine benzoylecgonine (BE) specimen (> 300 ng/ml)during intake, and are assigned medications or placebo, will be the candidates for this study. In addition, the included subjects must simultaneously meet the following criteria: (a) Be between 18 and 50 years of age; (b) Right handed; (c) Have no past history of Psychiatric or non-Psychiatric medical disorders which could affect the central nervous system as assessed by SCID and physical examination; (d) Be in acceptable health on the basis of interview,

medical history, labs, and physical exam; (e) Be able to understand the consent form and provide written informed consent; (f) Have no metal fragments or other bodily metal (e.g., pacemaker) or significant claustrophobia that would put the subjects at risk for MRI scanning. (For a complete list of inclusion and exclusion criteria see Human Subjects section below).

C.4.4 Coordination between the Proposed Study and the Clinical Trial: For eacCD, the MRI session will be conducted in Week 1, which is before the treatment is initialized (baseline pretreatment). The treatment outcome (see Section C.3.4 Treatment Outcome) will be shared for the snDCM study.

C.4.5 Working Memory Task for fMRI: The *immediate memory task/delayed memory task (IMTIOMT)* fMRI protocol [35, 138, 165] is used as the working memory test. The IMT and DMT are delayed-matching-to-sample tasks [166]. In both IMT and DMT, each stimulus consists of a string of numbers that is displayed for 0.5 s, followed by an inter-stimulus interval of 0.5 s, at a rate of 1 stimulus per second. In DMT, the target and probe stimuli are separated by distracter stimuli, consisting of a string of all zeros (e.g., 00000) that is repeated three times at the same rate and duration as the target and probe stimuli. Thus, in DMT, the memory delay between the end of the target stimulus and beginning of the probe stimulus is 3.5 s. In IMT, there are no distracter stimuli, and thus the memory delay between target and probe is 0.5 s. A nonsalient stimulus consisting of all ones (e.g., 11111) is presented once after each DMT trial and four times after each IMT trial. Therefore the sum of the distracter stimuli and the nonsalient intertrial stimuli is the same (four) during DMT and IMT conditions, and hence the number of trials is same (seven) for IMT blocks and DMT blocks. In both IMT and DMT, the probability of a match and the probability of a catch are 50%. In a catch, the probe differs from target in only one of the digits. Subjects are instructed to press a button using the right index finger only when the probe matches the target The A' score [167] is used as ari accuracy measure. The A' ranges from 0.5 to 1.0, corresponding to chance to perfect discriminability, respectively. There are 12 blocks alternating between IMT and DMT within each run. The number of digits in the stimulus string can be 3, 5, or 7 and is held constant within each block. The digit-length conditions represent three levels of digit load. All digit conditions are presented within each run, in counterbalanced order between runs and subjects. Each block is 42.5 s; there is 10 s rest between blocks and 20 s rest at the beginning of each run.

C.4.6 Response Inhibition Task for fMRI: An event-related Go/NoGo task [1] is used for fMRI of response inhibition. In each run, the subjects look at a total of 224 visual stimuli that are sequentially presented in random order. Each stimulus is displayed for 500 ms, and any two neighbor stimuli are separated by a blank screen lasting 1900, 2100, or 2300 ms Uittered randomly). Each visual stimulus consists of lines enclosed within two boxes that are presented simultaneously side by side. The subjects are instructed to look at both boxes and to discriminate the direction of the lines. The subjects are instructed to press a button using their right index finger only when they see that both boxes show diagonal lines in the same direction in both boxes (Go trials). The subjects are instructed not to press any button when they see that both boxes show horizontal lines (easy-NoGo trials), or when they see that one box contains diagonal lines that are in the opposite direction of the diagonal lines in the other box (hard-NoGo trials). For Go trials, a correct response is defined as a key-press completed within 600 ms. For NoGo trials, an incorrect response is defined as a key-press completed within 600 ms. There are 168 Go trials (75%), 28 easy-NoGo trials (12.5%), and 28 Hard-NoGo trials (12.5%) in each fMRI run (10 min 40 s per run). For detailed descripton, please see reference [1].

C.4.7 Pre- and Post-treatment Behavioral Performance: Because fMRI sessions in this project are conducted only during pretreatment baseline, this project will also acquire out-of-scanner pre-treatment and post-treatment (week 12) behavioral data for the cognitive tasks. Pre-treatment performance will be considered as a possible confounding factor and will be controlled as a covariate in the statistical analysis (Section C.4.20). Post-treatment behavioral performance is a treatment outcome and will be included in the treatment prediction analysis (C.4.20). In addition, Dr. Scott Lane, who is an expert in the behavioral science of drug addiction, will be a Consultant on this project to advise on the behavioral data implementation and analysis.

C.4.8 MRI Scanning Site and Devices: MRI sessions are conducted at the Magnetic Resonance Imaging Center of the UTHSC-H, using a research-dedicated Philips Intera 3.0 T MRI scanner with an 8-channel receive head coil (Philips Medical Systems, Best, Netherlands).

C.4.9 Task Practice and Mock MRI Simulation: Prior to the real MRI scan, the subjects undergo at least 10 min of practice of the fMRI tasks until it is clear that the procedures are understood and that the majority of the practice responses are correct. After that, the subjects undergo a mock MRI scan using a Philips product MRI

Simulator. Lying in the mock scanner, the subject performs the fMRI tasks as in the real scan, while listening to a recording of MRI sounds. The fMRI tasks are written in E-Prime 1.2 (Psychology Software Tools).

C.4.10 Pre-scan Screening: Immediately prior to the real MRI scan, all subjects undergo urine drug screen and breath alcohol screen. Female subjects also undergo urine pregnancy screen. The subjects with a breath alcohol result of greater-than-zero do not proceed for the MRI scan that day, and are rescheduled. The control subjects with a positive urine drug screen are excluded from the study. The female subjects with a positive urine pregnancy test are excluded from the study. Subjects who regularly smoke tobacco are asked to smoke two hours prior to the first fMRI scan, and not to smoke after that until the fMRI scans are finished.

C.4.11 MRI Scans: The MRI session is approximately 90 min, with a 10 min break in the middle. The first 2 series are a T1-weighted spin echo 3-plane localizer (scout), and a SENSE localizer. A high resolution T1-weighted 3D-MPRAGE series is acquired with the following parameters: 256 x 256 acquisition and reconstruction matrix, in-plane resolution= 0.94 mm, 170 sagittal slices, 0.94 slice thickness. This is followed by 3 working memory (IMT/DMT) **and 3 response inhibition (Go-NoGo)** fMRI series (runs) with approximately 2 minutes rest between runs. Each fMRI series is a single shot spin-echo echoplanar (EPI) pulse sequence (see next section for rationale), with SENSE factor= 2.0, repetition time= 2212 ms for IMT/DMT **and 2500 ms for Go-NoGo,** echo time= 75 ms (optimized for spin echo), flip angle= 90 degrees, number of axial slices = 22 for IMT/DMT **and 25 for Go-NoGo,** field-of-view= 240 mm x 240 mm, in-plane resolution 3.75 mm x 3.75 mm, slice thickness= 3.75 mm, gap= 1.25 mm, repetitions= 294 for IMT/DMT **and 256 for Go-NoGo** after 10 dummy acquisitions, total duration = 10 min 47 s for IMT/DMT **and 10 min 40 s for Go-NoGo.** A 3D-FLAIR and T2-weighted fast spin echo are acquired to rule out incidental intracranial findings.

C.4.12 Spin-Echo EPI: Spin-echo EPI, rather than gradient-echo EPI, is used for fMRI in this study to avoid signal losses caused by through-slice dephasing in regions that are affected by strong susceptibility gradients at 3 T [168-170]. The regions affected by strong susceptibility gradients near air-tissue interfaces include medial orbitofrontal cortex and medial temporal areas that are important for drug addiction research. In addition to its advantage of eliminating signal dropout, spin-echo EPI has other advantages over gradient-echo EPI including greater extravascular signal component, less spatial distortion, and more specific spatial localization at 3T [169, 171]. Although the magnitude of the BOLD effect is somewhat lower in spin echo EPI compared to gradient echo EPI at 3T (in areas of the brain that are not affected by susceptibility dropout), a cognitive fMRI study using spin-echo EPI at 3T [169] found significant activation in the same cortical areas that were found using gradient echo EPI (i.e., in the areas not affected by susceptibility dropout). Importantly, that study found significant activation in predicted medial orbitofrontal areas that were not found with gradient echo EPI [169]. Likewise, our fMRI [35] and fMRI-based DCM [138] studies, which used the same working memory task and scanning parameters as in the proposed project, found significant findings in medial orbitofrontal cortex, as well as in expected cortical areas that are not affected by magnetic susceptibility dropout.

C.4.13 fMRI presentation system: Stimulus presentation and recording of behavioral performance during fMRI scanning is managed through the Eloquence fMRI System (Invivo Corporation, Orlando, Florida), an update of the Integrated Functional Imaging System-Stand Alone system *(IFIS-SA,* InVivo Corporation, Orlando, Florida). The subject views the task on an LCD video display unit that is built into the head coil. The subject responds to the task by pressing a keyboard-like button with the right index finger.

C.4.14 Flowchart and Timeline of Procedures for All Aims:

Procedure	Location	Timing
Consent for participation, screening (physical examination, routine blood work, urinalysis, EKG, metal screening, SCID, ASI, HIV, and urine drug screen)	VCU Collaborative Advanced Research Imaging (CARI) program	Day 1
Training session on the cognitive tasks, mock scan, pre-treatment behavioral performance	MR Imaging Center Mock Scanner at CARI	Day 2
Urine drug screen, urine pregnancy screen, breath alcohol screen	CARI	Day 3
Sagittal and SENSE localizers, 3-D MPRAGE, IMT/DMT fMRI (x 3), **Go/NoGo fMRI** (x 3), 3D-FLAIR, T2-weighted fast spin echo	CARI Research Dedicated 3T MRI Scanner	Day 3

C.4.15/ncJdental Fmdmgs on MRI: The FLAIR 1mages and T2 weighted spm echo 1mages from all subJects will be read by the study Radiologist. The subjects who have clinically significant abnormalities will be excluded from the study and referred for appropriate treatment.

C.4.16 FMRI Preprocessing: The fMRI data is preprocessed using Statistical Parametric Mapping (SPM8, http://www.fil.ion.ucl.ac.uk/spm/) software from the Wellcome Department of Cognitive Neurology, London, UK, implemented in Matlab (Mathworks Inc.). After slice-timing correction, each fMRI time series is realigned to correct for head motion. Runs with head motion greater than 1 voxel (3.7S mm translation on any axis) or rotation greater than 3.7S degrees are eliminated from analysis. After coregistering the high-resolution 30-MPRAGE to the fMRI images, the 30-MPRAGE is warped to the coordinates of the Montreal Neurological Institute (MNI) standard space [172, 173] using the SPM8 Normalisation procedure, consisting of affine followed by non-linear transformations. These transformations are applied to the fMRI series to convert the fMRI images to MNI space, and the fMRI images are then resliced to 2 mm isotropic resolution and spatially smoothed with a Gaussian filter of 8 mm isotropic full width at half maximum (FWHM).

C.4.17 SPM univariate analysis of working memory fMRI data: First-level voxel-wise univariate statistical analysis of the fMRI data is conducted using SPM8. The IMT and DMT blocks for each digit condition are modeled by boxcar functions convolved with the SPM8 canonical hemodynamic response function. The parameters for each condition are estimated using the General Linear Model (GLM) [174] at each voxel without global normalization. The fMRI time series is high-pass filtered with an optimized cut-off period of 330 s determined by the Fourier transformation of each condition's time model, which shows that the signal from the experimental condition is retained at this value but not at shorter cut-offs. Activation for each digit condition is defined as the contrast of DMT minus IMT parameter estimates ("DI") for that digit condition (i.e., 013, DIS, and 017 for 3 digits, S digits, and 7 digit conditions respectively). In addition, the following contrast images are defined to determine the interaction effects between the memory delay (IMT and DMT) and the digit load (3 digits, S digits, and 7 digits): DIS minus 013, 013 minus 015, 017 minus 013, 013 minus 017, 017 minus DIS, and DIS minus 017. The following contrast images are defined to determine the main effects of memory delay (pooled across 3 digits, S digits, and 7 digits): combined 013, DIS, and 017. The summary contrast images are output for each subject for entry into the second-level analysis. Because a between-group DCM study focuses on the characterization of abnormal connectivity in a common network of regions, Seghier et al. [79] recommended that only commonly activated regions in patients and controls should be included as nodes in DCM [79]. Accordingly, a second-level Random Effects [17S] SPM8 GLM analysis is conducted in order to determine the nodes of the working memory system that are common to both groups for purpose of DCM. analysis. For each type of summary contrast image, the group difference and the main effects across the means for both groups are determined with the default non-sphericity correction for unequal variance between groups. The cluster-defining threshold is voxel t = 2.S. Statistical significance of the fMRI analysis is false discovery rate (FOR) corrected cluster P less than 0.0S.

C.4.18 SPM univariate analysis of response inhibition fMRI data: **This processing is similar to that for working memory, except for the following.** (a) **The cut-off period of the high-pass temporal filter is 128 s, which is an optimized setting determined by Fourier transform of the *GoiNoGo* task time-series model. (b) The summary contrast images of the first level fixed effects analysis are: correct Easy NoGo trials minus correct Go trials, correct Hard NoGo trials minus correct Go trials, correct Hard NoGo trials minus correct Easy NoGo trials and combined correct NoGo trials (correct Hard NoGo trials plus correct Easy NoGo trials) relative to correct Go tri()ls (in brain regions *not* showing significant interaction of activation with task difficulty). The last contrast image is used to investigate the overall effects of the NoGo trials.**

C.4.19 Effective Connectivity Analysis: The effective connectivity analysis, described below, is conducted using *stochastic and nonlinear DCM (snDCM)* as implemented in the latest update to SPM8.

C.4.19.1 Working memory DCM analysis

Regions-of-interest (ROis): L IFC, L DLPFC, L PPC, R PPC, LR dorsal striatum, LR ventral striatum, and LR thalamus are the ROis. The LR dorsal striatum (or LR ventral striatum) is treated as a single ROI because we found that the DCM parameters on the connections from or to the striatum were similar for left and right striatum [37]. The ROis are based on the following criteria: (a) these regions showed significant activation in the SPM second-level analysis of the main effect of memory delay for the combined groups in our previous study [37]; (b) meta-analyses have shown that these regions activate consistently during working memory tasks [176, 177]; (c) these regions have been implicated in working memory processes, e.g., encoding [178], storage [179], maintenance [177-179], and executive control [179]; and (d) some regions (e.g., thalamus) have been implicated in treatment response in COs [3S].

Volumes of interest (VO!s) and time series extraction: We follow the method of constructing the VOis as described in Ma et al. [138]. *The significant group level activation clusters form the VO/s, which are further constrained [141] to be within the boundaries of the aforementioned seven ROts,* as defined by anatomical atlases. The atlas-derived binary masks of the regions are obtained from the Anatomical Automatic Labeling atlas [180] as implemented in the WFU (Wake Forest University) PickAtlas SPM toolbox [181, 182]. The binary mask of DLPFC is the middle frontal gyrus. The binary mask of IFC is the set-theoretic union of the atlas-based binary masks of inferior frontal gyrus pars opercularis and inferior frontal gyrus pars triangularis. The binary mask of PPC is the union of the atlas-based binary masks of superior parietal lobule and inferior parietal lobule. The binary mask of dorsal striatum is the union of the atlas-based binary masks of dorsal caudate and dorsal putamen, and the binary mask of ventral striatum is the union of the atlas-based binary masks of ventral caudate, ventral putamen, and nucleus accumbens. Following Postuma and Dagher [183], z = 7 mm (Talairach space) is used as the border between dorsal caudate and ventral caudate, and z = 2 mm (Talairach space) is used as the border between dorsal putamen and ventral putamen. The atlas-derived binary mask of the nucleus accumbens is obtained from the Harvard-Oxford Subcortical Structural atlas (http://www.cma.mgh.harvard.edu/) as provided in the FSL software [184]. Each VOI is obtained by the intersection of the atlas-based binary masks with the significant activation clusters in common for both groups, as determined by the SPM8 second level random effects univariate analysis. For each subject, the time series from the first-level univariate analysis for that subject is extracted from all voxels within the VOI, and the principal eigenvariate [141] of this time series, which is adjusted for effects of interest, is input into DCM for model estimation [141]. The same VOis are used for all subjects.

Striatal gating DCM: Based on Landau et al. [64] showing that striatal DA function is related to working-memory capacity and activation in left inferior PFC, and Honey et al. [185] showing that working memory capacity is related to effective connectivity from L IFC to L PPC, we use L IFC and L PPC as the prefrontal and posterior regions, respectively, in the striatal gating model proposed by O'Reilly and Frank [22]. The activity in the dorsal striatum is hypothesized to gate the connectivity via thalamus, between the L IFC and the L PPC, based on the striatal gating models [22, 23] and our pilot study results (section C.2.4).

C.4.19.2 Response Inhibition DCM analysis. ROis are: R bilateral OFC, LR pre-supplementary motor area (pre-SMA), R PPC, LR dorsal striatum, LR ventral striatum, and LR thalamus are the ROis, which are based on our pilot data (Section C.2.5); and consistent with: (a) existing striatal gating models [22, 23] and theoretical models on impulsivity [24-26, 186]; (b) review articles on Go-NoGo fMRI studies [27-30] and cocaine addiction Go-NoGo fMRI studies [7, 8, 31, 187, 188]; and (c) DA modulation effect on response inhibition [15). Except for the striatum, our proposed ROis are different from those used in a published effective connectivity study [188] using Go/NoGo task, possibly due to different subject samples (children were studied in [188] and we studied adults) and different fMRI design (a block design was used in [188] and we used event-related design). Since these regions are based on our pilot data, other regions, if they show significant activation on fMRI using the larger sample size that is proposed in the grant, win be considered as ROis in the future. *VOis and time series extraction:* This part of processing is essentially same as that in the working memory DCM analysis. *Striatal gating DCM:* Based on the study [83] showing significant correlation between dopamine 02 receptor function and glucose metabolism in orbitofrontal cortex, and the study [15] showing that levodopa affected the magnitude of Go/NoGo task-elicited fMRI response in right parietal cortex, we use R lateral OFC and R PPC as the prefrontal and posterior regions, respectively, in the striatal gating models [22, 23]. The activity in the dorsal striatum is hypothesized to gate, via thalamus, the connectivity between the R lateral OFC and the R PPC, based on the striatal gating models [22, 23).

C.4.19.3 Inference on DCM model structure: In DCM analysis, the number of candidate DCMs, after permutation and combination of possible endogenous connections and exogenous inputs, is very large. Therefore, the network discovery technique (NDT) [189], as implemented in the latest release of DCM, is used as a first step to determine the model structure for each subject. The NDT scores the relative posterior probability of each candidate DCM in order to evaluate efficiently the large number of possible DCMs and to find the optimal model for each subject. The initial full model to be reduced by NDT includes all possible bi-directional endogenous connections between each node (see C.4.19.1 and C.4.19.2 VOis). The contextual conditions of the cognitive tasks are putative exogenous inputs and bilinear modulators at each connection in the initial full model. In addition, nonlinear gating by dorsal striatum on PFC-PPC is hypothesized in the initial full model (see section C.4.19.1 and C.19.2). The NDT results for each subject are checked first to see

whether the causal (directional) relationship between the OLPFC and the striatum has been determined and whether the striatal gating OCM is optimal for all subjects in each group. The inference on model structure is further conducted using Bayesian model selection (BMS) [190] and Bayesian family level inference (BFLI) [191], as described in detail in Ma, et al. [138]. BMS is an established procedure for selecting an optimal OCM from alternative OCMs. This is realized by comparing model evidence, which simultaneously quantifies how accurately a model explains the data and model complexity [190]. An "optimal" model is reflected by the Bayesian exceedance probability [190], which denotes the probability that a model is more likely to have generated the observed data than the other models considered. BFLI is an extension of BMS that can be used to select the optimal family from candidate families of models [138, 191].

C.4.19.4 Inference on DCM parameters: The inference on OCM parameters is conducted using Bayesian model averaging (BMA) [191]. BMA is a Bayesian approach that averages each OCM parameter across subjects and across models such that the contribution of each model (of each subject) for that parameter is weighted by each model'$ posterior probability for that subject. Thus, BMA avoids the rigid assumption of an identical single OCM for all subjects that is required in classical "Frequentist" analysis of OCM parameters [191]. The same model space, comprised of the optimal OCM for the COs group plus the optimal OCM for the control group, is used in the BMA analysis of each group to determine the posterior distribution of each OCM parameter for each subject within the group [138].

C.4.20 Statistical analysis:

Specific Aim 1: To conduct an snOCM study of working memory to determine the baseline effective connectivity between OLPFC and striatum in treatment-seeking COs compared to non-drug using controls.

Initial analyses will evaluate group differences on demographic and baseline variables, will use contingency tables with chi-square testing, ANOVA's, and examination of correlations between baseline variables and snOCM results. The Friston et al. [189] network discovery technique, BMS, and BFLI (C.4.19.3) will be used to select the optimal model based on the exceedance probability of the OCM (or family of OCMs) in which OLPFC causally affects ventral striatum vs. exceedance probability of the OCM (or a family of OCMs) in which the ventral striatum causally affects the OLPFC. The parameter estimates for the endogenous OLPFC-striatal connectivity will be compared between groups using BMA and also compared using analysis of covariance.

Specific Aim 2: To determine whether the pretreatment gating effect by dorsal striatum on IFC-PPC effective connectivity (reflecting low OA) predicts treatment response to OA pharmacotherapy in COs.

Urn randomization [153], a form of stratified randomization, will help ensure comparability of treatment groups on baseline severity and duration of cocaine abuse. Pretreatment task behavioral performance or demographic variables on which group differences are detected, and which are correlated with outcomes, meet the definition of confounders [192, 193]. In such cases, analyses will evaluate the robustness of findings after statistically controlling for the confounder. If conclusions remain unchanged after covarying for the confounder, the more parsimonious model will be reported along with a statement indicating its robustness to covariation for potential confounding. If inclusion of the covariate alters the conclusions of the analysis, both models will be reported. Treatment success is a function of two elements: (a) retention and (b) demonstrating cocaine-free status using the SRPHK1 criteria (see section C.3.4). The Treatment Effectiveness Score (TES) provides a metric for this composite outcome [194]. TES is the sum of all cocaine-negative tests (according to SRPHK1 rules). Higher TES indicates that participants both continue to participate in the study and are negative for cocaine. For each group, BMA (section C.4.19.4) is conducted across all OCMs in the model space. Group difference in the gating effect by the dorsal striatum is assessed using Bayesian confidence probability [195]. In addition, BMA is conducted across all OCMs in the model space for each subject in the medication group and in the placebo group. Evaluation of differential treatment response, measured by the TES, as a function of the averaged (across the OCM model space) gating effect, will use Poisson regression. Violations of assumptions of dispersion will result in the use of more appropriate approaches such as zero-inflated Poisson, negative binomial, or zero-inflated negative binomial models. Regression of treatment, averaged gating, and their interaction will permit evaluation of differential treatment response as function of gating. The critical element in doing so is the evaluation of the interaction term. Given that the current study is powered to detect group differences, it is likely underpowered to detect an interaction. Brookes, et al. [196] demonstrate that for studies with 80% power to detect a between-groups effect, power to detect an interaction effect of the same magnitude as the main effect is approximately 29%. One solution is to adopt a Bayesian subgroup analysis approach [197-201]. This approach utilizes the posterior distribution of the interaction parameter to estimate the

probability that it exceeds some number (e.g., for Poisson regression a Risk Ratio of 1.0). Specification of vague neutral priors will reflect uncertainty regarding parameter values, utilizing prior distributions for the lower order effects that are neutral and diffuse with specification -N(O, 1 x 10^{-6}) on the log scale (i.e., centered at the null hypothesis with a 95% Credible Interval of± 1960) for all coefficients, and -U(0,100) for any required dispersion terms. Examination of the interaction of treatment and the subgroup index forms the crux of the evaluation of the subgroup effect. For evaluating the interaction representing the differential subgroup effect, analysis will be conducted in two ways: (a) utilizing diffuse, indifferent priors (-N(O, 1 x 10^{-6}) on the log scale for the interaction coefficients, and (b) using informative skeptical priors for the coefficients will evaluate sensitivity of statistical conclusions to specification of priors. Informative, skeptical priors will be derived via the method first proposed by Dixon and Simon [200]. Evaluation of resulting posterior distributions will permit conclusions regarding the probability that subgroup/interaction effects of varying magnitudes obtain. Examples of this use of the posterior distribution may be found in Green et al. [197]. Bayesian modeling will use SAS v9.2 (Proc Genmod and Proc Monte-Carlo Markov chain [MCMC]), and in WinBugs 1.4.3 (http://www.mrc-bsu.cam.ac.uklbugs). Convergence of Bayesian analyses on the posterior distributions via MCMC will be assessed via graphical (Trace Plot, Autocorrelation Plot) and quantitative (Geweke Diagnostics, Gelman-Rubin Diagnostics, and Heidelberger-Welsh Diagnostics) evidence. Evaluation of posterior distributions will permit statements regarding the probability that effects of varying magnitudes exist, given the data.

While the use of urn randomization [153] will likely result in comparable groups, substantial heterogeneity may still exist within conditions on baseline variables such as severity, duration of use, and impulsivity. We propose subgroup analyses following the procedures described above. This will determine the degree to which baseline variables moderate the interaction of treatment and snOCM.

Specific Aim 3: To conduct an snOCM of impulsivity to test Hypotheses 1 and 2 (above).

The statistical analysis is essentially the same as in Specific Aims 1 and 2.

C.4.21 Power Analysis: The power analysis was conducted using StudySize 2.0 software (http://www.studysize.com/). The power analysis for the correlation analysis between the DCM striatal gating effect and the subsequent treatment outcome (Specific Aim 2) is based on the preliminary data (Section C.2.4). We have shown an effect size of 0.69 in our correlation analysis, but this is probably inflated due to the small sample size. Therefore we have conservatively used a more modest effect size of 0.60 when calculating the power. For an effect size d = 0.60 and 2-tailed alpha = 0.05, a sample size of 24 subjects would achieve Cl power of 86% for the correlation analysis. For Specific Aim 1, our pilot data (Section C.2.4) showed an effect size of 1.89 for the between-group comparison of the strength of the endogenous connection from DLPFC to ventral striatum. Based on this effect size, we have calculated that 24 subjects per group would achieve a power of 99.9% at a 2-tail alpha = 0.05. Previous studies by our group [35, 37] (section C.2) found significant fMRI activation in predicted regions with 19 CDs and 14 controls. Thus we expect significant fMRI activation in the current project using the same fMRI task and scanning parameters with 24 subjects per group.

C.4.22 Limitations: OCM is based on several assumptions, especially in its original form [75]. However, we will use the OCM extensions, nonlinear [76] and stochastic [77] OCM, to reduce the dependence on these assumptions. OCM is a relatively new approach to study effective connectivity. However, OCM is being established as a primary effective connectivity technique in the neuroimaging literature, as evidenced by rapidly increased citation rates [78], overall publication number [203], and publication number in other patient populations [79], and high reliability has been shown in healthy subjects [80]. This project will only study COs who are treatment-seeking individuals, which may affect generalization of the findings to all COs. We believe that this is an acceptable trade-off because the major objective of this study is to develop a novel biomarker to predict treatment response in treatment-seeking COs.

1. Be between 18 and 50 years of age;
2. Right handed;
3. Have no past history of Psychiatric or non-Psychiatric medical disorders which could affect the central nervous system as assessed by SCID and physical examination.
4. Be in acceptable health on the basis of interview, medical history, labs, and physical exam;
5. Be able to understand the consent form and provide written informed consent;
6. Have a medical history and physical examination demonstrating no clinically significant contraindications for study participation;
7. Have no metal fragments or other bodily metal (pacemaker) or significant claustrophobia that would put the subjects at risk for MRI scanning.

Cocaine-dependent subjects with medical conditions contraindicated to, or taking medications known to have significant drug interactions with the study medication(s) will be excluded from the ongoing clinical trial, and therefore will not participate this study. In addition, a subject will be excluded from this study if any of the following conditions apply:

1. Current DSM-IV diagnosis of any psychoactive substance dependence other than cocaine, marijuana, or nicotine (for cocaine-dependent subjects only);
2. Current DSM-IV diagnosis of any psychoactive substance dependence (for non-drug using control subjects only);
3. Have a DSM-IV axis I psychiatric disorder (other than psychoactive substance abuse or dependence as described in criteria 1 for cocaine dependent subjects) or neurological disease or disorder;;
4. Have cognitive impairment due to non-substance related factors (e.g., history of stroke, transient ischemic attacks, mental retardation, epilepsy, head injury);
5. Significant current suicidal or homicidal ideation;
6. Take CNS active concomitant medications;
7. Pregnant or nursing (for female subjects only);
8. Inability to read, write, or speak English;
9. Unwillingness to sign a written informed consent form;
10. Have evidence of clinically significant heart disease or hypertension.

1.b Sources of Materials

Urine, blood, and breath samples will be collected from all recruited subjects. We will also collect demographic information as well as other information needed for screenings, e.g., family history information, and drug use information, from all recruited subjects. In addition, MRI data will be collected from all recruited subjects. All data collected from the subjects will remain confidential. Data recorded by paper will be kept in a locked cabinet in the Principal Investigator's laboratory. Electronic data will be stored in computer, COs or hard disks with password protection. Only the Principal Investigate and trained research assistants will have access.

1.c. Potential Risks

Phlebotomy: There is the potential risk of bruising at the site of the blood draw for the HIV test and for the blood chemistries and complete blood count.

Contraindication to MRI scanner: Individuals who have pacemakers, metal or electromechanical implants or metallic foreign bodies can be injured if they undergo an MRI scan.

Claustrophobia: Some individuals become anxious due to claustrophobia during MRI scans.

Privacy of individuals: There is potential risk to privacy of individuals.

Pregnant women: There is unknown risk of MRI in pregnancy.

2 Adequacy of Protection Against Risks

2.a Recruitment and Informed Consent

Participants for the Ongoing Clinical Trial (R01 DA030787; Dr. Joy M. Schmitz, EJj will be self-referred in response to various study advertisements. Individuals who call for information will be given a brief description of the study. Those interested will then be asked to answer questions about their current substance use. A trained research assistant will conduct this telephone-screening interview. Interviewee responses will be evaluated by the Principal Investigator of the ongoing clinical trial (Dr .Joy Schmitz) , who is Co-Investigator on the current proposal, and a decision regarding study eligibility will be made. Eligible subjects will be scheduled for an in-person visit at the CNRA. The first appointment will begin with the presentation of the informed consent form for the ongoing clinical trial. The consent form will detail the requirements of study participation (e.g., number of visits, type of data collected, time commitment, etc.). Information about the potential risks and how these risks are minimized will be informed. Other information on the consent form will include a full description of study requirements, reimbursement, risks, benefits, alternatives, and the role of the local Institutional Review Board (IRS). All questions will be answered before written consent is requested.

A copy of the signed consent form for the ongoing clinical study is made and given to the subject, another copy is held in the Principal Investigator's (Dr. Joy M. Schmitz) records, and the original signed consent is kept in a separate, locked file accessible to the IRS upon request.

Subjects for the current DCM MRI proposal will be recruited from participants in the ongoing clinical trial (above). Subjects will be told by the P.I. (Dr. Liangsuo Ma, or trained research assistant) that the purpose of the current proposed study is to evaluate the mechanism of pharmacotherapeutic response for cocaine addiction treatment using MRI. Information about the potential risks and how these risks are minimized will be given. Other information on the consent form will include a full description of study requirements, reimbursement, risks, benefits, alternatives, and the role of the local Institutional Review Board (IRS). All questions will be answered before written consent is requested.

A copy of the signed form is made and given to the subject, another copy is held in the Principal Investigator's (Dr. Liangsuo Ma) records, and the original signed consent is kept in a separate, locked file accessible to the IRS upon request.

2.b Protection Against Risk from the proposed DCM MRI study

Phlebotomy: This risk will be minimized by having blood drawn by a trained phlebotomist or nurse.

Contraindication to **MRI** scanner: The individuals under this risk will be carefully screened out prior to participation in the MRI experiment by careful history and physical examination.

Claustrophobia: This risk will be minimized by having all subjects undergo a "mock" MRI scan using a simulator prior to the actual scan. Subjects with significant claustrophobia during the mock scan will be excluded from the actual MRI study. Subjects will be monitored and can communicate with the scanner operators at all times during scanning. The scanning will be stopped immediately at the subject's request or in case the subject appears uncomfortable.

Privacy of individuals: All subject related information will be coded and de-identified. Any information about the subjects gained during the course of the study will remain confidential. Data recorded by paper will be kept in a locked file cabinet in the principal investigator's laboratory. Electronic media (i.e., computer-stored data, and data stored on COs) will be password protected via encrypted code key known only to the study personnel.

Pregnant women: Pregnancy will be carefully screened for female subjects. Pregnant women will be excluded from the study.

3 Potential Benefits of the Proposed Research to the Subject and Others

All subjects will undergo a free thorough psychiatric and non-psychiatric medical screening during the study. Any clinically relevant abnormalities discovered during this screening will be discussed with subjects and they will be referred for treatment for these abnormalities. All subjects who undergo an MRI scan will have a structural scan read by a Radiologist. Any clinically significant abnormalities on the MRI scans will be discussed with the subjects by the study physician and will be referred for treatment. The optimal measurement of the mechanism of pharmacotherapeutic response has significant implications in cocaine addiction treatment, and would be a great benefit to society.

4 Importance of the Knowledge to Be Gained

This study aims to optimally measure the mechanism of pharmacotherapeutic response in dopamine-related neuronal systems underlying impaired cognitive function in cocaine dependence. The completion of the proposed study is expected to improve our understanding of the dopaminergic mechanism and pharmacotherapeutic mechanism in cocaine dependence, and ultimately allow development of a clinically useful biomarker that could be predictive of more time-consuming and costly clinical trial results. Compared to above-stated risks that are minimal, the knowledge to be gained outweighs the risks.

5 Data and Safety Monitoring Plan

The Principal Investigator will be responsible of monitoring the safety and efficacy of this study, executing the data and safety monitoring (DSM) plan, and comply with the reporting requirements. The P.I. will provide a summary of the DSM report to NIDA on an annual basis as part of the progress report. The DSM report will include the participants' sociodemographic characteristics, expected versus actual recruitment rates, treatment retention rates, any quality assurance or regulatory issues that occurred during the past year, summary of adverse events (AEs) and serious (SAEs), and any actions or changes with respect to the protocol. The DSM report to NIDA will also include, if applicable, the results of any efficacy data analysis conducted.

All data will be obtained for the specific purposes of this research. We will obtain information about subjects from structured interview evaluations, physical examinations, self-report measures, and collateral informants. The biological specimens obtained from all subjects will include urine, blood, and breath samples. Brain imaging studies will also be performed on all subjects. Collected data will be identified with the study's ID of the participant. The codes that link the name of the participant and the study ID will be kept confidential in a secured cabinet. Data entered by trained research staff will be verified via second entry by independent staff. The study statistician will analyze the data, using SAS software. Primary outcome variables will be derived through vital signs, self-reported behavioral effects, and brain imaging measures.

The following procedures will be taken to safeguard against AEs: (1) careful initial screening to determine eligibility based on inclusion/exclusion criteria; (2) thorough physical evaluation prior to exposure to study drugs consisting of physical examination, standard laboratory tests, electrocardiogram, toxicology screen, pregnancy test and vital signs; (3) careful screening for presence of a pacemaker, metal or electromechanical implants or metallic foreign bodies. Specific criteria will be used to exclude potential subjects for whom MR imaging is contraindicated.

All AEs occurring during the course of the study will be collected, documented, and reported to the Principal Investigator. The occurrence of AEs will be assessed at baseline, on each day of testing, and on visit during the chronic administration phase of the study.

AE's deemed to be serious, as defined by the FDA, will be systematically evaluated on each day of study participation. Any SAE, whether or not related to study, will be reported to the IRB and NIDA. A full written report to all institutions will follow as soon as possible but in no more than three days. The written report will be in the format required by the local IRB and will contain information regarding the date of the SAE, description of the SAE, severity rating (Grade 1 to 4), assessment of cause, whether the SAE indicates an increased risk for current or future subjects, and whether changes to the informed consent form are necessary.

In cases of early termination from the study due to SAE, the participant will have appropriate follow-up medical monitoring. Monitoring will continue until the problem has resolved or stabilized with no further change expected, is clearly unrelated to study medication, or results in death. Outcome of SAEs will be periodically reported to NIDA. A summary of the SAEs that occurred during the previous year will be included in the annual progress report to NIDA.

Inclusion of Women and Minorities

Men and women of all ethnic backgrounds will be recruited to participate. It is anticipated that the subject demographic profile will closely mirror the larger population from which they are recruited. In the previous cocaine clinical trials at our center, the percentage of females and males is approximately 30% and 70% respectively. We will continue recruitment efforts to achieve a 50 50 balance and thus permit meaningful analyses by sex across groups.

In the ongoing clinical trial, efforts are made to have the outreach coordinator present educational material and referral information at women's clinics in Houston and the surrounding communities. Advertising in papers especially directed to women, such as SingleFile (a publication with 53% female readership), and the Health and Beauty section of the Greensheet has helped in the past to enhance recruitment of women with cocaine dependence.

The ethnic representation has been 55% African-American, 44% Caucasian, 15% Hispanic, and <1% Asian. In the absence of data on the prevalence of cocaine dependence disorder in the Houston area, we cannot define ethnic base rates. The medical center environment is not known to be uniquely avoided by any particular ethnic group and patients view it as favorable and neutral. Nevertheless, we are prepared to implement recruitment procedures to ensure a more diverse patient population. These procedures include: 1) Targeted advertising in newspapers which serve minority communities (e.g., Chance, a Hispanic publication). 2) Distribution of flyers and notices in neighborhoods known to have a high minority population. 3) Engaging in outreach activities on an ongoing basis, e.g., contacting church and community leaders in the Hispanic communities to provide educational material about cocaine dependence and its consequences; providing contact information to aid inreferrals to clinic at the CNRA.

Inclusion of Children

Children between the age of 18 and 21 years will be included in this study. This age range was chosen as the vast majority of subjects who meet criteria for cocaine dependence are over 18 years of age. In addition, brain function in children under 18 is significantly different from that of adults. The inclusion of younger subjects than this age level will increase variance in brain function.