Detailed protocol
Drugs Brain and Behavior (DA02812)
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Specific Aims.

Addiction, especially addiction to stimulant drugs, remains an urgent public health problem. In 2016-7 as many as 5 million Americans reported misusing prescription stimulants, and 0.4 million had stimulant use disorders. In a recent survey of 5,585 young women aged 10-18, 6.6% reported nonmedical use of stimulants. Of all the people who try stimulant drugs, only a subset go on to develop dependence. The risk factors for escalating use are poorly understood. One biologically plausible, but understudied, source of risk for repeated use of drugs is individual variation in brain reward circuitry. Stimulant drugs have primary actions on the dopaminergic mesolimbic reward system, which also mediates sensitivity to other rewards. Individuals vary in their subjective and behavioral responses to stimulants and these variations may be related to brain reward function. We recently found that individuals who exhibited *greater* striatal brain activity during anticipation of a monetary reward experienced greater euphoria and drug liking after a single dose of amphetamine (determined on a separate session). Studying effects of drugs on brain reward circuits in relation to behavioral indices of reward offers a promising new avenue for identifying a biological basis for risk for stimulant drug use.

In this project, we will examine individual differences in the effects of a stimulant drug, methamphetamine (MA), on mesolimbic reward function using fMRI. We will determine how individual differences in neural activity after the drug are related to its behavioral, rewarding effects. The effects of MA on mesolimbic reward function will be examined using both reward-task-related activation and resting state functional connectivity. On separate sessions, we will examine the effects of MA on behavioral reward in the same subjects, using ratings of drug liking and euphoria, as well as measures of drug seeking (drug and dose choice). Our goal is to identify neural processes that predict MA liking and 'seeking' behaviors. We have preliminary evidence that amphetamine increases striatal brain activity during a nondrug reward task, and that it facilitates frontal-striatal resting state functional connectivity. We will extend these observations to a larger sample using a more sensitive task, more refined imaging techniques, more comprehensive behavioral assessment and sufficient power to compare men and women. Healthy young adults will first undergo two fMRI scans with placebo and MA (20 mg), while performing the Monetary Incentive Delay and the Doors task. Then, they will participate in two behavioral sessions conducted in our laboratory. We will examine correlations between the neural responses and behavioral responses to MA.

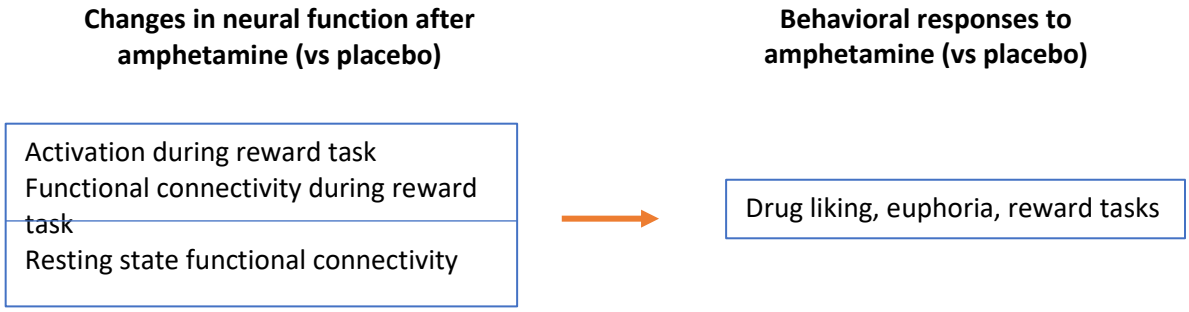
Specific Aim 1. To determine the effects of MA on regional brain activity during anticipation of reward, task-related functional connectivity and resting state connectivity. We will examine the direct effects of a moderate dose of MA (20 mg) vs placebo on mesolimbic circuits. Imaging measures will include on regional activation during two reward tasks, task-related changes in functional connectivity, and resting state functional connectivity. These measures of assess different dimensions of brain function impacting reward processing. We hypothesize that MA will increase activation in striatal regions during anticipation of reward, and enhance both task-related and resting state functional connectivity in fronto-striatal reward circuits. We also expect that there will be inter-subject variability in these effects.

Specific Aim 2. To examine the relationship between neural effects of MA and behavioral rewarding effects of MA. We will examine the relationship between the neural effects of MA (task-related and resting state) and rewarding behavioral responses to the drug. Neural effects will be assessed as in Aim 1. Behavioral responses to MA will be assessed in a 2-session procedure providing drug liking ratings as well as measures of reward learning. We hypothesize that greater magnitude of effects of MA on brain reward function (regional activation and connectivity) will be related to greater behavioral rewarding effects. Specifically, we hypothesize that MA-induced increase in fronto-striatal resting state connectivity will be linked more strongly to tonic increases in drug liking, whereas task-related striatal activation will be associated with greater reward learning on the learning task.

The project will advance our understanding of how stimulants alter neural reward processes, and how individual differences in brain function predict variation in the rewarding and cognitive effects of drugs. The findings will help to develop novel treatment approaches, including medications or noninvasive

techniques such as transcranial magnetic stimulation, that target reward substrates in addicted individuals.

Background.
This project will investigate the effects of methamphetamine (MA) on brain reward circuits in relation to its effects on behavioral indices of reward. Healthy volunteers will first engage in two fMRI imaging sessions in which they will receive MA (20 mg oral) or placebo (PL) under double-blind conditions. These scanning sessions will assess i) regional activation and connectivity in mesolimbic circuits during anticipation and receipt of reward, and ii) resting state functional connectivity in fronto-striatal circuits. Then, during two behavioral sessions, subjects will receive MA or PL during two sampling sessions on which they will complete mood and drug liking questionnaires and complete simple learning tasks.



Methods.
We address the following questions: i) does MA alter either regional activation or task-related connectivity in mesolimbic regions during anticipation of reward, ii) does MA alter resting state functional connectivity in mesolimbic and frontostriatal circuits, iii) are the neural effects of MA on reward circuitry (task-related or resting state connectivity) related to the rewarding effects of the drug, as measured by drug liking, or to its effects on simple learning? We will utilize several indices of drug reward: ratings of drug liking or euphoria. We have shown that ratings of drug liking are highly related to measures of drug seeking. We will also examine sex differences, and control for menstrual cycle phase in women (White, Justice et al. 2002).

MA is a prototypic stimulant drug with a well-defined mechanism of action. We have conducted many studies with MA at the doses proposed here, and its effects on mood and cardiovascular function are robust and reliable (Wachtel, Ortengren et al. 2002; Soderpalm, Nikolayev et al. 2003; Mayo, Fraser et al. 2013; Ballard, Weafer et al. 2015; Bershad, Kirkpatrick et al. 2015). MA produces effects that are very similar to d-amphetamine (Childs and de Wit 2013; Weafer and de Wit 2013; Hart, Gamazon et al. 2014; Wardle, Marcus et al. 2015; Childs, Bershad et al. 2016). Both drugs increase synaptic levels of dopamine and norepinephrine, and to a lesser extent serotonin (Moore 1977) by releasing neurotransmitter from the vesicles in the pre-synaptic neuron, as well as blocking re-uptake and inhibiting degradation by monoamine oxidase. We selected MA because it has a slightly faster onset of effects. There is evidence that the rewarding effects of amphetamines are mediated by its actions on dopamine, with some role also for norepinephrine (Laruelle, Abi-Dargham et al. 1995; Kumari, Corr et al. 1997; Mattay, Callicott et al. 2000; Drevets, Gautier et al. 2001; Navarra, Clark et al. 2017).

Participants will be healthy young men and women (N=80, 80). They will first participate two fMRI scans, when they receive oral MA (20 mg) and PL, in random order under double blind conditions. During the scan they will complete the Monetary Incentive Delay task (MID) (Knutson, Adams et al. 2001) and the Doors task (Carlson et al, 2011) and resting state data will be obtained. The primary outcome measures during these sessions will be task-related (anticipation and receipt of reward) regional brain activation, task-related connectivity, and resting state functional connectivity. After the two scans, subjects will participate in two behavioral sessions during which they receive MA (20 mg oral) or PL in mixed order, in color-coded capsules. Subjects will complete mood and drug effects questionnaires, and cognitive tasks.

Subjects. Healthy men and women aged 18-35 years will be recruited from the university and surrounding community without regard to race or ethnicity, according to Protocol 13681B. Initial

eligibility will be ascertained in a telephone interview (age, current drug use, medical conditions), and appropriate candidates scheduled for an in-person interview with a physical examination, EKG and a structured clinical psychiatric interview (First, Williams et al.). Their screening information will be approved by a physician. Subjects must have a high school education, fluency in English, body mass index between 19 and 26, and good physical and mental health. Exclusion criteria are serious psychiatric disorder including psychosis, severe PTSD or depression, any regular prescription medication, contraindications for fMRI scanning, history of cardiac disease, high blood pressure, consuming >4 alcoholic or caffeinated beverages a day, or working night shifts. Subjects will be excluded either if they report never having used any substance for nonmedical purposes, or if they have experienced adverse responses to any of the drugs they might receive. This information is obtained during in-person screening by a trained clinical interviewer. Women who are not on oral contraceptives will be tested only during the follicular phase (1-12 days from menstruation) because responses to stimulant drugs are dampened during the luteal phase of the cycle (White, Justice et al. 2002). We recognize that the 5 sessions will need to be scheduled across two months for women not on oral contraceptives. Before participating subjects will complete several questionnaires: a psychiatric symptom checklist, the BIS/BAS, the Depression, Anxiety and Stress Scale (DASS) (Lovibond and Lovibond 1995) and the Multidimensional Personality Questionnaire (Patrick, Curtin et al. 2002).

Consent. Qualifying subjects will attend an orientation session to familiarize them with the methods and obtain informed consent. Subjects will be told that the purpose of the study is to investigate the effects of psychoactive drugs on mood, brain and behavior. To reduce expectancies, they will be told that they might receive a placebo, stimulant/anorectic, opioid, alcohol or sedative/tranquilizer. They agree not to use any drugs except for their normal amounts of caffeine for 24 hours before and 6 hours following each session. Subjects are informed that drug testing will occur before each session. Pregnancy and drug tests are conducted before each session using urine drug tests (ToxCup, Branan Medical Corporation) and breathalyzer tests (Alcosensor III, Intoximeters), and pregnancy tests (AimStickPBD, hCG professional, Craig Medical Distribution). Positive tests lead to rescheduling or dismissal from the study.

Testing environments. Screening and behavioral testing will be conducted individually in comfortably furnished rooms with a couch, easy chair, side table, coffee table, television and video player. Subjects may relax, read or watch neutral movies when they are not completing study measures. Scanning will be conducted in the Magnetic Resonance Imaging Research Center (MRIRC) located a 3-min walk from the behavioral testing rooms.

Procedures

Session	**Orientation**	**Scan 1**	**Scan 2**	**Behav Session 1**	**Behav Session 2**
Drug	Practice	MA*	PL*	PL*	MA*
Measures	MID Doors	Self-report Mood drug, cv	Self-report mood drug, cv	Self-report Mood drug, cv	Self-report mood drug,cv
	EEfRT	fMRI with MID, Doors and motor	fMRI with MID, Doors and motor	EEfRT	EEfRT
	Stop Task			Stop Task	Stop Task
	Simple reaction time			Simple reaction time	Simple reaction time
	Go-No-Go			Go-No-Go	Go-No-Go
	Continuous performance task gradCPT			Continuous performance task gradCPT	Continuous performance task gradCPT
	Weather Prediction Task			Weather Prediction Task	Weather Prediction Task
	Learning task			Learning task	Learning task

*PL=placebo, MA=methamphetamine, *orders counterbalanced*

(i) Imaging (2). The two fMRI sessions will assess brain activity under the influence of MA and PL. After drug and pregnancy tests, we obtain baseline measures of mood, heart rate and blood pressure. At 9:15 am subjects ingest a capsule (PL or MA 20 mg) under double-blind conditions. The scan will be conducted from 60-120 min after ingestion of the capsule at the time of peak drug effect. During the scan subjects will complete the MID and the Doors task and have a resting state scan. We will obtain several indices of the effects of MA on reward circuit function: i) for reward anticipation: regional brain activation during the MID, MID task-related functional connectivity, ii) for reward receipt, regional brain activation during the Doors task, Doors task-related functional connectivity and iii) resting state functional connectivity. Subjects will also complete one other task assessing motor reactivity.

(ii) Behavioral sessions (2): Sessions are conducted from 9 am to 1 pm, at least 48 hours apart. Upon arrival drug and pregnancy tests are obtained, and subjects complete baseline measures of mood. Heart rate and blood pressure are measured. At 9:15 am they ingest capsules (PL or MA 20 mg, in color-coded capsules) under double-blind conditions. Oral MA (Desoxyn, 5 mg per tablet) will be placed in opaque size 00 capsules with dextrose filler. PL capsules will contain only dextrose. Subjects will complete mood and drug effects questionnaires at regular intervals, cognitive tasks and a learning task. At the end of the sessions subjects complete a questionnaire rating their overall drug experiences. The primary subjective measures during these sessions are ratings of drug liking and drug-induced euphoria. Secondary measures include cognitive tasks.

(iv) End of study Interview When subjects have completed both phases of the study the PI will fully inform subjects of the study aims and drugs administered.

Primary measures

Imaging: Scanning sessions will be conducted at least 48 h apart under the direction of Dr. Keedy at the UC MRI Research Center (MRIRC) on a 3T Philips Ingenia scanner with a 32 channel headcoil. Subjects first complete a scanner safety questionnaire and baseline mood questionnaires. Participants will be familiarized with the tasks before scanning, and once in the scanner, made comfortable and head stabilized with foam padding. We have observed no difference in head motion in association with a similar dose of d-amphetamine (unpublished data from Weafer et al (Weafer, Van Hedger et al. 2019)). Physiological sensors for heart rate and respiration will monitor patient status during scanning, and will be used in fMRI data analyses to account for physiological noise.

Imaging tasks and data collection.

MID TASK: Following Knutson (Knutson, Adams et al. 2001), the MID task assesses neural responses for both reward anticipation and consumption, and offers actual small and large monetary gains and losses based upon performance. This optimized task version requires participants to respond rapidly during an individually determined response window. Response windows are titrated after a pre-scan practice and during imaging to ensure optimal performance, e.g., 50-80% success rates. There will be 2 runs consisting of 24 trials each. For each trial, a 500 ms anticipatory cue alerts the subject to the opportunity to either win money or avoid losing money, specified as +/-$0.30 or $3.00, or a neutral cue is given ($0.0) indicating that no money is at stake. Win/avoid loss and $0.30/$3.00 are presented pseudorandomly and at equal frequency across 64 of the trials; 32 trials are the neutral cue condition interspersed with the other trials. After the cue there is a 2-4s (jittered) interval and then a stimulus (a white box) appears. The stimulus duration is derived from data collected during the pre-scan practice. Subjects are required to press their thumb button as quickly as possible while the stimulus is visible. Visual feedback is provided indicating whether the participant responded within the time frame. Pre-scan practice (PSP) assesses individual-specific reaction time (RT) for correct responses. PSP mean RT and standard deviation are used to calculate a response window for the stimulus cue during the first run of the MID inside the scanner. For example, a subject with a mean RT of 180ms and SD of 20 will have a stimulus duration/response window of 220ms (180+ 2*20), and a delay jitter screen of 780ms (1000ms-220). The technician tracks performance after each run and uses the number of total correct responses to modify the response time for the next run. If a participant's accuracy falls below 50%, the stimulus duration/response window increases by 0.5 SD (and the delay jitter screen conversely). If the participant's accuracy exceeds 80%, then the stimulus duration/response window decreases by 0.5 SD. The subjects are told they will receive the money they win based on their performance, added to their payment for the scan. They cannot lose money, in accordance with the success rate calibration. The task takes ~20 minutes. For this study, cue conditions (receive/avoid losing $0.30/$3.00; or no

money at stake) primarily enhance subject task engagement and will be collapsed for primary analyses, in accordance with limited clear knowledge of neural system differences distinguishing such conditions (Oldham, Murawski et al. 2018).

Doors Task. Participants will complete the doors task (Carlson et al. 2011) during BOLD fMRI to assess brain activation during monetary reward and loss. Briefly, two doors are presented on the screen, and participants are told that behind one of the doors is a monetary prize of $.50 (signaled by '↑') and behind the other door is a loss of $.25 (signaled by '↓'). For each trial, participants are instructed to use a button box to choose one of the two doors. They are told that they will either win or lose money based on their choices, and that they had a chance of winning between $0 and $15 total based on their performance. In actuality, the subjects' performance has no impact on outcomes. The task consists of 30 predetermined Wins and 30 Losses presented in a pseudorandom order over two runs. The task requires 15 minutes to complete, and all subjects earn $10.

Motor task: Subjects will complete a motor task to provide information on the effect of the drug on activation of brain regions controlling motor function. As in an early study from our laboratory Uftring (Uftring, Wachtel et al. 2001), subjects will complete a finger tapping task in which they touch their right thumb and index finger together repeatedly for three 20s blocks, alternating with 4 20s rest blocks. We will assess for motor system changes during this task.

The scan will include a 10-minute resting state sequence, eyes open fixating on a light crosshair on a dark background, a high-resolution T1-weighted image for alignment and spatial standardization, and an Arterial Spin Labeling sequence to assess blood flow (described below).

Imaging data analysis. Imaging data from fMRI tasks will be preprocessed and analyzed using AFNI tools, as in Van Hedger (Van Hedger, Keedy et al. 2018). Preprocessing steps include alignment of each time series, spatial registration of the aligned time series to the anatomical scan, anatomical scan warped to standard space and warp applied to functional data, functional data spatially smoothed, and intensity normalization. Neural activation for conditions of interest from each task will be estimated using AFNI's 3dDeconvolve program, incorporating motion and heart rate/respiration as regressors. For MID, we will use a gamma response function to create activation maps for each task element: cues, motor response, and feedback. For the secondary tasks, we will use block functions and create contrast maps between the two block types. Mean activation for each subject at each scan session will be extracted from regions of interest (ROI). For MID, ROIs will be 8mm spheres created around coordinates reported in a recent meta-analysis of 50 MID fMRI studies (Oldham, Murawski et al. 2018). For Cue: caudate, putamen ventral striatum, thalamus, and anterior insula; Feedback: ventromedial prefrontal, orbitofrontal cortex, ventral striatum, amygdala, and will be a subtraction of mean activation of loss trials from win trials in each ROI (this contrast is the most common and more sensitive approach, per Oldham); Motor: all prior ROIs (to confirm lack of drug effect), primary sensorimotor cortex. ROI activation will be a subtraction of mean activation of loss trials from win trials in each ROI (this contrast is the most common and more sensitive approach, per Oldham). We will also look specifically at win vs neutral and loss vs neutral to confirm whether findings are driven by sensitivity to reward or avoiding loss. Tapping: primary motor cortex: dorsal striatum (caudate, putamen). For connectivity analyses of the MID task and of resting state, we will use the CONN toolbox (Whitfield-Gabrieli and Nieto-Castanon 2012) which employs similar preprocessing steps (alignment and spatial standardization, intensity normalization, motion estimation, white matter and CSF-based regressors) implemented through SPM12. We will use a 2mm displacement cutoff. For MID task-related connectivity, we will conduct an ROI-to-ROI analysis, using the spherical masks described previously. The toolbox allows comparison of correlation strengths (Pearson r converted to Fisher z) of the timeseries between each pairing of the ROIs. We will compare these correlations between drug and placebo sessions, as well as extract ROI-to-ROI correlation values for assessment in relation to behavioral measures. For resting state, we will calculate seed-based connectivity maps separately for MA and placebo, as per our prior study (Weafer, Van Hedger et al. 2019). Seeds will be bilateral caudate, putamen, and ventral striatum. We will conduct paired t tests within CONN to compare connectivity following MA and placebo. As our hypotheses are specific to frontal and subcortical reward circuitry, statistical inferences will be made within an anatomically-focused frontal-insular-striatal partial brain mask. The mask will include medial and lateral frontal and orbital regions, bilateral precentral gyri, anterior and middle cingulate cortex, anterior insula and ventral striatum. Drug order will be included as a covariate in all analyses.

Arterial Spin Labeling (ASL) data will be preprocessed and analyzed using FSL analysis tools (Smith, Jenkinson et al. 2004). Quantification of CBF will be completed using FSL's BASIL tool (Chappell, Groves et al. 2009). CBF will be quantified in units of ml/100g/min using a kinetic model and defaults for the parameters consistent with recent consensus (Alsop, Detre et al. 2015). For the within group analysis between drug and PL, quantified CBF images will be normalized to MNI space and adjusted to account for individual differences in global CBF. We will assess for spatial overlap of significant changes in CBF and regions of interest from the fMRI outcomes to inform interpretation of our findings. If there are significant changes in CBF following methamphetamine, we will also include CBF as a covariate in each of our fMRI task-based and connectivity analyses.

Mood Effects of Drug: We will utilize standardized self-report questionnaires to assess the mood-altering effects of the drug during the scanning sessions (abbreviated questionnaires) and the behavioral sessions (Carter and Griffiths 2009). Participants will complete the Drug Effects Questionnaire (DEQ) (Morean, de Wit et al. 2013) which consists of 5 questions; "Do you feel any drug effect?", "Do you like the effects you are feeling now?", "Do you dislike the effects?", "Are you high?" and "Would you like more of what you consumed, right now?" each rated on a 100 mm visual analog scale. Ratings of liking will be one of our measures of mood effects of the drug. The Profile of Mood States (POMS) (McNair D. 1971), an adjective checklist, will be used to assess momentary mood states. Subjects indicate how they feel at the moment in relation to each of 72 adjectives on a 5-point scale from "not at all" (0) to "extremely" (4). The 72 adjectives form 8 scales: Anxiety, Depression, Anger, Vigor, Fatigue, Confusion, Friendliness, Elation. They will also complete the Addiction Center Research Inventory (ARCI) (Martin, Sloan et al. 1971), a 49-item true-false questionnaire that is sensitive to psychoactive drugs (Haertzen 1966), with scales measuring stimulant-like effects (Amphetamine; A, and Benzedrine Group; BG) and euphoria (Morphine-Benzedrine Group; MBG). The drug liking scale and the ARCI MBG scales will be the primary measures of positive mood effects of the drug. Subjects will also complete ratings of drug liking and drug identification during the debriefing session after the last session.

Cardiovascular measures. During sampling sessions we will measure heart rate and blood pressure at 30 min intervals using portable monitors (Omron 10 Plus, Omron Healthcare).

Cognitive and Motor Tasks. We will also obtain secondary measures of the effects of MA during sampling sessions. These will include standardized measures of attention and cognition, most of which are available on the public site Psychology Experiment Building Language; PEBL: Stop Task, Simple reaction time, Go-No-Go, continuous performance vigilance task, EEfRT), a simple finger tapping task and measures of reward and learning (Pessiglione et al, 2006; Jocham et al, 2014), and the Weather Prediction Task (WPT; PEBL)). The Stop Task and Go-No-Go assess inhibitory control, the simple reaction time and continuous performance tasks assess attentional control. The motor task measures psychomotor performance and fine motor speed, which may reflect effects of the drug independent of its rewarding effects. Individuals are instructed to tap the letter "A" on their keyboard with the index finger as rapidly as possible for eight trials of 10 seconds, once with the dominant hand and once with the non-dominant hand. Mean number of taps is the outcome measure. The EEfRT measures willingness to expend effort (number of key clicks) for varying amounts of reward (hypothetical money). The Gradual-Onset Continuous Performance Task (gradCPT; Esterman et al. 2013 Rosenberg et al. 2013) is a test of sustained attention and inhibitory control. Stimuli are grayscale images of cities and mountains. On each trial (800 ms), one image gradually transitions to the next using linear pixel-by-pixel interpolation. Participants are instructed to respond via button press to city scenes (90%) and to withhold response to mountains (10%). Performance is measured with response time variability and sensitivity (d'). We will use two measures of simple reward learning, an instrumental approach and avoidance learning task used by Pessiglione and colleagues (Pessiglione et al., 2008; Fischer and Ullsperger, 2013) and the WPT, a standardized measure of learning. In the Pessiglione task subjects acquire associations between neutral symbols and either gain or loss of points.

C6. Statistical Plan

Aim 1 is designed to determine the effects of MA on regional brain activity during anticipation of reward, as well as changes in connectivity both during the task and at resting state. We hypothesize MA will increase activation in mesolimbic reward regions during the task and increase both task-related and resting state functional connectivity in reward circuitry. For task-related activation we will use SPSS to conduct, for each MID task component separately (cue, motor response, feedback), within-group contrasts of the MA vs PL sessions using the extracted activation from the ROIs. Based

on our preliminary data, we expect greater activation in ROIs, specifically dorsal and ventral striatal reward regions (i.e., caudate, putamen, and nucleus accumbens) following MA compared to PL for cue and feedback. Given that we did not see any effect of methamphetamine on motor cortex in our preliminary data, we do not expect to see a drug effect on motor here. Secondary, exploratory whole brain analyses will also be conducted comparing MA vs PL in each task component. To examine the effects on task-related connectivity, we will use the CONN toolbox to conduct within-group ROI-to-ROI contrasts, e.g., we will compare correlation strengths of the seed regions to one another during the MA vs. the PL scan, and expect stronger connectivity (correlation) from the MA session, specifically between striatal (i.e., caudate, putamen, and nucleus accumbens) and medial frontal (i.e., ventromedial prefrontal and orbitofrontal cortex) reward regions. To assess resting state connectivity drug effects, we will use CONN to contrast the seed-based connectivity maps from the MA vs PL sessions. CONN analyses will be evaluated at $p < .05$, familywise, within the fronto-insular-striatal mask. Based on our preliminary findings (Weafer, Van Hedger et al. 2019), we hypothesize greater connectivity between striatal seed regions and medial frontal reward regions (i.e., ventromedial and orbitofrontal cortex). All analyses will include drug order, age, and sex as covariates.

Power analysis for Aim 1: Our preliminary data indicate a large effect size for the effect of MA compared to placebo on resting state fronto-striatal (NAcc to OFC) functional connectivity ($d = 0.94$). The proposed sample of N=160 would provide over 99% power (alpha = .05) to detect an effect of MA on resting state functional connectivity.

Aim 2 is to determine the relationships between MA-induced changes in cortico-striatal function and positive behavioral effects of the drug (liking, and task performance). We hypothesize that MA-induced frontal-striatal activation and connectivity in reward circuits will be positively associated with behavioral measures of reward, including ratings of drug liking and attentional bias to cues. For the behavioral measures, we will first examine the degree of concordance among the different measures of MA reward (liking and attentional bias). We expect that these measures will be moderately correlated, but we also believe they measure separate components of drug reward. We will then create summary scores of drug effects (MA minus placebo) for each of the ROI's implicated in drug reward. For MID task activation, we will subtract activation during Win – Loss following placebo from activation following MA within each ROI that showed a significant drug effect. For MID task connectivity, we will subtract connectivity estimates following placebo from those following MA for each significant ROI-to-ROI connectivity pathway. For resting state connectivity, we will extract connectivity estimates from 10mm regions surrounding significant peaks for each seed, and again subtract placebo estimates from MA. These neural drug effect scores will then be analyzed in relation to each of the behavioral measures. The hypotheses are based on preliminary data (Weafer, Van Hedger et al. 2019).

a. For drug liking, we will perform linear mixed effects models for repeated measures (Hedeker and Gibbons, 2006) within SPSS25 to test the degree to which neural drug effects interact with drug and time to predict subjective measures of drug liking, measured during the behavioral phase of the study. The models will include random intercept, drug and time effects to allow for individual differences in subjective drug response and time trends, and to account for the correlation between repeated measurements. Drug order, age, and sex will be included as model covariates. The effects of interest are the two- and three-way interactions among neural drug effects, behavioral drug effects (MA vs. placebo) and time (linear and quadratic trends) on subjective liking. We hypothesize that drug liking will be correlated with NAcc-OFC connectivity.

Power analysis for Aim 2: For correlational analyses, the proposed sample size of N=160 yields power over 80% to detect correlations of 0.22 and greater for a two-tailed alpha=.05 test. This corresponds to a small to medium effect size (Cohen's $f2 = .051$), and so the sample size will allow us to detect reasonable associations with good statistical power.

Ancillary analyses. The design includes several ancillary measures that will serve as important controls. During the fMRI scan, subjects will complete a simple motor task and we will examine neural responses to this task, in both reward-related and other brain areas. During the MID task, we will collect data during receipt of reward, as well as our primary measure of anticipation of reward. On the behavioral sessions, subjects will also complete cognitive tasks (Stop Task, Go No Go and Simple Reaction time), a simple motor task, and and two reward-learning tasks. Cardiovascular measures will be obtained during the drug and MA sessions. These measures will allow us to investigate

individual differences in neural function (as determined by the scan) and behavioral measures, both without drug (PL session) and after drug. We will examine the specificity of any observed correlations to reward-related tasks. We will also assess MA vs PL changes identified in fMRI data against changes noted in the ASL data.
Sex differences. We will compare each of the measures in men and women (N=80, 80), and if sex differences are detected they will be included as covariates in the correlational analyses. We do not expect significant sex differences especially because women will be tested during the follicular phase of the cycle (White, Justice et al. 2002) and (Wilkinson, Khan et al. 2009). Nevertheless, we believe it is important to investigate drug responses in men and women, with sufficient statistical power.

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