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Study title: Nicotinic agonist effects on BMI and neuronal response in overweight/obese adults
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I. Hypotheses and Specific Aims:

Overall Objective: The overall goal of this application is to examine the effects of an α7 nicotinic receptor partial agonist (DMXB-A) on neuronal, physiological and behavioral mechanisms of obesity. This will be accomplished by evaluating the following outcomes following approximately 12 weeks of treatment with either DMXB-A or placebo, in a randomized, double blind, parallel design study:
Aim 1. To determine if approximately 12 weeks of treatment with the α7 nicotinic partial agonist DMXB-A, as compared to placebo, alters measures of neuronal response to a meal in overweight/obese individuals.
Aim 2. To determine if approximately 12 weeks of treatment with the α7 nicotinic partial agonist DMXB-A, as compared to placebo, alters measures of food intake behavior, body weight and fat mass in overweight/obese individuals.

II. Background and Significance:

The prevalence of obesity in the United States has drastically increased in recent decades[10], with current estimates of 68% of adults being overweight or obese[11]. Obesity is associated with increased mortality and risk for multiple diseases, including cardiovascular disease (especially stroke and heart disease), diabetes, musculoskeletal disorders (especially osteoarthritis) and cancer (especially breast and colon cancer)[12-15]. Weight loss in obese individuals reduces many of these risk factors[16-20].

A possible novel mechanism for treating obesity is activation of the α7 nicotinic cholinergic receptor. Nicotine has long been known to affect energy balance and weight. Smokers, for example, weigh less than their age- and sex-matched non-smoking counterparts[1], while smoking cessation is associated with increased food intake and weight gain[2, 21, 22]. Given the strong link between smoking and reduced weight, many individuals report using smoking as a weight control strategy, or avoid smoking cessation due to fears related to weight gain[3, 23, 24]. Experimentally, nicotine has been shown to suppress appetite, increase energy expenditure, and alter feeding patterns, which can lead to weight loss[25, 26].

Nicotine acts on both high affinity nicotinic cholinergic receptors, such as the α4-β2 receptor, and the low affinity homomeric α7 receptor, both centrally and peripherally. Recent studies have suggested that the α7 nicotinic acetylcholine receptor may play a particularly prominent role in energy balance effects. Administration of an α7 nicotinic agonist was recently found to reduce weight gain and food intake in a mouse model of diabetes[9]. Furthermore, a recent human study demonstrated downregulation of α7 nicotinic receptors in obese compared to lean subjects[8]. One pathway for α7 nicotinic mediation of eating behaviors involves hypothalamic cholinergic input. The hypothalamus contains rich cholinergic innervation and some of the highest levels of α7 nicotinic receptor expression in the brain[4]. Appetite-related circuits within the hypothalamus can be modulated by activation of nicotinic receptors, where a complex network of hormone and neuropeptide signals exerts neuronal effects to regulate eating behaviors.

III. Preliminary Studies/Progress Report:
There are no preliminary data to report. It is hoped that results of this study will inform our knowledge of nicotinic cholinergic involvement in obesity, potentially leading to novel treatment strategies to address this critical problem.

IV. Research Methods

A. Outcome Measure(s):

a. Neuronal response to visual food cues
b. Intrinsic resting state network activity (default network)
c. Body weight and fat mass
d. Appetite ratings

The primary outcome measure in this study is neuronal response, to both food cues and during rest.

B. Description of Population to be Enrolled:

Subjects included in the study will be overweight/obese, with a BMI > 27 kg/m^2. To be included in the study, subjects must have (and provide contact information for) a primary care provider. Subjects over 65 are excluded because the cardiovascular effects of nicotinic agonists may exacerbate underlying heart disease. Similarly, individuals with known cardiovascular disease are excluded. This includes coronary artery disease, stable angina, and uncontrolled hypertension (>150/95 mm Hg). We will also exclude individuals with a marked baseline prolongation of QT/QTc interval (e.g., repeated demonstration of a QTc interval > 450 ms for men and >460 ms for women), a history of additional risk factors for torsade de pointes (e.g., heart failure, hypokalemia, family history of Long QT Syndrome), and the use of concomitant medications that prolong the QT/QTc interval (e.g., quinidine, erythromycin, risperidone, citalopram). Use of antidepressant medications is an exclusion criterion unless the following 3 criteria are all met: (1) the antidepressant is not associated with a lengthened QT interval, (2) the type/dose the participant is taking has been stable for the past year, and (3) taking the medication has not been associated with participant weight fluctuations (plus or minus more than 5%) within the past year.

An electrocardiogram (ECG) will be administered as part of screening procedures, to assess QT/QTc interval. Also excluded are individuals with uncontrolled diabetes (defined as A1c value >9), individuals taking insulin, GLP-1 agonists, or any other injectable diabetes treatment, and illicit drug use, excluding marijuana use per self-report

Women who are capable of conception are excluded because the potential for teratogenicity of DMXB-A is not fully established. Incapable of conception is defined as post menopausal, surgical sterilization, or adherence to an anti-contraception birth control regimen (for at least 6 months if hormonal contraceptive). Women of child-bearing potential will be given a pregnancy test as part of screening procedures, at baseline, and on a monthly basis throughout the study. For this initial study, individuals who use nicotine, assessed by blood cotinine levels and self-report, will be excluded to preclude interactions between nicotine and the study drug. Subjects will be excluded for history of non-compliance in previous studies, and any form of nicotine use, including cigarettes, cigars, pipes, chewing tobacco, e-cigarettes, or nicotine gum. Medical history, physical examination, and blood screening tests (metabolic panel, lipid panel, complete blood count, TSH, A1c) will be performed to exclude for significant endocrine/metabolic disease (e.g., uncontrolled hypertension, severe hypertriglyceridemia, and diabetes) and kidney disease, neurological illness or injury that would be anticipated to affect subject safety or MRI data. UCH normal cutoff values will be used for exclusion criteria for blood screening tests for AST (>39 U/L), ALT (>52 U/L), TSH (0.5-5.0 uIU/mL), BUN (>25 mg/dL), and Cr (M: >1.3 mg/dL; F: >1.2 mg/dL). Serum CR below UCH normal values (<0.6 mg/dL for women, <0.7 mg/dL for men) will

not be considered exclusionary unless the Cr level is judged to be clinically significant by study physicians. AST, ALT, and BUN levels below UCH low cutoff values (AST< 12 U/L, ALT < 7 U/L, BUN < 7 mg/dL) will not be considered exclusionary unless judged to be clinically significant by study physicians. Subjects will also be excluded if triglyceride levels are >400 mg/dL. Potential participants will be asked to report concomitant medications at screening, at each check-in visit, and post-intervention. Participants taking medications that can affect appetite and metabolism (e.g., oral steroids) will not be included in the study. BMI will be determined by measuring height and body weight. MRI-specific exclusion criteria include claustrophobia, metal in the body, weight in excess of 500 lbs, largest body circumference > 155 cm, sagittal diameter of > 35 cm anywhere from the top of the head to 70 cm from the top of the head, and/or shoulder width > 52 cm. In our previous studies, approximately 5% of subjects have been excluded due to size restrictions.

C. Study Design and Research Methods

Participants will complete two study phase assessments: (1) at baseline and (2) post-intervention.

Study Phase Assessments: The following evaluations will be completed during both study phases: After a 1-day run-in eucaloric diet period to ensure energy and macronutrient balance, subjects will arrive the morning after an overnight fast. Prior to each study phase assessment day, participants will wear an activity monitor for 5-7 days (ActivPal, Glasgow, Scotland), to assess any activity differences between groups. Sleep habits will be assessed via brief self-report questionnaire (Pittsburgh Sleep Quality Index; PSQI) at both assessment days to determine if group differences in sleep habits are a possible confounding variable. A blood draw will not be completed at the baseline study day, unless it has been more than 60 days since the screening day. This blood draw will measure the complete metabolic panel to reassess liver function (i.e., we will not repeat all screening blood labs, only the complete metabolic panel). On the post-intervention study day, a nurse will insert an IV for blood draws in the morning. The initial blood draw will be used to measure DMXB-A levels, lipids, complete metabolic panel, and leptin. Three more blood draws will be completed on the post-intervention study day, each of which will be used to measure DMXB-A levels. If the nurse/phlebotomist has difficulty inserting the IV, a single blood draw for lipids, complete metabolic panel, and leptin will be considered acceptable. Following the first blood draw on the post-intervention study day, subjects will take their final pill capsule, prior to completing any other study measures. The remaining three blood draws will be taken approximately 1 hour after taking the pill, 2 hours after taking the pill, and 6 hours after taking the pill. During both study days, subjects will complete assessments of body composition, food and eating-related behaviors (Three Factor Eating Questionnaire (TFEQ[51]); Power of Food Scale[52]; Food Craving Inventory[53]), cognitive and mood measures (Digit Span task; Coding task; Comprehensive Trail Making Test [CTMT]; Center for Epidemiological Studies Depression Scale (CES-D); State Trait Anxiety Index [STAI]), and appetite evaluations (hunger, appetite, satiety). Following these measures, subjects will be escorted to the Brain Imaging Center for fMRI measures (visual food cue task and resting-state), which will first be performed in the fasted state, then again in the fed state (approximately 30 minutes following a breakfast meal, described below). Appetite evaluations will be performed at approximately 30, 90, 120, 150,180, and 210 minutes post-meal. Subjects may then complete a measure of resting metabolic rate, followed by a buffet-style lunch at the Anschutz Health and Wellness Center to measure ad libitum food intake.

Intervention:

Following baseline assessments, subjects will be randomized to DMXB-A or placebo in a 1:1 ratio. The randomization scheme will be prepared by a study biostatistician. Only the study pharmacy (Belmar Pharmacy) will be aware of drug assignment. They will have no direct interaction with study participants. A pharmacist at Belmar Pharmacy will dispense biweekly pill

vials to the study staff for distribution to subjects. Subjects will receive the drug for approximately 12 weeks, with biweekly visits (at weeks 2, 4, 6, 8, and 10; visits may be conducted remotely via phone or video call when possible) for pill box administration and side effect evaluation. The final pill capsule will be taken at the AMC on the morning of the post-intervention study visit, after the first blood draw. Women of child bearing potential will complete pregnancy tests as part of screening procedures, at baseline, and monthly throughout the study (weeks 4, 8, and 12). Blood tests (comprehensive metabolic panel, lipid panel, CBC, TSH, A1c and leptin) will be completed at screening. The comprehensive metabolic panel will be repeated at weeks 4, 8 and 12. The lipid panel and leptin will also be repeated at week 12. A blood draw will not be completed at the baseline study day, unless it has been more than 60 days since the screening day. This blood draw will measure the complete metabolic panel to reassess liver function. On the post-intervention study day, a nurse will insert an IV for blood draws in the morning. The initial blood draw will be used to measure DMXB-A levels, lipids, complete metabolic panel, and leptin. Three more blood draws will be completed on the post-intervention study day, each of which will be used to measure DMXB-A levels. If the nurse/phlebotomist has difficulty inserting the IV, a single blood draw for lipids, complete metabolic panel, and leptin will be considered acceptable. Each group will receive one capsule per day. The drug dose to be used for this study will be 50 mg, qd. The formulation will use a methylcellulose filler (Dow Chemicals) and be prepared by a compounding pharmacy. Placebo pills identical in appearance will be given to those in the placebo group.

Drug Formulation: The DMXB-A to be used in the trial is synthesized as a dihydrochloride salt by reaction of synthetic anabaseine dihydrochloride with 2,4-dimethoxybenzaldehyde[54]. The manufacture of DMXB-A by our collaborator William Kim, Ph.D. at the University of Florida and its use here at the University of Colorado for phase-1 and phase-2 studies has been granted an Investigational New Drug exemption (IND# 126803).

D. Description, Risks and Justification of Procedures and Data Collection Tools:

After screening and baseline measures, subjects will receive approximately 12 weeks of 50mg qd DMXB-A. Subjects will be exposed to the risk of a new drug, which has been given to fewer than 1000 people. However, DMXB-A has had low toxicity in pre-clinical trials. The Ames test was negative, there were no significant pathological findings in rats or dogs, and there were no significant alterations in serum chemistries, EKG, or hematological analyses. DMXB-A has been given to over 80 normal volunteers without adverse effects (Kitagawa et al., 2003). The only effect noted in the Kitigawa et al. report (Kitagawa et al., 2003) was elevation in liver enzymes to 5 times normal value in several subjects, including those who received only placebo. There were no similar liver enzyme elevations in our Phase 1 trial (Olincy et al., 2006). There were lesser enzyme elevations in the Phase 2 trial, only rarely over 3 times normal values, but they occurred with equal frequency on DMXB-A and placebo. There was no overall trend for liver enzyme effects and none of the enzyme effects were seen at repeat testing (Freedman et al., 2008). In a Phase 1 trial in schizophrenia, one patient had a transient decrease in white blood cell count that reversed within 2 days and was related to an increase in olanzapine dose (Olincy et al., 2006). The chief symptoms have been nausea and headache, which have not significantly exceeded placebo rates. The risks of treatment with nicotinic agonists generally include cardiovascular toxicity, seizures, and gastrointestinal distress.

The subject care protocol protects subjects against risk by ensuring that they see or virtually interact with an investigator biweekly (weeks 2, 4, 6, 8, and 10) for pill counts and/or pill dispensing. If interactions are conducted virtually (via phone or video call) to minimize contact (as part of safety precautions surrounding COVID-19), pills can be delivered to participants

(while following precautions that will include wearing a mask, gloves, and practicing appropriate social distancing). During each biweekly interaction, the side effects questionnaire and the Columbia Suicide Severity Rating Scale will be completed. Visits for weeks 2, 6, and 10 may be conducted virtually (via phone or video) to minimize the number of visits to campus. Vitals will be measured as part of screening measures, at baseline, and at weeks 4, 8, and 12. Physical exams will be conducted at screening, week 8 and week 12. Subjects will be evaluated further for any adverse event, with a physical exam if indicated. A clinical laboratory assessment including comprehensive metabolic panel, lipid panel, CBC, TSH, and A1c will be completed at screening. The comprehensive metabolic panel will be repeated at weeks 4, 8 and 12. The lipid panel will also be repeated at week 12. Leptin will be measured at screening and week 12. ECG monitoring will also be conducted every 4 weeks (screening and weeks 4, 8 and 12). Women of childbearing potential will complete urine pregnancy tests at screening, baseline, and monthly throughout the study (weeks 4, 8, and 12). As part of COVID-19-related precautions, we may take a measure of the participant's temperature at the beginning of each visit to campus. If they have a fever greater than 100.4 °F, the participant will be advised to seek medical care and the visit will be rescheduled for a later time. We will also use the most recent University COVID-19 screening questions to screen for COVID-19 symptoms/risk in advance of their scheduled visit and upon their arrival to campus. If the participant endorses any symptoms, has been in unprotected contact (within 6 feet for > 30 minutes) or caring for someone who has been diagnosed with COVID-19 within the last 30 days without wearing proper PPE, or has had a diagnosis of COVID-19 in the last 30 days, the visit will be rescheduled for a later time. If they endorse symptoms of COVID-19, they will be advised to seek medical care. Additionally, we will follow space plan guidelines and any instructions from COVID-19 officials regarding physical distancing and participant contact. Research staff and participants will be required to follow current University guidelines regarding PPE (e.g., wearing masks, gloves, face shields when necessary). If the participant will be traveling to campus via public transportation or cab/rideshare, they will be instructed to follow current University guidelines regarding PPE during transportation. Subjects enrolled in the study during the COVID-19 pandemic campus closure will be contacted via email to re-start the study if they are interested. Participants that are interested will be re-consented and informed of the changes made to the consent form. Since participants already passed the screening for the study, only safety measures including a CMP and ECG will be collected to ensure participants still qualify prior to repeating the baseline measures and intervention.

Run-In Diet: A 1-day eucaloric, run-in diet will be performed prior to the study day during each of the study phase assessments. The caloric value of the diet will be determined by taking into account age, gender and weight, adjusted for physical activity, and will have a macronutrient composition of approximately 50% carbohydrate, 30% fat, and 20% protein. This approach has been used effectively by our group in a number of prior studies[42, 44, 57]. All meals will be prepared by the metabolic kitchen of the Anschutz Health and Wellness Center (AHWC) or the CTRC. Subjects will report to the AHWC or CTRC in the morning, be weighed, eat breakfast on the unit, and pick up food for the remaining meals (lunch, dinner, snack). Subjects will be asked to return containers and uneaten food from the previous day when they arrive the next day for the study day. Subjects will be asked to not consume any other food or beverages and to maintain usual activities.

Resting Metabolic Rate (RMR): After approximately 30 minutes of quiet rest, RMR may be measured prior to lunch on each study day, using standard indirect calorimetry with the ventilated hood technique (Parvo Medics Truemax 2400). It is acceptable to omit this measure if data cannot be collected in accordance with COVID-19-related University and CTRC guidelines, or if participants/staff feel it would be safer to omit it.

Body Composition: Body composition will be measured using BOD POD air displacement plethysmography (Life Measurement, Inc., Concord, CA) at baseline and after the intervention. This measure takes approximately 2 minutes to complete.

Blood Analyses: Blood analyses will take place as follows (also see study procedures table, p.7): Complete metabolic panel at screening and at weeks 4, 8 and 12. Lipid panel and leptin at screening and week 12. CBC, TSH, A1c, and cotinine at screening. At the post-intervention study day (week 12 visit), we will utilize an IV catheter to facilitate 4 separate blood draws to measure DMXB-A blood levels. These draws will take place immediately prior to morning study drug dosing, and approximately 1 hour post-dose, 2 hours post-dose and 6 hours post-dose. If the nurse/phlebotomist has difficulty inserting the IV, a single blood draw for lipids, complete metabolic panel, and leptin will be considered acceptable.

During the first draw of the post-intervention study day, blood for CBC, lipid panel, and leptin measures will be collected at the same time as blood for DMXB-A measurement.

Appetite Evaluations: Subjects will complete visual analog scale (VAS) questions (hunger, appetite, satiety) as we have used in a number of prior studies[44, 57-59]. Hunger will be rated on a line preceded by the question, "How hungry are you right now?" and anchored on the left by "not at all hungry" and by "extremely hungry" on the right. Other questions will address appetite and satiety, with the anchors "not at all..." and "extremely..." Subjects will complete these VAS questions at approximately 30, 90, 120, 150,180, and 210 minutes post breakfast meal on each study day.

fMRI Tasks: The primary imaging measure for this study will be neuronal response to visual food cues. Subjects will undergo fMRI while viewing images of food and nonfood objects described previously. Two runs, each ~7 minutes, will be performed. Each run will consist of a blocked design with 6 blocks of pictures of “high-calorie” foods , 6 of “low-calorie” foods, 6 of nonfood related objects, and 6 blocks of a low-level baseline fixation condition, consisting of 3 crosses centered in a black screen. Conditions will be pseudo-randomized with presentation order balanced across each run. All blocks will be 16 s in duration. Four additional images will be acquired in the beginning of each run to eliminate saturation effects. Following the visual food cue task, subjects will perform resting state imaging while approximately 10 minutes of EPI data are acquired.

Visual Stimuli Evaluations: Subjects will be asked to rate ‘food appeal’ and ‘desire to eat’ of the visual stimuli presented to them during the fMRI sessions after the fMRI session is completed (outside of the MRI scanner). Images of food will be randomly displayed and subjects will be asked to rate each on a visual scale of 0-100.

Breakfast on Study Day: Following the fasting fMRI session, subjects will consume a standard breakfast meal over approximately 20 minutes. The caloric content will equal approximately 25% of the total daily requirement and will have a macronutrient composition identical to the run-in diet. After about 30 minutes of rest, subjects will undergo a repeat fMRI to measure neuronal activity in the acutely fed state.

Ad-Libitum Buffet Lunch: On each study day after the final blood draw and appetite evaluations, subjects will be offered an ad libitum lunch. The purpose of this will be to quantify food intake and macronutrient content using weigh and measure methods. A research dietician will work with the subject to provide a meal diet that replicates, to the extent practical/possible, a usual lunch consumed by the subject. The meal will be consumed at the Anschutz Health and Wellness Center or CTRC and offered in a ‘buffet’ style with approximately 15% more food than predicted and the option to get more as desired. Subjects will be instructed to eat what they want and that they can request more of any food. This design should neither restrict intake, nor encourage over consumption.

Plans to Minimize Risk:

DMXB-A

The subject care protocol protects subjects against risk by ensuring that they see an investigator biweekly for pill counts and pill dispensing. At each visit, the side effects questionnaire and Columbia Suicide Severity Rating Scale will be completed. The subject is evaluated further for any adverse event, with a physical exam if indicated. A comprehensive metabolic panel will be repeated at screening and at weeks 4, 8 and 12, to assess liver function. CBC, TSH and A1c tests will be completed at screening. A lipid panel will be completed at screening and week 12. Women will complete pregnancy tests at screening, baseline, and monthly throughout the study (weeks 4, 8, and 12).

Criteria for a subject to continue in the study will include the ability to attend biweekly appointments. Subjects who cannot comply or who express desire to leave the study because of diminished tolerability will be withdrawn. The stopping criteria are well defined. Specifically, any laboratory or vital sign value that is over five standard deviations from the mean, or a significant abnormality on physical exam, will lead to discontinuation of that subject. Changes over two times normal in laboratory values will be followed up immediately with repeat assessment. If the change persists, the subject will be stopped. An increase of serum transaminases to > 3x ULN will be followed by repeat testing of ALT, AST, ALP, and TBL within 48 to 72 hours. If repeat testing still shows levels >3x ULN, close observation will be initiated to determine whether abnormalities are improving or worsening. This threshold was chosen as per the stated guidelines in the FDA Guidance for Industry Drug-Induced Liver Injury: Premarketing Clinical Evaluation, which suggest >3x ULN as a reasonable threshold, as lesser elevations are common and nonspecific. If close monitoring is not possible, the subject will discontinue the drug and stop the study. Close observation will include repeating liver enzyme and bilirubin tests 2 times per week. Frequency of retesting can decrease to one per week or less if abnormalities stabilize. In addition, close monitoring will include obtaining a more detailed history of prior or concurrent diseases, repeated administration of the concomitant medications questionnaire, obtaining a history of exposure to environmental chemical agents, and ruling out acute viral hepatitis types A, B, C, D, and E, autoimmune or alcoholic hepatitis, and cardiovascular disease. As per the Guidance for Industry Drug-Induced Liver Injury, the subject will discontinue the drug and stop the study if any of the following criteria are determined:
-ALT or AST >8xULN
-ALT or AST>5xULN for more than 2 weeks
-ALT or AST >3xULN and (TBL >2xULN or INR>1.5)
-ALT or AST >3xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and or eosinophilia (>5%)

During close observation in the event of potential liver effects, we will also evaluate data for alternative causes. If elevated levels persist, we will rule out acute viral hepatitis (via serological markers), alcoholic and autoimmune hepatitis (via history, physical examination, and serologic testing), cardiovascular causes (via history and physical examination), and concomitant treatments (via assessment of concomitant medications).
If a subject experiences a prolonged QT interval >500 ms or of > 60 ms over baseline the subject will discontinue the study and will be referred to their primary care provider for further evaluation (subjects must list a PCP to enroll in the study). If a subject experiences an adverse event suggestive of torsade de pointes, this will be treated as a medical emergency, upon which we will call 911 and the patient will be transported by ambulance to the University of Colorado Hospital emergency room. A significant adverse effect—myocardial ischemia or infarction, syncope, stroke, or seizure or other loss of consciousness, gastrointestinal blockage or severe diarrhea or emesis, or urinary retention, suicide attempt—will also lead to discontinuation of a subject. If any one of these criteria occurs in two subjects or a death in one subject while they

are being treated with DMXB-A, then the entire trial will be discontinued until the FDA, NIDDK, and the IRB, in conjunction with the investigators, have determined whether or not the trial should continue.

Resting Metabolic Rate
The test will be aborted if the subject feels too claustrophobic to continue.

BodPod
Some subjects may find this procedure slightly claustrophobic. However, due to the short measurement time (less than 2 minutes total inside the BodPod), this risk is minimal.

fMRI
If the subject becomes too anxious during MR scanning, the session will be stopped. Subjects with claustrophobia and paramagnetic metal in the body will be excluded. In addition to the initial metal screening, subjects will fill out the standard MR screening form, which contains a detailed checklist to ensure subjects are free of paramagnetic metal, prior to scanning. This form will be reviewed by the MR technologist immediately prior to scanning.

Blood Draw
Using a phlebotomist with sufficient experience in hygiene practices and proper technique minimizes these risks.

IV Catheter
The risks are minimized by using highly trained staff.

Confidentiality
To address confidentiality risks, the research team will take great care in protecting participant confidentiality, by (1) storing all data in locked file cabinets in the PI's office (which is locked when unoccupied), (2) password-protecting all electronic data, (3) identifying all data by numerical code with no personal identifying information, and (4) restricting access to all data to the research team.

D. Potential Scientific Problems:
We felt that the proposed 12 weeks would be sufficiently long to detect effects on weight, in addition to neuronal effects, while minimizing dropouts that may occur with longer durations.

F. Data Analysis Plan:
A mixed model repeated measures analysis of variance will be used to assess group differences (DMXB-A vs. placebo) in intervention-associated change from baseline to post-intervention. Potential covariates will include age, sex, and DMXB-A plasma level. Missing data will be considered missing at random. Missing data generally occur from subjects who cannot complete one arm of the test or from a technical failure. fMRI data will be processed with Statistical Parametric Mapping (SPM12, Wellcome Trust Centre for Neuroimaging) in Matlab. All data initially will be visually inspected for motion, field homogeneity and reconstruction errors. Data from each participant will be realigned to the first volume, normalized to the Montreal Neurological Institute (MNI) template using a gray-matter-segmented IR-EPI as an intermediate to improve registration, and smoothed with an 8-mm full width at half maximum Gaussian kernel to improve signal to noise ratio and account for the non-independence on neighboring voxels. For the visual food-cue task, after preprocessing, a 128-second high-pass filter will be applied to remove low-frequency fluctuation in the BOLD signal. To account for both intersubject and intrasubject variability, a random effects statistical model will be utilized. To generate the random effects model in SPM8, statistical parametric maps will first be generated for each subject using the general linear model to describe the variability of the data on a voxel by voxel basis. The hemodynamic response will be modeled with a double gamma function, without

temporal derivatives. Because the first level analysis only considers a single source of error variance, in this case scan-to-scan residual error, a second level analysis will be performed to incorporate both within subject and between subject variance, thus allowing inference to the population. This will be accomplished by summarizing each individual subject's data with one parametric map (accounting for within subject variance), and then assessing these measures across subjects (accounting for between subject variance), thereby implementing a random effects model. Single subject SPM contrast images will be entered into a second-level repeated measures ANOVA in which contrasts of interest will be evaluated. For the resting state network analysis, group ICA analyses will then be performed using the GIFT toolbox (http://icatb.sourceforge.net)[65]. The dimensionality of the data from each subject first will be reduced using principle component analysis (PCA). Data sets will then be concatenated into an aggregate data set and the number of spatially independent sources will be estimated with a minimum description length (MDL) algorithm[66]. The data will be further reduced to the number of independent components indicated by MDL. An independent component analysis (ICA) then will be performed using the infomax algorithm[67]. Individual ICA maps will then be back-reconstructed. The default network component will be identified by selecting the independent component with the highest spatial correlation to a default network mask[39, 68]. This component will be entered into a second-level model in SPM12.

G. Summarize Knowledge to be Gained:

Obesity is a serious and growing health problem in the United States. Obesity is associated with health problems such as type 2 diabetes and cardiovascular disease, leading to substantially diminished quality of life and increased mortality. Given the health and quality-of-life effects of obesity, developing effective treatments clearly is an important goal.

H. References:

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